

Quantitative Microbiology in Food Processing

Modeling the Microbial Ecology



Edited by **Anderson de Souza Sant'Ana**

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Modeling the Microbial Ecology

EDITED BY

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PART I

Introductory section

CHAPTER 1

Introduction to the microbial ecology of foods

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1.1 Introduction

Food products become a microbial ecosystem when they are contaminated and colonized by microorganisms. Fresh foods allow rapid microbial growth due to a high content of nutrients whereas processed foods correspond to a harsher environment for growth, reducing the natural microbial population associated with raw food. In addition to natural microbiota related to its origin and environmental conditions, food may be contaminated from outside sources during production, processing, storage, transport, and distribution. Hence, growth and activities of microorganisms (bacteria, yeasts, and molds) are some of the major causes of food spoilage. However, few microorganisms are pathogens while many are useful in producing desirable changes during food fermentation. A large number of microorganisms can simultaneously grow in food if the abundance of nutrients is sufficient. As a consequence, the diversity and occurrence of microorganisms present depend on the composition of food, the extent of microbial contamination, and the treatments applied. Finally, intrinsic and extrinsic factors such as temperature, water content, and oxygen content have a considerable influence on the growth of microorganisms, depending on the properties of the microorganisms and on the interactions among them.

Microbial ecology of food concerns the study of the type of microorganisms present

(diversity and structure), their rate of occurrence, activities (functionality), and interactions with each other (microbial communities) and their environment. Ecological studies also help to understand the transmission and dissemination of pathogens and toxins. Microbial ecology is intimately connected with microbial physiology as ecophysiological parameters determine the activities within individual cells and thus the responses of microbial populations to environmental influences. These combined effects control the type of microorganisms capable of growth in a particular food ecosystem (Leistner, 2000; McMeekin *et al.*, 2010).

Quantitative microbial ecology relies on predictive microbiology to forecast the quantitative evolution of microbial populations over time, using models that include the mechanisms governing population dynamics and the characteristics of food environments. In this respect, the diversity of the microbial community of a food ecosystem must be assessed, along with the identification of species and their comparative quantification. Traditional microbiological techniques (culture-dependent methods) have been used for decades for this purpose. However, these methods give a single viewpoint for describing a portion of the microbial dynamics and estimating microbial diversity. Culture-independent techniques based on direct analysis of genetic materials (DNA or RNA) are increasingly being used for characterization of microbial diversity structure and function. The

development of these molecular methods and their applications in the field of microbial ecology of food has transformed our understanding of the nature and evolution of microbial populations and their metabolic activities (Ndoye *et al.*, 2011).

This introductory chapter aims at providing some background in order to set the stage for further study of predictive microbiology, unit operations, processes, and the microbial ecology of specific categories of food products in the subsequent chapters.

1.2 Role of food characteristics and environment on microbial fate

Foods are classified as non-perishable for those that do not need time/temperature control, semi-perishable for those that remain unspoiled for a prolonged period and perishable for those that need time/temperature control to kill or prevent the growth and activities of microorganisms in order to extend their shelf life.

In 1971, Mossel defined four groups of ecophysiological parameters that influence the survival or growth of the microorganisms contaminating a raw or processed food: (i) intrinsic factors that are essentially chemical but with some important intrinsic factors that are physical and structural (e.g., pH, water activity, redox potential, available nutrients, presence of antimicrobial substances, food matrix); (ii) extrinsic factors that include the externally applied factors (e.g., temperature, relative humidity, etc.); (iii) implicit factors that are mostly dependent on the physiological properties of the microorganisms and microbial interactions; and (iv) processing factors (heat destruction, smoke, salts, organic acids, preservatives, and other additives) and conditions affecting foods (slicing, mixing, removing, washing, shredding, etc.) as well as influencing transfer of microorganisms (cross-contamination events) (Gould, 1992; ICFMS, 1980; Mossel, 1971; McMeekin and Ross, 1996).

In the context of quantitative microbial ecology, the growth of microorganisms could be modeled and then predicted as a function of only a few ecophysiological parameters such as temperature, pH, and water activity (a_w), sometimes with other factors such as the presence of preservatives and oxygen. Growth of a specific microorganism also depends on the initial microbial load, the sources of nitrogen and carbon, the processing method used in the food production, and the external environment of the food during storage, distribution, sale and handling. The physicochemical properties of foods in association with environmental conditions determine the selection of microorganisms capable of growing and multiplying at the expense of other less competitive species. As a result, the whole microbial ecology of the food system should be considered to accurately predict food spoilage (Braun and Sutherland, 2006). Such an integrated microbial model must take into consideration all these factors as input variables along with modeling parameters representing the processes applied during food manufacture and storage (Figure 1.1).

1.2.1 Temperature

The lag period and growth rate of a microorganism are affected by temperature as growth can be inhibited by decrease or increase of temperature below or above the optimum growth range. Indeed, every microorganism has a defined temperature range in which they grow, with a minimum, maximum, and optimum within the extended range of -5 to 90°C (Table 1.1). Organisms causing food spoilage can be grouped by temperature preference as (i) mesophiles (optimum temperature 30 – 45°C , minimum growth temperature ranging from 5 to 10°C and a maximum of 50°C); (ii) psychrophilic organisms (optimum growth range temperature of 12 to 15°C with a maximum range of 15 to 20°C); (iii) psychrotropes (formerly called psychrotrophs with an optimum temperature 25 – 30°C with a minimum of -0.4 to 5°C); and (iv) thermophiles (optimum temperature 55 – 75°C with a maximum as high as 90°C and a minimum of around 40°C).

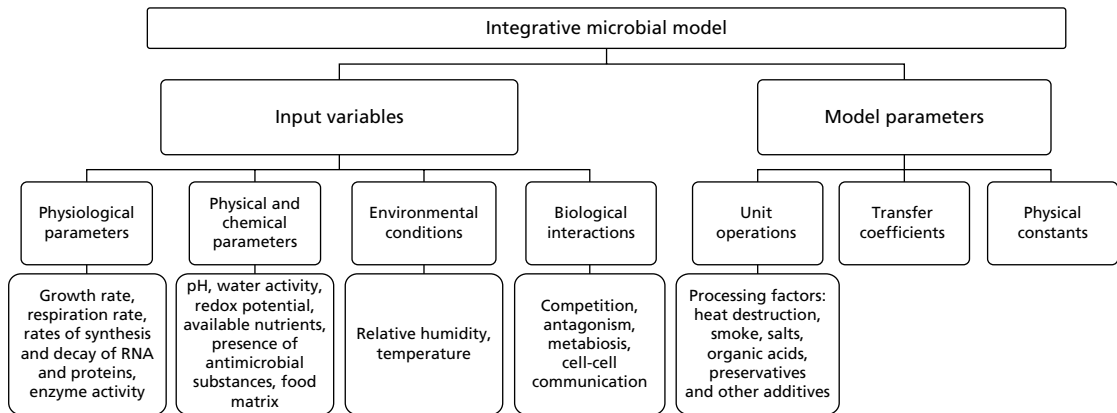


Figure 1.1 Integrative parameters affecting the development of microbial ecosystems in food.

Table 1.1 Psychrophilic, psychrotropic, mesophilic, and thermophilic microorganisms of importance in food.

Group	Temperature (°C)	Examples of bacteria (genus name only)
Psychrophiles	–5 to 20	<i>Acinetobacter</i> , <i>Bacillus</i> , <i>Clostridium</i> , <i>Flavobacterium</i> , <i>Vibrio</i>
Psychrotropes	–5 to 35	<i>Pseudomonas</i> , <i>Enterococcus</i> , <i>Alcaligenes</i> , <i>Shewanella</i> , <i>Brochothrix</i> , <i>Corynebacterium</i> , <i>Lactobacillus</i> , <i>Listeria</i> , <i>Micrococcus</i> , <i>Moraxella</i> , <i>Pectobacterium</i> , <i>Psychrobacter</i>
Mesophiles	5 to 47	<i>Bacillus</i> , <i>Carnobacterium</i> , <i>Clostridium</i> , <i>Corynebacterium</i> , <i>Escherichia</i> , <i>Lactobacillus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> , <i>Listeria</i> , <i>Hafnia</i> , <i>Pseudomonas</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Staphylococcus</i> , <i>Vibrio</i> , <i>Yersinia</i>
Thermophiles	40 to 90	<i>Bacillus</i> , <i>Paenibacillus</i> , <i>Clostridium</i> , <i>Geobacillus</i> , <i>Alicyclobacillus</i> , <i>Thermoanaerobacter</i>

Adapted from ICMFS (1980) and Jay (2005).

Changes in storage temperature as well as the time–temperature relationship have an impact on the evolution of these different groups. Refrigeration and chill temperatures promote growth of psychrophilic microorganisms, of which there are few that affect food spoilage, and psychrotrophic spoilage organisms such as *Pseudomonas*, yeasts, and molds as well as pathogens such as *Listeria monocytogenes*. At high temperatures, spore-forming bacteria and lactic acid bacteria are able to multiply. Thus, temperature changes have an influence on the metabolic activities of some microorganisms and consequently on the biochemistry of the spoilage process. Time has an impact in relation to the storage temperature because it is a factor that influences

the rate of growth of microorganisms: extended storage at low temperatures allows the growth of some psychrotrophic microorganisms. Further discussion on food safety and the role of quantification in microbial risk assessment will be given in Part IV of this book.

1.2.2 pH and acidity

The pH is a measure of acidity of a food that influences microbial growth and survival, as every microorganism possesses a minimum, an optimum, and a maximum pH for growth. Most bacteria exhibit an optimum pH near the neutral point (pH 7.0) although acetic and lactic acid bacteria are able to survive at reduced pH levels. Molds and yeasts are generally more

acid-tolerant than bacteria and therefore acidic foods are more susceptible to spoilage by these types of microorganisms.

Low pH values and associated high acid concentrations inhibit microbial growth and survival in foods due to the acid-induced denaturation of cell wall proteins. A decrease of pH also reduces the heat resistance of microorganisms. Moreover, the pH can interact with water activity, redox potential, salt, and preservatives to inhibit growth of food-borne pathogens and spoilage microorganisms. The undissociated form of weak acids shows antimicrobial activity because they pass freely through the cell membrane and then dissociate, as the cytoplasmic pH is usually higher than that of the growth medium. This leads to the release of protons, which in turn results in an acidification of the cytoplasm. Bacterial growth can be prevented by addition of weak organic acids alone or in combination with other preservatives as well as by production of lactic and acetic acids by fermentation.

Food products can essentially be divided into three types according to their pH: (i) low-acid foods where pH is greater than 4.6 and less than 7.0, (ii) acid foods that have a pH lower than 4.6, and (iii) acidified foods obtained by addition of acids into low-acid foods. This classification is based on the fact that pathogenic microorganisms generally cannot grow at pH values below 4.6. Low-acid foods can be fermented (fermented foods) by acid-producing bacteria that reduce the pH below 4.6. Foods can also be characterized by their buffering capacity, which is defined as the ability to resist changes in pH. The pH of foods with a low buffering capacity in the presence of acidic or alkaline compounds produced by microorganisms will change quickly, whereas foods with a high buffering capacity are more resistant to pH changes.

1.2.3 Water activity

Water is a requirement for growth and metabolic activities of microorganisms in a food product. However, microbes can only use water

in an available form. Free water that is not in the bound state participates in many chemical and biochemical reactions, supports microbial growth, and acts as a transporting medium for compounds (sugars, salt, organic acids) in the food system. Water activity (a_w), defined as the free or available water in a food, is therefore a better indicator for microbial growth than the water content. In a food matrix, the requirements for moisture by microorganisms are expressed in terms of a_w (the a_w of pure water is 1.00 and the a_w of a completely dehydrated food is 0.00) and the lower limit for microbial growth in a food product will be determined by the a_w . Food products can thus be broadly classified by water activity into (i) high a_w (>0.92), (ii) intermediate a_w (0.85 to 0.92), and (iii) low a_w (<0.85). Fresh foods (meat, vegetables, and fruits) generally have a_w values higher than 0.97. By reducing water activity below 0.7, osmotic pressure is increased, thus inhibiting microbial growth and maximizing the shelf life of the food product. This reduction can be accomplished by adding sugars or salt, removing water by drying or baking, and binding the water to various macromolecular components such as cellulose, protein, or starch in the food.

Microorganisms exhibit optimum and minimum levels of a_w for growth, depending on a number of other ecophysiological factors (pH, temperature, oxido-reduction level, and nutrients). Bacteria are more sensitive than yeasts and molds, and Gram-positive bacteria are more resistant to lower values of a_w than Gram-negative bacteria. The growth of food-borne pathogens is inhibited below a_w 0.86, except that *Staphylococcus aureus* can grow down to a value of 0.83 and produces toxin below a_w 0.90. Growth of molds will be controlled at a_w 0.80 and mycotoxin production requires a higher a_w than that of growth.

1.2.4 Oxygen and redox potential

Based on their oxygen requirements and tolerance, microorganisms are classified into the following groups: (i) obligate aerobes are microorganisms that require oxygen for growth;

(ii) obligate anaerobes are microorganisms that do not need or use oxygen, which is toxic for them; and (iii) facultative aerobes (or facultative anaerobes) are microorganisms that can grow in the presence and absence of oxygen, switching to aerobic respiration in the presence of oxygen but under anaerobic conditions they grow by fermentation or anaerobic respiration.

The oxidation–reduction or redox potential is an intrinsic factor that influences the growth of microorganisms in foods. The redox potential of the food varies according to the physico-chemical characteristics, partial pressure of oxygen, and the presence of other gases in the storage atmosphere (water vapor, nitrogen, CO_2). The presence of substances that are highly hydrogenated, that contain SH radicals, reducing sugars, or other compounds such as ascorbic acid (vitamin C) and tocopherols (vitamin E) in a food creates a reducing environment. When the redox potential (E_h) is negative in terms of millivolts, this means a reduced state, while the presence of oxygen at the surface or in the bulk has an oxidizing effect (an oxidized state). Aerobic organisms require a food environment with a positive redox potential (+500 to +300 mV) whereas anaerobes require a negative potential (+100 to less than –250 mV) and facultative anaerobes tolerate a range in potential between +300 and –100 mV.

1.2.5 Nutrient content

The nutritional needs of microorganisms can usually be met in foods due to the presence of water, carbohydrates (sources of carbon and energy), fats, proteins, vitamins, and minerals. However, these nutrients must be available in an easily digestible form, such as simple sugars and amino acids, for many microorganisms. Some microorganisms possess specific enzymes that allow them to degrade more complex structures such as proteins and fibers. Most spoilage microorganisms have no fastidious nutritional requirements and possess essential metabolic activities such as glycolysis and proteolysis. In this way, a complex microbial community capable of degrading the nutrients present will

colonize any type of food. Therefore, it is practically impossible to predict the microbial ecology of a food based on its nutrient composition.

1.2.6 Physical structure and microenvironments

The physical barriers to food spoilage by microorganisms are: (i) the skin of fish and meats, (ii) the shell of nuts and eggs, (iii) the external layers of seeds, and (iv) the outer covering of fruits and vegetables such as the husk or rind. These protective biological structures are usually composed of macromolecules that are relatively resistant to penetration or degradation. They constitute hostile microenvironments for the growth of microorganisms by having a low water activity, a lack of readily available nutrients, and a presence of antimicrobial compounds such as short-chain fatty acids (on animal skin) or essential oils (on plant surfaces). During the preparation of foods, processes such as cutting, grinding, and heating break down the biological barriers and change microenvironments, thus favoring contamination and proliferation of microbes inside the food product. The impact of these unit operations on specific microorganisms will be further detailed in Part II of this book. Most microorganisms will grow in the majority of foods, as individual free-floating (planktonic) cells in the aqueous phase or as an association of microbial cells with a solid substrate either through entrapment, constrained growth, attachment, or a combination of these factors (Skandamis and Nychas, 2012).

1.2.7 Food preservation processes (antimicrobials, preservatives)

Food preservation mainly involves a prevention or exclusion of microbial activity. This may be achieved: (i) by inhibiting the growth or shortening the survival of microorganisms, (ii) by excluding or removing microorganisms, and (iii) by killing the microorganisms. Some plant- and animal-based foods contain natural antimicrobial compounds such as essential oils and lysozyme, respectively, that inhibit the growth of spoilage microorganisms. Some chemical

food additives such as salts, sugars, and organic acids are commonly applied for creating a hostile environment in food products. The presence of gases (carbon dioxide, ozone, and oxygen) is also able to inhibit the growth and proliferation of microorganisms by direct toxic effects and by indirect inhibitory effects, by modifying the gas composition and thus altering the ecology of the microbial environment. Various types of food processing such as heating, smoking, and fermentation are also used for the formation of antimicrobial substances in food. Part II of this book contains more information on specific food preservation operations, including fermentation. Food fermentation is one of the oldest food processing technologies that can suppress the growth and survival of spoilage microorganisms in food products. This process depends on the biological activity of microorganisms that produce a large range of metabolites (acids, alcohols, and carbon dioxide) by fermentation or oxidation of carbohydrates or derivatives. For example, among members of competitive microbiota, lactic acid bacteria (LAB) exhibit unique metabolic activities and are employed as starters for the fermentation of milk, meats, cereals, and vegetables and are used as probiotic cultures (Champagne *et al.*, 2005). In addition to the production of lactic, acetic, and propionic acids leading to an acidic environment appropriate for controlling the growth and metabolic activity of many pathogenic and spoilage microorganisms, these beneficial bacteria are also able to produce ethanol, hydrogen peroxide (H_2O_2), diacetyl, and bacteriocins. Bacteriocins are protein or peptide antimicrobial substances that inactivate other bacteria through depolarization of the target cell membrane or through inhibition of cell wall synthesis. In addition, LAB can also produce antifungal compounds, including reuterin, carboxylic acids, cyclic dipeptides, and fatty acids (Crowley *et al.*, 2013).

Antimicrobial substances produced by microorganisms provide an additional hurdle for keeping the natural population of microorganisms under control. Indeed, traditional food

preservation has often been achieved by the combination and interactions of pH, a_w , atmosphere, numerous preservatives, and other inhibitory factors, referred to as the “hurdle effect”. These preservative factors (hurdles) temporarily or permanently disturb the homeostasis of microorganisms, defined as the tendency to uniformity and stability in the internal status of an organism; microorganisms remain in the lag phase or even die before homeostasis is re-established (Leistner, 2000).

1.3 Understanding microbial growth, death, persistence, competition, antagonism and survival in food

1.3.1 Principles of microbial growth

In a food environment where nutrients are not limiting, microbial cells increase in number in a characteristic manner and at a specific rate as determined by their genetic traits. It is well known by microbiologists that the growth curve exhibits four different phases: (i) the lag phase in which microorganisms, by a series of biochemical activities, acclimate to their environment and initiate cell reproduction and growth; (ii) the exponential or log phase, where cell components are synthesized in order to allow cell replication at a logarithmic rate determined by their generation time and ability to assimilate the substrate; (iii) the stationary phase, which begins when a microbial population tends to stabilize due to accumulation of metabolic end-products and limitations in substrates necessary for growth, leading to reduction of the growth rate; and (iv) the decline or death phase, when microorganisms die and lyse (autolysis) due to nutrient depletion and the toxic effects of metabolic end-products.

1.3.2 Survival

Microbial populations in foods are subjected to stressful conditions such as low or high temperatures, acidity, low water activity, modified

atmospheres, or nutrient deprivation. By a variety of strategies, microorganisms attempt to resist and adapt to these hostile conditions, constantly switching between growth and merely surviving. The stress response, which results in a characteristic change in the pattern of gene expression, helps to restore cellular homeostasis and increase resistance to subsequent stressful conditions. Although death is an irreversible state, bacterial cells can be sublethally injured or enter a dormant state. These cells may repair the damage caused by the hostile environment and survive, even growing when conditions become favorable (Aertsen and Michiels, 2004; Wesche *et al.*, 2009). The stress response in relation to microbial ecology of food will be discussed in Part IV of this book.

1.3.2.1 The viable but non-culturable state

Many stressed organisms may regain the characteristics of normal cells, but some severely injured cells remain metabolically active but cannot be resuscitated under routine laboratory conditions, entering a viable but non-culturable (VBNC) state (Wesche *et al.*, 2009). The VBNC state can be a significant means of survival if the cells have the ability to increase metabolic activity and become culturable once resuscitated (Oliver, 2005).

A large number of non-spore-forming bacteria are capable of entering the VBNC state. Although the exact role of this state in bacteria is yet to be elucidated, it can be induced by stressful conditions such as nutrient starvation, temperature, osmotic concentration, oxygen concentration, and food preservatives. Hence, the VBNC state might be an adaptive strategy for long-term survival of bacteria under unfavorable environmental conditions. In contrast to dead cells that have a damaged membrane, VBNC cells have an intact membrane retaining chromosomal and plasmid DNA and differ from “injured” bacteria that are unable to grow on selective media. VBNC cells do not grow on any medium, even if non-selective. However, VBNC bacteria have higher resistance to physical and

chemical stresses than culturable cells and can resuscitate when environmental conditions become favorable (Li *et al.*, 2014; Oliver, 2005, 2010).

1.3.3 Strategies for persistence

Many microorganisms associated with food survive treatments such as heat and disinfection, so they persist during storage and their numbers remain unchanged.

1.3.3.1 Sporulation

Some bacteria can form spores as a defense mechanism against unfavorable environmental conditions (e.g., Gram-positive bacteria such as *Bacillus* and *Clostridium*). Indeed, endospores are very resistant structures with no measurable metabolism but can confer a great advantage for these bacteria to persist for prolonged periods of time and endure extreme stress conditions (high temperatures and UV irradiation, extreme freezing, desiccation, chemical damage by disinfectants, and enzymatic destruction). Under favorable environmental conditions, the endospore can undergo activation and germination. Hence, metabolic activity is restored and the cell becomes vegetative.

1.3.3.2 Biofilm formation

Generally, bacteria do not live freely in suspension as planktonic cells and biofilms protect them from desiccation, bacteriophages, and sanitizing agents. Biofilm formation thus constitutes one of the survival strategies of microorganisms in hostile environments. The persistence of food-borne pathogens and spoilage microorganisms on foods and food contact surfaces often adversely affects the quality and safety of raw and minimally processed foods.

1.3.4 Competition

The composition of the microbial community of a food varies according to many ecophysiological factors that have been described so far. However, the ecosystem is also altered by the interactions among microbes themselves.

Mixed cultures in food fermentation processes represent some of the best examples of microbial interactions. Microbe–microbe interactions can be classified as positive (+), neutral (0), and negative (–). These interactions can be further subdivided into: (i) mutualism (+/+ interaction: both microbes involved benefit from the interaction, e.g., synergism or proto-cooperation among yogurt bacteria or mutualism between yeast and bacteria during sourdough fermentation); (ii) commensalism (+/0 interaction: one organism benefits from the interaction while the other is not affected, e.g., cultivation of propionic bacteria in the presence of LAB in Swiss-type cheese); (iii) amensalism (–/0 interaction: interspecies interaction in which one organism adversely affects the other without being affected itself, e.g., bacteriocin production by LAB and ethanol production by yeasts); (iv) parasitism (+/– interaction: one species benefits at the expense of another, e.g., bacteriophages in fermentations); and (v) competition (–/– interaction: two or more species, strains, or subpopulations of microbes compete for energy sources and nutrients) (Ivey *et al.*, 2013; Sieuwerts *et al.*, 2008; Smid and Lacroix, 2013).

Microorganisms compete for nutrients, adhesion/attachment sites, as well as by their ability to alter the environment by producing metabolites. Preservation methods combined with ecophysiological factors and the genetic characteristics of each microorganism (lag phase, growth rate, and total cell biomass yield) lead to selection of microbial associations of a particular food at any given point in time during production and storage. For example, psychrotrophic bacteria such as *Pseudomonas* spp. dominate proteinaceous foods (meat, poultry, milk, and fish) stored at refrigeration temperatures under aerobic conditions. In meat and fish products, a change in the atmosphere (e.g., vacuum packaging) promotes LAB at the expense of *Pseudomonas*. Microbial ecology of food provides a comprehensive overview of the dominance of an organism based on its origin, substrate composition, temperature,

pH, a_w , and atmosphere, regardless of raw material and processing.

In addition to these conditions determining the association of microbiota in food, there are three aspects of microbial interaction that must be taken into consideration according to Gram *et al.* (2002), namely: (i) antagonism, (ii) metabiosis, and (iii) cell-to-cell communication.

1.3.4.1 Antagonism

In addition to changes in environmental conditions such as lowering pH by acid-producing microorganisms, antagonistic abilities include competition for nutrients. Scavenging growth-limiting compounds such as iron represents one type of nutritional competition. Microorganisms with higher metabolic activity may selectively consume required nutrients, resulting in growth inhibition of other organisms with lower activity. The growth rate of particular microorganisms may also be affected by an overgrowing microbiota in a phenomenon described as the “Jameson effect”, which is essentially non-specific nutrient competition (Gram *et al.*, 2002).

1.3.4.2 Metabiosis

The microbial profile of a food evolves over time because of the changes in environmental conditions caused by the action of the community on the supply of nutrients from limiting metabolic compounds. This refers to the term “metabiosis”, which describes the interrelationships among microorganisms to produce a given environment (Gram *et al.*, 2002).

1.3.4.3 Cell-to-cell communication

The role of quorum sensing (QS) or cell-to-cell communication in food microbial ecology is now considered a microbial behavioral pattern that is correlated with the density of the microbial population and with the ability to regulate gene expression as a function of cell density (Gram *et al.*, 2002; Skandamis and Nychas, 2012). In Part IV of this book, the quantitative aspects of quorum sensing will be applied to microbial ecology.

The food matrix is composed of interconnected microenvironments where the levels of intrinsic ecophysiological factors (oxygen, pH, a_w , nutrients, preservatives, and antimicrobial compounds) may change. A large variety of microorganisms proliferates as microcolonies or biofilms and reaches high densities (10^7 – 10^9 cfu/g) in these *in situ* environments. The growth and activity of any one species or strain will be determined by the presence of other species since we can assume that quorum or other sensing molecules are released (*in situ* cell-to-cell ecological interactions). Microorganisms interact and influence the growth of one another by synthesizing specific low-molecular-weight diffusible signaling molecules as a function of population density: (i) Gram-negative bacteria produce and utilize *N*-acyl homoserine lactones (AHLs) or autoinducer-1 (AI-1) for intraspecies communication; (ii) autoinducing peptides (AIPs) are produced and used by Gram-positive bacteria for intraspecies communication; and (iii) furanosyl borate diester derivatives or autoinducer-2 (AI-2) are produced by both Gram-positive and Gram-negative bacteria and seem to serve as a universal language for inter- and intraspecies communication (Bai and Rai, 2011).

1.4 Methods to study the microbial ecology of foods

The aims of microbial ecology studies are to determine changes in microbial populations by characterizing community structure, diversity, activity, and interactions in their natural environments. The three basic questions that detection methods must answer are: (i) “who is there?” by identifying the types of microorganisms such as food-borne pathogens, spoilage microorganisms, starter cultures, or potentially probiotic and beneficial microorganisms present in the specific food environment; (ii) “who is doing what?” by assigning functional roles to these microorganisms; and (iii) “how do the activities of these microorganisms contribute to

specific ecosystem functions or processes” (Ndoye *et al.*, 2011; Ercolini, 2013).

Culture-independent methods can circumvent the limitations of traditional microbiological methods for the analysis of complex microbial ecosystems. These methods have been used in various types of foods, especially for cheese in recent years (Ndoye *et al.*, 2011). In contrast to conventional microbiological techniques based on cultivation of the microorganisms on media and phenotypic or genotypic characterization of a fraction of the community (culture-dependent methods), culture-independent techniques are based on direct analysis of DNA or RNA for efficient characterization of whole microbial communities, evaluation of *in situ* gene expression and determination of metabolic activities of microbial populations present in a particular food product. However, both culture-dependent and culture-independent methods have limitations and should be combined as much as possible through polyphasic approaches to undertake analysis of both the community and activity of natural microbiota and spoilage microorganisms (Cocolin *et al.*, 2013; Ercolini, 2013; Ndoye *et al.*, 2011).

1.4.1 Culture-independent analysis of microbial communities

The application of molecular techniques has modified our understanding of the microbial ecology of food, allowing significant insights into all aspects of microbial populations (identification of specific isolates, changes in microbial communities, nature of the functional groups). These techniques have been classified into two major categories: (i) partial community analysis approaches and (ii) whole community analysis approaches (Rastogi and Sani, 2011).

1.4.1.1 Partial community analysis approaches

These approaches are based on the direct extraction of total DNA or RNA from the food product. Then, the genetic materials extracted from food samples, either DNA or cDNA (after

reverse transcription of the total RNA), are amplified by polymerase chain reaction (PCR)-based methods. The application of the various molecular culture-independent tools allows: (i) direct identification of members of a community and assessment of their abundance; (ii) reliable fingerprinting of complex bacterial communities; (iii) analysis of the diversity and dynamics of the dominant microbial community; (iv) comparison of spatial and temporal changes in bacterial community structure; and (v) accurate quantification of target species (Ndoye *et al.*, 2011).

1.4.1.2 Whole community analysis approaches

Next-generation DNA sequencing (NGS) or high-throughput sequencing is a hundred times faster and cheaper than the conventional Sanger approach and is already considered as the most powerful culture-independent method for analysis of all the genetic information present in total DNA/RNA extracted from food samples or pure cultures. The NGS approach provides a more global perspective on food microbial communities, including molecular mechanisms of metabolically active microorganisms in food ecosystems. Metagenomics is defined as the investigation of the collective microbial genomes retrieved directly from environmental samples. When combined with other “omics” (functional genomics, transcriptomics, proteomics, and metabolomics), these data provide deeper insight into microbial diversity and the metabolic potential of microbial communities as well as predictive models of the contribution of individual microorganisms to the development of food quality and safety (Rastogi and Sani, 2011; Solieri *et al.*, 2012).

1.5 Perspectives on applying food ecosystem modeling

Predictive microbiology, which will be detailed in Chapter 2, was originally conceived for analyzing the behavior of pure cultures of

food-borne pathogens, and then spoilage bacteria, in order to develop food processes that adequately control microbial growth throughout the shelf life of food products. For example, the growth boundary models for *L. monocytogenes* erroneously predict the growth of this pathogen, as it does not take into account the biofilm microbiota interactions (Guillier *et al.*, 2008). In addition, pH and a_w could not solely account for growth arrest in the stationary phase, without including non-specific competition for nutrients (Jameson effect). Considering factors that determine enzyme production has revealed crucial restraints on litter decomposition rates in soil (Allison *et al.*, 2012), so these metabolic factors have great potential for application to food products as well. Genome-scale metabolic models are becoming useful in analyzing interactions in multispecies microbial systems from a metabolic standpoint, requiring the integration of the ecological concept of trade-offs between individual and community fitness criteria (Zomorroddi *et al.*, 2012).

Advances in our understanding of microbial interactions will allow us to envisage more complex predictive models (Figure 1.2). Complex system science is a process of integrating a multiplicity of variables and knowledge from an array of disciplines (Perrot *et al.*, 2011). In the case of food, this means joining together the skills of mathematicians, physicists, and computer scientists with those of microbiologists to complete food science and engineering. First used in environmental ecosystem modeling, this approach is beginning to be applied in order to comprehend the Camembert cheese ripening process (Sicard *et al.*, 2012). Viability theory from complex science was employed to define an optimal trajectory for Camembert cheese ripening, which was validated through pilot studies by manipulating cheese size, relative humidity, and temperature controls. As a result, the cheese ripening process was shortened by four days without significant changes in the microorganism kinetics. The quality target was reached and the sensory properties of the cheeses produced were similar to those obtained under

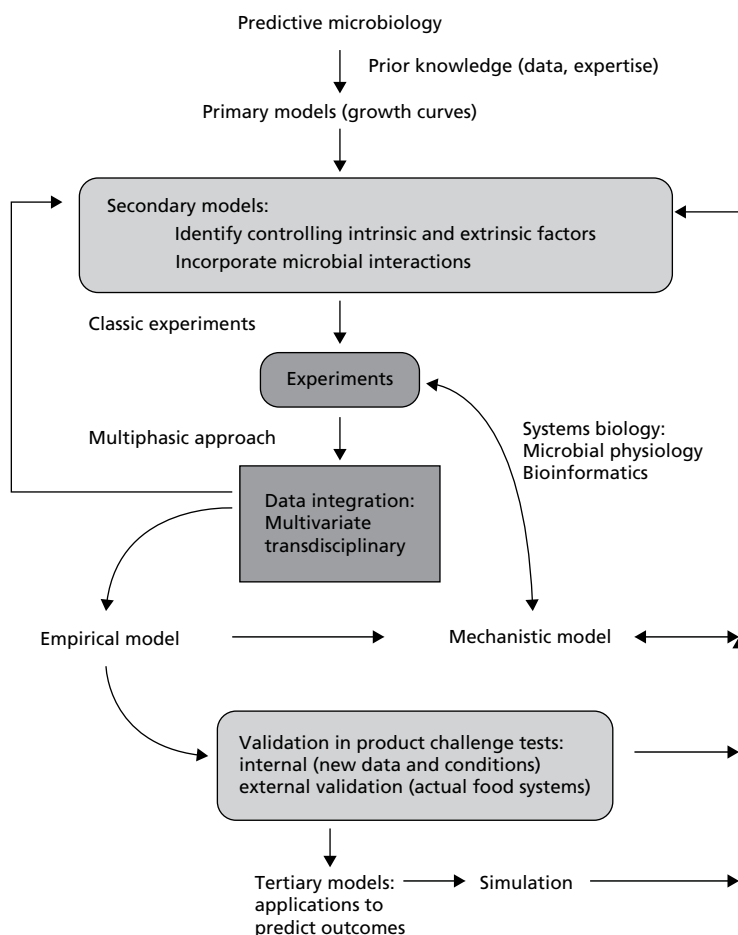


Figure 1.2 Towards developing models from predictive microbiology to quantitative microbial ecology and systems biology.

standard conditions (Sicard *et al.*, 2012). This is one example of how microbial community modeling can have a concrete impact on developing more efficient and less costly food processes.

Multiple species community modeling is still in its early stages and faces many challenges, especially applied to food. The next step, microbial community engineering, has even greater challenges and potential rewards in ensuring food quality. Continual innovation in analytical methods will contribute to improve the prospects of microbial community modeling in the future.

References

- Aertsen, A. and Michiels, C.W. 2004. Stress and how bacteria cope with death and survival. *Critical Reviews in Microbiology*, **30**, 263–273.
- Allison, S.D. 2012. A trait-based approach for modelling microbial litter decomposition. *Ecology Letters*, **15**, 1058–1070.
- Bai, A.J. and Rai, V.R. 2011. Bacterial quorum sensing and food industry. *Comprehensive Reviews in Food Science and Food Safety*, **10**, 183–193.
- Braun, P.G. and Sutherland, J.P. 2006. Modelling microbial food spoilage. In: *Food Spoilage*

- Microorganisms* (ed. Blackburn, C. de W.), pp. 86–118. Woodhead Publishing, Cambridge.
- Champagne, C.P., Gardner, N. J., and Roy, D. 2005. Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition*, **45**, 61–84.
- Cocolin, L., Alessandria, V., Dolci, P., Gorra, R., and Rantsiou, K. 2013. Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. *International Journal of Food Microbiology*, **167**, 29–43.
- Crowley, S., Mahony, J., and Sinderen, D. 2013. Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives. *Trends in Food Science and Technology*, **83**, 93–109.
- Ercolini, D. 2013. High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology*, **79**, 3148–3155.
- Gould, G.W. 1992. Ecosystem approaches to food preservation. *Journal of Applied Bacteriology*, **73**, 59S–68S.
- Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., and Givskov, M. 2002. Food spoilage – interactions between food spoilage bacteria. *International Journal of Food Microbiology*, **78**, 79–97.
- Guillier, L., Stahl, V., Hezard, B., and Briandet, R. 2008. Modelling the competitive growth between *Listeria monocytogenes* and biofilm microflora of smear cheese wooden shelves. *International Journal of Food Microbiology*, **128**, 51–57.
- ICMSF 1980. *Microbial Ecology of Foods V1: Factors Affecting Life and Death of Microorganisms*, pp. 1–37. Academic Press, New York, USA.
- Ivey, M., Massel, M., and Phister, T.G. 2013. Microbial interactions in food fermentations. *Annual Review of Food Science and Technology*, **4**, 141–162.
- Jay, J.M. 2005. Intrinsic and extrinsic parameters of food that affect microbial growth. In: *Modern Food Microbiology* (eds Jay, J.M., Loessner, M.J., and Golden, D.A.), 7th edn, pp. 39–56. Springer Science, New York, USA.
- Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, **55**, 181–186.
- Li, L., Mendis, N., Trigui, H., Oliver, J.D., and Faucher, S.P. 2014. The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology*, **5**, 258.
- McMeekin, T.A. and Ross T. 1996. Shelf life prediction: status and future possibilities. *International Journal of Food Microbiology*, **33**, 65–83.
- McMeekin, T.A., Hill, C., Wagner, M., Dahl, A., and Ross, T. 2010. Ecophysiology of food-borne pathogens: essential knowledge to improve food safety. *International Journal of Food Microbiology*, **139**, Suppl., S64–78.
- Mossel, D.A.A. 1971. Physiological and metabolic attributes of microbial groups associated with foods. *Journal of Applied Bacteriology*, **34**, 95–118.
- Ndoye, B., Rasolofo, E.A., LaPointe, G., and Roy, D. 2011. A review of the molecular approaches to investigate the diversity and activity of cheese microbiota. *Dairy Science and Technology*, **91**, 495–524.
- Oliver, J.D. 2005. The viable but nonculturable state in bacteria. *The Journal of Microbiology*, **43**, 93–100.
- Oliver, J.D. 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiology Reviews*, **34**, 415–425.
- Perrot, N., Trelea, I.C., Baudrit, C., Trystram, G., and Bourguine, P. 2011. Modelling and analysis of complex food systems: state of the art and new trends. *Trends in Food Science and Technology*, **22**, 304–314.
- Rastogi, G. and Sani, R. 2011. Molecular techniques to assess microbial community structure, function, and dynamics in the environment. In: *Microbes and Microbial Technology* (eds Ahmad, I., Ahmad, E., and Pichtel, J.), pp. 29–35. Springer, New York, USA.
- Sicard, M., Perrot, N., Reuillon, R., Mesmoudi, S., Alvarez, I., and Martin, S. 2012. A viability approach to control food processes: application to a camembert cheese ripening process. *Food Control*, **23**, 312–319.

- Sieuwert, S., de Bok, F. a M., Hugenholtz, J., and van Hylckama Vlieg, J.E.T. 2008. Unraveling microbial interactions in food fermentations: from classical to genomics approaches. *Applied and Environmental Microbiology*, **74**, 4997–5007.
- Skandamis, P.N. and Nychas, G.-J.E. 2012. Quorum sensing in the context of food microbiology. *Applied and Environmental Microbiology*, **78**, 5473–5482.
- Smid, E.J. and Lacroix, C. 2013. Microbe–microbe interactions in mixed culture food fermentations. *Current Opinion in Biotechnology*, **24**, 148–154.
- Solieri, L., Dakal, T.C., and Giudici, P. 2012. Next-generation sequencing and its potential impact on food microbial genomics. *Annals of Microbiology*, **63**, 21–37.
- Wesche, A.M., Gurtler, J.B., Marks, B.P., and Ryser, E.T. 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *Journal of Food Protection*, **72**, 1121–1138.
- Zomorroddi, A.R. and Maranas, C.D. 2012. OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities. *PLoS Computational Biology*, **8**, e1002363.

CHAPTER 2

Predictive microbiology: mathematics towards understanding the fate of food-borne microorganisms in food processing

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2.1 Introduction

Predictive food microbiology is a subdiscipline of food microbiology that uses models (i.e., mathematical equations) to describe the growth, survival, or inactivation of microbes in food systems. Mathematical growth and inactivation models are increasingly used in the context of the hazard analysis and critical control point (HACCP) system for food safety and as a means to demonstrate compliance of a product with microbiological criteria enforced by food safety and hygiene legislation.

Three ideal uses of predictive models are: (i) to narrow the choices for processing steps to be considered in an HACCP plan as having a major impact of final product safety, (ii) to assist in choosing the most resistant biological hazards (i.e., microorganisms) controlled at a specific processing step, and (iii) to define a safe operational design space without the need for additional data generation (e.g. when a model has been appropriately validated in representative food–model systems and deemed to provide ‘fail-safe’ predictions). When using predictive models in HACCP plan development the intrinsic and extrinsic factors (pH, a_w , temperature, etc.) used as inputs for the model should be chosen with care. If the conditions modeled suggest that growth could occur or that there is

limited lethality for the product/process, then additional studies, product reformulation, or modification of the target shelf life would be warranted. If there is less confidence in the model, then limited challenge studies may be warranted to verify the prediction from the model.

The long roadmap of predictive modeling over the last four decades, along with the advances towards understanding and quantifying microbial responses down to the single cell level, have led to the appointment of predictive modeling as one of the most promising decision–support methodologies for food safety assessment by the Food Industry and competent authorities. Predictive modeling has been greatly benefitted by the technological and scientific evolution in both collection and processing of data, through the introduction of –omics, the deployment of advanced microscopy techniques (e.g., confocal laser microscopy and fluorophores fused in the genome), the application of chemometrics, data mining, and the emergence of advanced data modeling techniques (e.g., artificial neural networks). As a next step, the rising trend for application of predictive modeling in daily practice has intensified the need to systematically exploit the vast number of available predictive models so far. Meeting

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this demand is being markedly achieved through the development of collective predictive modeling repositories (e.g., ComBase, Pathogen Modelling Program, iRisk, Food Spoilage and Safety Predictor, Sym'Previus, etc.) equipped with search engines for guided retrieval of the appropriate food-specific or generic models (i.e., not food-specific) associated with particular hazards and built-in fitting or simulation modules, in order to visualize and numerically express the model outputs in comprehensive and ready-to-use formats. Such a variety of predictive model and risk assessment/risk ranking software tools may indeed help the food producers, researchers, and food safety inspectors to apply the concepts of predictive modeling in quality-by-design, identification of safe product formulations, and evaluation of product compliance with safety standards and microbiological criteria. A comprehensive and quite extensive review of the available software tools can be found in the study by Tenenhaus-Aziza and Ellouze (2014) and thus the underpinning principles and validity range of these tools will not be further analyzed in the present chapter.

Models alone should be applied with caution and with proper disclaimers in the context of decision-making during HACCP plan development. Use of models requires experience and judgment, both in modeling and food microbiology. Therefore, it is of vital importance to be clearly perceived that the predictive models and associated software tools should not replace the expert opinion, but rather assist the experts (and sometimes even the non-experts) to elicit a food safety plan. When models alone are used to make a decision, those models must be shown to be valid for the food in question and should take into consideration lot-to-lot variation. Validation may be based on published or unpublished data for very similar or identical foods. Nonetheless, even in cases when the available predictions are obtained from lab-media-based models, which may potentially overlook some significant food-specific impacts on microbial behavior, such

predictions are still very useful in guiding more focused and targeted challenge testing.

Based on microbial responses, expressed as a change in numbers and stress tolerance, the combinations of intrinsic and extrinsic environmental determinants to which microorganisms may be exposed are divided into the following major domains: the growth era and the domain including the combinations that allow survival or cause death of microorganisms (Booth, 2002). The conditions that lie between these two domains refer to a zone where microbial responses are uncertain and characterized by the growth/no growth interface (Le Marc *et al.*, 2005). This zone is strongly associated with the so-called cardinal values (T , pH, a_w , etc.) for growth and outlines the biokinetic range of microbial proliferation. Such values are species- or even strain-dependent and thus introduce significant variability in the assessment of the impact of marginal growth conditions on microbial growth, an issue commonly encountered in quantitative microbial risk assessment. To remedy that, models have been proposed that embed the theoretical growth-limiting values for critical hurdles, such as temperature, a_w , pH, %CO₂, and preservatives, as biological meaningful parameters in the model structure. Notably, a theoretical interface also exists between survival and inactivation separating combinations that cause growth cessation but not cellular death from those that are lethal (McKellar *et al.*, 2002).

Different processes and product formulations determine the dominant phenotype (i.e., growth or inactivation) and proper mathematical models should be retrieved to accurately (or, better to say, realistically) describe microbial dynamics. In this chapter, the available classical and modern modeling approaches are categorized according to the existing food processes and associated microbial responses, starting from below the growth boundaries and extending to the superoptimal growth limits. The available model structures are detailed in each category.

2.2 Probability and kinetic models for food processing and HACCP

The probability of growth models constitutes the cornerstone of predicting microbial dynamics, acting as the filter of the primary microbial response (growth or inactivation) and guiding the selection of the subsequent modeling tool, i.e., growth or inactivation model, for predicting the change in microbial numbers along processing or distribution. Such models together with cardinal growth models may be of great assistance to HACCP, by offering science-based numerical evidence for setting critical limits, establishing process or product criteria, and assessing the compliance of a given process to these limits.

2.2.1 Probability of growth models

Probability models can be used to predict the likelihood of the occurrence of a microbial response as a function of intrinsic and extrinsic factors of foods and processing environment (Ross and Dalgaard, 2004). Microbial responses that have been modeled using this approach include spore germination, toxin formation by *Clostridium botulinum*, growth initiation, and survival or death of bacteria as a result of lethal pH and organic acid combinations. The logistic model was the first to be used for fitting data on the probability (%) of one of the above responses over time at a given set of conditions. Common dependent variables that were monitored over time include the fraction of positive (turbid) tubes, the probability of a single spore to give growth, or the probability of toxigenesis (Table 2.1). Parameters of the logistic model, such as $P_{\max}$, probability rate (time^{-1} ; i.e., the slope of the tangent through the inflection point, commonly symbolized as k), and mid-time point τ , were then expressed as a quadratic expression of the explanatory variables, such as T, pH, organic acid salt, preservatives, and CO_2 concentration (Table 2.1). The evolution of these models has led to the non-linear and ordinary logistic regression models, which describe

the growth/no growth (G/NG) interface of microbial growth or death (McKellar *et al.*, 2002) as a function of intrinsic and extrinsic factors of foods (Table 2.2). These models are fitted to dichotomous (binary) datasets generated by storage of inoculated media or foods for a long period of time, e.g., 60 days. Microbial responses are then recorded by visible inspection of biomass precipitation and measurement of optical density (OD) (in liquid cultures), or by comparing the final and the initial counts of the microorganism (e.g., in foods). Probability values of 1 and 0 are assigned to cases that gave growth and no growth, respectively. Logistic regression models may assist the Food Industry in the establishment of critical limits and product criteria, as part of HACCP. Furthermore, they may be used as a reliable proof of whether a product complies with microbiological criteria and also contributes to the achievement of a Food Safety Objective (FSO) (van Schothorst *et al.*, 2009).

G/NG models were first developed for pathogenic bacteria, such as *Escherichia coli* O157:H7 (Presser *et al.*, 1998; McKellar and Lu, 2001), pathogenic *E. coli* (Salter *et al.*, 2000), and *Listeria monocytogenes* (Tienungoon *et al.*, 2000), describing the interface of these pathogens as a function of pH, T, a_w , and organic acids. The concept of interface in microbial responses has also been extended to fungi, spoilage yeasts, and lactic acid bacteria (LAB) in shelf-stable acidified sauces and fruit juices (Vermeulen *et al.*, 2007a, 2008). In the special case of mycotoxigenic fungi, the prediction of growth initiation eventually represents the risk of toxigenesis (Garcia *et al.*, 2011). Similarly, the assessment of growth of spoilage molds and yeasts or LAB in acetic acid-based sauces and fruit juices characterizes the microbial stability of their formulation (Vermeulen *et al.*, 2007a, 2008; Dang *et al.*, 2010; Panagou *et al.*, 2010). Therefore, proper models may be used for optimization of product development, considering that once spoilage organisms initiate growth, then spoilage is inevitable. For this reason, the initial G/NG models for shelf-stable acidified

Table 2.1 Probability models for *Cl. botulinum* growth from spores and toxigenesis.

Response variable	pH	T °C	a _w /NaCl	Other	Inoculum size	Product	Reference
Spore germination %	6.9–7.0	35 °C	0.5%	N ₂ CO ₂ + H ₂ H ₂ N ₂ + H ₂	10 ⁶ spores/ml	Peptone yeast extract broth	Feoeding and Busta (1983)
Time to swelling	Not determined	27 °C	2.3–2.4%	0, 0.02% sodium isoascorbate	100 spores/can	Perishable canned cured pork	Tompkin <i>et al.</i> (1978)
Time to turbidity ($\ln T_m$)	5.5 6.5	5–12 °C	0.5–2.5%	0–90% CO ₂ CO ₂ : H ₂ N ₂	10 ⁶ spores/ml	PYGS	Fernandez <i>et al.</i> (2001)
Lag phase: longest incubation showing no toxic samples		4–30 °C	0–2%	0–3% NaCl	10 ⁻² –10 ⁴ (7) spores/ml	Cooked turkey meat Chicken beast	Meng and Genigeorgis (1993)
Log <i>P</i> : probability of toxigenesis LP: lag phase		5–25 °C	Natural NaCl content of the product	Irradiation 0.5–1.0 kGy 0–20% O ₂ 15–60% CO ₂	Undefined $p\% = 1/(1 + e^{-p})$	Pork chops μ =polynomial	Dodds (1993)
Log <i>P</i> : probability of a single spore giving growth LP: lag phase		1–30 °C	Natural NaCl content of the product	Vacuum 70% CO ₂ –30% N ₂ 100% CO ₂	10 ⁻¹ –10 ⁴ /50 g Fillet(6)	Salmon fillets with 3 levels of microflora	Garcia and Genigeorgis (1987)
LP: lag times		4–30 °C	Natural NaCl content of the product	Vacuum 70% CO ₂ –30% N ₂ 100% CO ₂	10 ⁻² –10 ⁴ (7) spores/sample 0 log–4 log	<i>Fresh fish</i> Rockfish Salmon Sole	Baker and Genigeorgis (1990)
Log <i>P</i> (%) of a single cell of LM to initiate growth CN: cells needed for one cell to initiate growth	>5.9	4–30 °C	0.5–12.5%	Methyl paraben (0–0.2%) Na Propionate (0.3%) Na Benzoate (0.1%) K Sorbate (0.3%)	>10 ⁵ –10 ⁻² / 2.4 ml (8)	Broth	Razavilar and Genigeorgis (1998)
Fraction of positive tubes $P(P = P_{\max}/(1 + e^{k(t-t_d)}))$	5–7.0	4–28 °C	0–4%		10–10 ⁵ spores/tube	BAM broth	Whiting and Oriente (1997)
Time to turbidity	5.5–7.2	15–34 °C	0–2.5%	Expansion of time to turbidity	10 ⁰ –10 ⁴ spores/tube	RCM broth	Whiting and Strobaugh (1998)
Time to germinate under the microscope	7.0	20–37 °C	0.5%	Microscopic examination Slide-agar-cover slip sealed with paraffin wax	Single spores	Nutrient agar	Billon <i>et al.</i> (1997)

Table 2.2 Representative growth/no growth models. When inoculation levels are not specified, high population densities, e.g., $>4.5 \log_{10}$ cfu/ml were used.

Micoorganism	Substrate	Model type	Factors/ranges	Total data	Measured by	Reference
<i>Listeria monocytogenes</i>	Cooked meat product	Ordinary	a_w : 0.88–0.98 T: 0–10°C Inoc: 10 and 10^4 cfu/cm ²	4 replicates per conditions 36 combinations	Viable count data per 60 days	Mataragas <i>et al.</i> (2006)
<i>Listeria monocytogenes</i> (inoculum) 1 strain	TSB	Ordinary	a_w : 0.888–0.997 pH: 3.76–6.44(HCl) T: 4–30°C Inoc: 0.90, 2.58, 4.20, 6.01 log cfu/well	4 replicates per conditions 500 combinations	OD for 60 days	Koutsoumanis and Sofos (2005)
<i>Listeria monocytogenes</i> 5 strains	TSB TSB + 1.5% agar	Ordinary	a_w : 0.900–0.993 pH: 4.24–6.58 T: 4–30°C Inoc: broth on agar surface	4 replicates per combination 180 combinations	OD for 30 days	Koutsoumanis <i>et al.</i> (2004a)
<i>Salmonella typhimorium</i> 5 strains			a_w : 0.913–0.990 pH: 3.76–6.44 T: 10–35°C	4 replicates per combination 350 combinations	OD for 62 days	Koutsoumanis <i>et al.</i> (2004b)
<i>Listeria monocytogenes</i> 5 strains	Nutrient broth	Ordinary	a_w : 0.960–0.990 pH: 5.0–6.0 T: 7°C Acetic acid (AA): 0–0.8%	20 replicates per combination 180 combinations	OD for 30 days	Vermeulen <i>et al.</i> (2007b)
<i>Listeria monocytogenes</i> 5 strains	Nutrient broth	Square root cardinal model with ordinary terms for inoculum level (n , $\sqrt{n}$)	a_w : 0.960–0.990 pH: 5.0–6.0 T: 7°C AA: 0, 0.2 and 0.4% Inoculum size: 2×10^5 to 1 cfu/well	66 combinations and 20 to 40 replicates per inoculum size and condition (total of 47,808 cases)	OD and plating for 90 days	Gyssemans <i>et al.</i> (2007) Vermeulen <i>et al.</i> (2009)
<i>Zygosaccharomyces bailii</i>	Sabouraud and broth with glucose (7.5%) and fructose (7.5%)	Ordinary using undissociate and dissociate acid terms	a_w : 0.950 pH: 3.0–5.0 T: 30°C LA: 0–3% AA: 0–3.5%	12 replicates per combination 336 combinations	OD for 30 days	Vermeulen <i>et al.</i> (2008)

<i>Zygosaccharomyces bailii</i>	Sabouraud and broth with glucose (7.5%) and fructose (7.5%)	Ordinary using total AA (%)	a_w : 0.93–0.97 pH: 3.0–5.0 T: 22 or 30 °C AA: 0–2.5%	20 replicates per combination 150 combinations per temperature	OD for 45 or 60 days	Dang et al. (2010)
<i>Lactobacillus plantarum</i> <i>Lb. fructivorans</i>	MRS broth with glucose (7.5%) and fructose (7.5%)	Ordinary	a_w : 0.950 pH: 3.0–5.0 T: 30 °C LA: 0–3% AA: 0–3%	12 replicates per combination 180 combinations	OD for 30 days	Vermeulen et al. (2007 ^a)
<i>Listeria monocytogenes</i> 10 strains	TSBYE	Ordinary	<i>Experiment I</i> pH: 3.82–7.42 T: 10 and 30 °C SL: 0–10% SD: 0–0.5% <i>Experiment II</i> SL: 0–6% SD: 0–0.5% T: 4–30 °C NaCl: 0.5 and 2.5% Atmosphere: anaerobic and aerobic	8 replicates per combination 220 combinations 4 replicates per combination 346 combinations	OD for 40 days	Skandamis et al. (2007 ^c)
<i>Escherichia coli</i> 0157:H7 4 strains	TSB	Ordinary	<i>Experiment I</i> pH: 3.52–7.32 with lactic acid a_w : 0.955–0.995 T: 10–35 °C Inoculation levels: 3.2×10^1 , 3.6×10^3 , 3.4×10^5 cfu/ml <i>Experiment II</i> pH: 3.52–7.32 with lactic acid a_w : 0.938–0.995 T: 10–35 °C Inoculum preparations: 1. TSB-G 2. TSB-G 3. Water washings (pH 6.87) 4. LA-washings (pH 4.92)	4 replicates per combination 160 combinations per inoculum 4 replicates per combination 360 combinations per inoculum preparation	OD for 60 days	Skandamis et al. (2007 ^b)

(Continued)

Table 2.2 (Continued)

Micoorganism	Substrate	Model type	Factors/ranges	Total data	Measured by	Reference
<i>Listeria monocytogenes</i> 4 strains	Lightly preserved seafood	Square-root cardinal model	SD: 0–0.15% SL: 0–1.5% T: 8 and 15 °C Vacuum or 40% CO ₂ – : 60% N ₂	3 replicates	Viable counts	Mejlholm and Dalgaard (2007b)
<i>Listeria monocytogenes</i> 3 strains	TSBYE	Ordinary	pH: 4.8–6.6 a _w : 0.955–0.996 Inoculum : 10 ⁹ , 10 ⁶ cfu/ml Inoculum type: 1. Detached from SSG 2. Planktonic cells	3 replicates per combination 30 combinations	OD for 30 days	Belessi et al. (2011a)
<i>Escherichia coli</i> (STEC) 2 strains	BHI	Ordinary model per time to growth (TTG) Non-linear cardinal model	a _w : 0.945–0.995 Undissociated LA : 2–10 mM pH: 4.46–5.24 T: 27 °C	3 replicates per combination 30 combinations	OD for 14 days	Lindbland and Lindqvist (2010)
<i>Listeria monocytogenes</i> 2 strains	TSB, milk, cheese curd		pH: 5.5–6.5 (LA) NaCl: 0–8% Inoculation level: 10 and 100 cfu/ml T: 30 °C	6 replicates per combination 20 combinations	Viable counts for 8 h	Schvartzman et al. (2010)
<i>Listeria monocytogenes</i> 1 strains	BHI	Ordinary	pH: 5.5–6.5 Citric acid: 0–0.4% Ascorbic acid: 0–0.4% T: 4–30 °C	8 replicates per combination 232 combinations	OD for 21 days	Valero et al. (2006)
<i>Escherichia coli</i> 0158:H23 (selected as the serotype growing faster than 059:H21; 055:H6; 0157:H7)	TSB	Ordinary with variables scaled in the range 0.1–0.9 $x = (x - x_{min}) / (x_{max} - x_{min})$	pH: 5.0–7.0 a _w : 0.960–0.999 T: 8–16 °C Inoculum level: 2, 3, 4 log cfu/ml	8 replicates per combination 405 combinations	OD for 30 days	Valero et al. (2010)
<i>Staphylococcus aureus</i> 5 strains	TSB	ordinary	pH: 4.5–7.0 a _w : 0.856–0.999 T: 8–19 °C	30 replicates per combination 146 combinations	OD for 36 days	Valero et al. (2009)

<i>Aspergillus ochraceus</i> <i>Aspergillus parasiticus</i>	Malt extract agar	Ordinary	pH: 6.0–6.2 a_w : 0.80–0.93 (glycerol) T: 10–42 °C	10 replicates per combination 42 combinations	Visual inspection for 90 days	Garcia et al. (2011)
<i>Byssoscllamys fulva</i> <i>Byssoscllamys nivea</i>	Malt extract agar	Ordinary	pH: 5.5–6.0 a_w : 0.88–0.99 T: 10–45 °C	8 replicates per combination 48 combinations	Visual inspection for 30 days	Panagou et al. (2010)
<i>A. flavus</i>	3% chilli powder extract agar	Ordinary	a_w : 0.85–0.97 Time : 0–90 days	10 replicates × 3 times per combination 6 combinations	Visual inspection for 90 days Ergosterol analysis	Marín et al. (2009)
<i>A. carbonarius</i>	Hulled pistachios	Ordinary	Moisture: 12.5–34.8% T: 10–42 °C Time: 17–90 days	10 replicates per combination 35 combinations	Visual inspection for 90 days	Marín et al. (2008)
<i>A. carbonarius</i>	Synthetic grape medium	Ordinary	a_w : 0.85–0.96 T: 10–40 °C Time: 0–25 days	4 replicates × 2 times per combination 42 combinations	Visual inspection for 25 days Ochratoxin A determination	Tassou et al. (2009)
<i>P. brevicompactum</i>	MY 50	Non-linear	pH: 5.0 Sugar: 500 g/l Sorbic acid: 0–1000 mg/l Benzoic acid: 0–300 mg/l	5 replicates per combination 24 combinations	Visual inspection for 75 days	Membré et al. (2001)

products predicted not only the probability of growth but also the time to growth (TTG) of spoilage organisms, such as *Zygosaccharomyces bailii* (Jenkins *et al.*, 2000). The latter response resembles that of the time to toxigenesis (also incorrectly termed “lag time”) by *Cl. botulinum*, whereas TTG has also been modeled for *E. coli* (Lindblad and Lindqvist, 2010) and *Geobacillus stearothermophilus* (Llaudes *et al.*, 2001).

Given the variety of existing G/NG models (Table 2.2), recent reports attempted to validate the most commonly used cardinal G/NG models against independent data from literature and international databases, such as ComBase. Representative initiatives include the validation of G/NG and growth models for *L. monocytogenes* in mayonnaise-based salads (Vermeulen *et al.*, 2007c) and ready-to-eat (RTE) meat products and seafood (Mejlholm *et al.*, 2010). More specifically, the collective validation dataset of Mejlholm *et al.* (2010) consisted of 1014 G/NG responses and six different models were evaluated. Notably, it was proven that the most complex models, which accounted for the effect of nine environmental factors, performed better than simpler models, suggesting the usefulness of complexity in model development, when necessary.

Judging by the overview of the existing G/NG models in Table 2.2, it is evident that ordinary models are the most commonly used. This was expected because ordinary logistic regression is often a built-in routine in most commercial statistical software, thereby requiring no code or advanced mathematical skills for their application. Although the high number of models published emphasizes their importance for food spoilage and safety, the marked diversity in strains, experimental conditions and protocols, culture preparations, inoculation levels, and duration of incubation underlines the difficulty in comparison of different models. Furthermore, overlapping between experimental conditions for the same microorganisms by different researchers is also evident. Therefore, systematic grouping of models and available G/NG responses would

greatly enhance the application of probability models in daily practice. In this regard, the Microbial Response Viewer (Koseki, 2009) constitutes an updated ComBase database of G/NG data for 29 microorganisms in response to temperature, pH, and a_w , fitted to ordinary and square-root cardinal models. An important advantage of this web-based application is the simultaneous illustration of the G/NG interface and the growth rate at growth-supporting conditions, through colored contour plots.

2.2.2 Growth kinetic models

2.2.2.1 Effect of temperature

As nicely detailed by Corkrey *et al.* (2012, 2014) in their seminal meta-analysis article, in their effort to thermodynamically describe the temperature dependence of the growth rate of multiple species from all three domains of life, namely Bacteria, Archaea, and Eukarya: “...temperature governs the rate of chemical reactions including those of enzymatic processes controlling the development and decline of life on earth from individual cells to complex populations and spanning temperatures from well below freezing (e.g., -2°C) to above the boiling point of water, such as at 122°C , which is the highest temperature so far known for biological growth”. Commonly, the change of growth rate of poikilothermic organisms in the biokinetic ranges has a U-shape characterized by a minimum (suboptimal), maximum (superoptimal) around the optimal for growth. In their work, Corkrey *et al.* (2012, 2014) concluded that the effect of temperature on poikilotherm growth rate is exerted through a single rate-limiting enzyme-catalyzed reaction, also associated with protein denaturation. In this way, the temperature dependence of the growth rate of various organisms can be described by an Arrhenius-type equation that uses the following thermodynamic components in the form of non-linear regression model parameters: (i) the enthalpy (ΔH) of activation of the rate-limiting reaction, (ii) the heat capacity change (ΔC), (iii) denaturation kinetics (D), and (iv) the critical temperature (T_{mes}) at which the protein denaturation

is minimized (i.e., corresponding to the theoretical optimal growth temperature).

Temperature is also known to control the shelf life of foods, either by determining the rate of microbial spoilage or the rate of microorganism-independent quality decay reactions, e.g., due to enzymes causing texture breakdown (e.g., pectinolytic, enzymes acting on myofibrillar proteins) and lipid or colour oxidation (e.g., polypheloxodases, esterases, etc.) A shelf life loss kinetic model is characteristic not only for the studied food but equally important to the set of environmental conditions of the experiment. These conditions can determine the reaction rates and have to be defined and monitored during kinetic experiments. Since most environmental factors do not remain constant the next logical step would be to expand the models to include them as variables, especially the ones that more strongly affect the reaction rates and are more prone to variations during the life of the food. The practical approach is to model the effect into the apparent reaction rate constant, i.e., expressing k as a function of E : $k = k(E)$ (Taoukis *et al.*, 1997):

$$r_A = \frac{-d[A]}{dt} = k[A]^m$$

Secondary models describe the mathematical dependence of parameters (e.g., lag time, growth rate, time-to-certain log increase or reduction) estimated by a primary model on the environmental or cultural conditions, such as pH, a_w , T , E_h , CO_2 concentration in the packaging atmosphere, preservatives, etc. Food product spoilage is dynamic and in some cases relatively small changes in environmental conditions cause a complete shift in the microflora responsible for product spoilage (i.e., the ephemeral or specific spoilage organisms) or in the non-microbial quality decay indices. The modeling procedure includes the mathematical description of growth/inactivation kinetics or the probability of growth for a microorganism of interest, as a function of a specific interpolation range of the environmental variables affecting

these kinetics. In 1982, Hauschild assessed the probability of spore germination and the production of toxin in vacuum-packaged bacon and liver sausage. Hauschild and other researchers (Roberts and Gibson, 1986; Tompkin, 1986) also examined the effects of some variables such as salt, nitrite, phosphate and sorbate concentration, formulation, processing techniques, and pH. The most common secondary models are the Arrhenius model, modified Arrhenius, polynomial and square root models. The Arrhenius is an empirical model, based on thermodynamic considerations (Labuza and Riboh, 1982):

$$k = k_0 e^{-E_A/RT}$$

The Arrhenius equation expresses the dependence of the rate (k) of chemical reactions on the temperature T (in absolute temperature kelvin) through the activation energy E_A . Parameter k_0 is the pre-exponential factor or simply the prefactor and R is the gas constant (8314 J/Kmol). In the above equation, if values of k are recorded at different temperatures and if $\ln k$ is plotted against $1/T$, a straight line is formed with slope $-E_A/R$ (Labuza and Riboh, 1982; Labuza *et al.*, 1992). If the objective is to get a rapid estimation of the expected shelf life of the product, then an accelerated shelf life test (ASLT) can be used in the range of interest. Taking into account the limitations or possible deviations from the Arrhenius law, the ASLT involves the use of the Arrhenius equation at higher testing temperatures in a thorough shelf life study and extrapolation of the kinetic results to normal, non-abusive storage conditions. This procedure is used to substantially reduce the experimental time, through the acceleration of the quality deterioration reactions. The successive steps that outline shelf life determination and ASLT methodology are presented in Taoukis *et al.* (1997). The Arrhenius relation developed theoretically for reversible molecular chemical reactions has been used to describe the effect of temperature on the rate of several reactions of quality loss. It should be noted that

the Arrhenius equation implies that k_A is the value of the reaction rate at 0K, which is of no practical interest. Alternatively, the use of a reference temperature, T_{ref} , is recommended, corresponding to a representative value in the temperature range of the process/storage of study. The Arrhenius equation is then mathematically transformed as follows:

$$k = k_{ref} \exp \left[\frac{-E_A}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}} \right) \right]$$

where k_{ref} is the rate constant at the reference temperature T_{ref} . The value of E_A is, in that case, calculated from the linear regression of $\ln k$ versus $(1/T - 1/T_{ref})$. Alternatively to isothermal kinetic analysis, the study at a single non-isothermal temperature profile is proposed, where temperature varies with time, following a predetermined function of $T(t)$ (Taoukis and Giannakourou, 2004). In that case, the above equation is modified as follows, assuming a first-order reaction:

$$A = A_0 \exp \left[-k_A \int_0^t \exp \left(-\frac{E_A}{R} \frac{1}{T(t)} \right) dt \right]$$

where the integral is calculated by numerical techniques. This approach requires a very strict temperature control and is very sensitive to experimental error in concentration measurements. Although the Arrhenius equation may be applied in most non-microbial quality decay indices, for the bacterial growth extrapolations plots may show non-linearity. Therefore, it cannot fit data well below optimum or above minimum temperatures for growth. The plots are normally only accurate over a limited temperature range for microbial growth (Labuza and Fu, 1993). Fu *et al.* (1991) illustrated this accuracy with Arrhenius plots for *Pseudomonas fragi*.

Schoolfield *et al.* (1981) reparameterized an earlier equation (Sharpe and De Michele, 1977) into a six-parameter non-linear model shown as follows:

$$\frac{1}{K} = \frac{\rho(25^\circ\text{C}) \frac{T}{298} \exp \left\{ \frac{\Delta H_A^*}{R} \left(\frac{1}{298} - \frac{1}{T} \right) \right\}}{1 + \exp \left[\frac{\Delta H_L}{R} \left(\frac{1}{T_{1/2L}} - \frac{1}{T} \right) \right] + \exp \left[\frac{\Delta H_H}{R} \left(\frac{1}{T_{1/2H}} - \frac{1}{T} \right) \right]}$$

where T is the absolute temperature, R is the universal gas constant, K is the response (e.g., generation time), $\rho_{(25)}$ a scaling factor equal to the response rate ($1/K$) at 25°C , H_A is the activation energy of the rate-controlling reaction, H_L is the activation energy of denaturation of the growth-rate-controlling enzyme at low temperatures, H_H is the activation energy of denaturation of the growth-rate-controlling enzyme at high temperatures, $T_{1/2L}$ is the lower temperature at which half of the growth-rate-controlling enzyme is denaturated, and $T_{1/2H}$ is the higher temperature at which half of the growth-rate-controlling enzyme is denaturated.

The Q_{10} approach in essence introduces a temperature dependence equation of the form:

$$k(T) = k_0 e^{bT} \quad \text{or} \quad \ln k = \ln k_0 + bT$$

which implies that if $\ln k$ is plotted versus temperature (instead of $1/T$ of the Arrhenius equation) a straight line is obtained. Such plots are often called shelf life plots, where b is the slope of the shelf life plot and k_0 is the intercept. The shelf life plots are true straight lines only for narrow temperature ranges of 10 to 20°C (Labuza, 1982). For such a narrow interval, data from an Arrhenius plot will give a relatively straight line in a shelf life plot, i.e., Q_{10} and b are functions of temperature:

$$\ln Q_{10} = 10b = \frac{E_A}{R} \frac{10}{T(T+10)}$$

The variation of Q_{10} with temperature for reactions of different activation energies is shown in

Table 2.3 Q_{10} dependence on E_A and temperature.

E_A (kJ/mol)	Q_{10}			Reactions in E_A range
	at 4 °C	at 21 °C	at 35 °C	
50	2.13	1.96	1.85	Enzymic, hydrolytic
100	4.54	3.84	3.41	Nutrient loss, lipid oxidation
150	9.66	7.52	6.30	Non enzymatic browning

Table 2.3. Similarly to Q_{10} the term Q_A is sometimes used. The definition of Q_A is the same as Q_{10} with 10 °C replaced by A °C:

$$Q_A = Q_{10}^{A/10}$$

Zwietering *et al.* (1991) compared the suitability and usefulness of the Schoolfield model to five other models using *L. plantarum* grown at various temperatures between 6 and 43 °C. It was found that the Schoolfield model described data satisfactorily. Davey (1989) used a modified Arrhenius-type model to describe the effects of water activity and temperature on microbial growth rates:

$$\ln k = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 a_w + C_4 a_w^2$$

where T is temperature (K), a_w is the water activity, and C_0, C_1, C_2, C_3, C_4 are coefficients to be determined. Products, such as dried or fermented meats, that have low water activity (e.g., $a_w < 0.90$) may find this equation applicable (Van Gerwen and Zwietering, 1998). However, when water activity is non-limiting the last two terms of the equation can be removed (McMeekin *et al.*, 1992). In the above equation, all parameters appear linearly and thus estimation can be made using multiple linear regression.

Ratkowsky *et al.* (1982) introduced the Bełehradek model for the first time in food microbiology. Until then, this model was unknown in the field of microbiology, although it has been published since 1926 (McMeekin *et al.*, 1993; Ross, 1993). This model, which is

also known as the “square root model” is shown below:

$$\sqrt{k} = b(T - T_{\min})$$

where k is the growth rate, b is a constant, and T is the temperature. The parameter $T_{\min}$, a theoretical minimum temperature for growth, is the intercept between the model and the temperature axis. Given that $T_{\min}$ is a model parameter (i.e., theoretical), its value can be 5 to 10 °C lower than the actual lowest temperature at which growth may occur. Ratkowsky tested 50 sets of growth data and found that this model described the microbial growth rate well (Ratkowsky *et al.*, 1982). Moreover, Pooni and Mead (1984) compared the results of the square root model with other models to data from 14 published studies on poultry spoilage and found that the above equation was the most appropriate for predicting spoilage from –2 up to 15 °C. The square root model was later extended in order to include the entire biokinetic temperature range (Ratkowsky *et al.*, 1982), as described by the following empirical non-linear regression model:

$$\sqrt{k} = b(T - T_{\min}) \{1 - \exp[-c(T - T_{\max})]\}$$

where b is the regression coefficient of k for temperatures $T < T_{\text{opt}}$, c is a parameter allowing the model to fit the data over the optimum temperature, T is the temperature, $T_{\min}$ is the theoretical minimum temperature below which no growth is likely, and $T_{\max}$ is the theoretical

maximum temperature beyond which growth is not possible.

The equation terms $T_{\min}$ and $T_{\max}$ can be used to classify the microorganisms into psychrophiles, mesophiles, or thermophiles (Ross, 1993). Other researchers have also shown that this equation is reasonably effective in predicting effects of constant storage temperatures on microbial growth rates (Chandler and McMeekin, 1985a, 1985b; Phillips and Griffiths, 1987). The effects of fluctuating storage temperature has been studied by Blankenship *et al.* (1988) who developed a dynamic model for predicting growth of *Cl. perfringens* in cooked meat with chilli during chilling using a time-explicit approach.

2.2.2.2 Effects of other environmental factors

Moisture content and water activity (a_w) are also important factors besides temperature that affect the rate of food deterioration reactions. Water activity describes the degree of bounding of the water contained in the food and its availability to act as a solvent and participate in chemical reactions or support microbial growth (Labuza, 1980). Critical levels of a_w can be recognized above which undesirable deterioration of food occurs. Controlling the a_w is the basis for preservation of dry and intermediate moisture foods (IMFs). Minimum a_w values for growth can be defined for different microbial species. For example, the most tolerant pathogenic bacterium is *Staphylococcus aureus*, which can grow down to an a_w of 0.85–0.86. This is often used as the critical level of pathogenicity in foods. McMeekin *et al.* (1987) modified the classical square root model linking growth rate to temperature by adding the a_w variable. The new model accurately predicted the effect of temperature and water activity on the growth rate of *Staphylococcus xylosum* and *Halobacterium* spp., respectively, on salted dried fish (McMeekin *et al.*, 1987; Chandler and McMeekin, 1989a). However, it was found that the effect of variables were additive, suggesting that T and a_w act independently (Chandler and McMeekin, 1989a):

$$\sqrt{k} = b(T - T_{\min})\sqrt{(a_w - a_{w\min})}$$

The pH of the food system is another determining factor. The effect of pH on different microbial, enzymatic, and protein reactions has been studied in model biochemical or food systems. Enzymatic and microbial activity exhibits an optimum pH range and limits above and below which activity ceases, much like the response to temperature. The functionality and solubility of proteins depend strongly on pH, with the solubility usually being at a minimum near the isoelectric point, having a direct effect on their behavior in reactions. Examples of important acid–base catalyzed reactions are non-enzymatic browning and aspartame decomposition. Non-enzymatic browning of proteins shows a minimum near pH 3–4 and high rates in the near neutral–alkaline range. Significant progress in elucidating and modeling the combined effect to microbial growth of factors such as T , pH, a_w , or salt concentration has been achieved in the field of predictive microbiology (Ross and McMeekin, 1994; Rosso *et al.*, 1995).

Adams *et al.* (1991) modified a square root model for the combined effects of pH and temperature using a variety of acidulants and showed that the growth rate under varying conditions of suboptimal temperature and pH can be predicted using this modified equation, which gave good fits for three serotypes of *Yersinia enterocolitica*:

$$\sqrt{k} = b(T - T_{\min})\sqrt{(pH - pH_{\min})}$$

McMeekin *et al.* (1992) suggested that the effects of temperature, a_w , and pH on microbial growth could be described together with the following equation that was subsequently used successfully on growth data for *Listeria monocytogenes* by Wijtzes *et al.* (1993):

$$\sqrt{k} = b(T - T_{\min})\sqrt{(a_w - a_{w\min})}\sqrt{(pH - pH_{\min})}$$

Gas composition also affects certain quality loss reactions. Oxygen affects both the rate and apparent order of oxidative reactions, based on

its presence in limiting or excess amounts. Exclusion or limitation of O₂ by nitrogen flushing or vacuum packaging reduces the redox potential and slows down undesirable reactions. Further, the presence and relative amount of other gases, especially carbon dioxide and secondly ethylene and CO, strongly affects biological and microbial reactions in fresh meat, fruit, and vegetables. The mode of action of CO₂ is partly connected to surface acidification but additional mechanisms, not clearly established, are in action. Different systems require different O₂:CO₂:N₂ ratios to achieve maximum shelf life extension and, often, excess CO₂ can be detrimental. Alternatively, hypobaric storage, whereby total pressure is reduced, has been studied. Modified atmosphere packaging is commonly used with many fresh meats. Recently, Devlieghere *et al.* (1998) replaced the pH terms in the square root model with terms to describe for dissolved CO₂ in modified atmosphere cooked meats. The following equation was proposed for modeling the effect of carbon dioxide-enriched (%CO₂) atmospheres on growth of the

specific spoilage organism *Photobacterium phosphoreum* on fish (Dalgaard, 1995; Dalgaard *et al.*, 1997):

$$\sqrt{k} = b(T - T_{\min}) \frac{(\%CO_{2\max} - \%CO_2)}{\%CO_{2\max}}$$

Later, similar but square-root-transformed terms were used to model the effect of CO₂ and sodium lactate (NaL) on the growth of *Lactobacillus sake* and *Listeria monocytogenes* at a given pH (Devlieghere *et al.*, 1998, 2000a, 2000b):

$$\sqrt{k} = b(T - T_{\min}) \sqrt{(a_w - a_{w\min})} \sqrt{(CO_{2\max} - CO_2)} \sqrt{(NaL_{\max} - NaL)}$$

A more comprehensive square-root-type model that includes the effects of temperature, pH, water activity, and lactic acid has been suggested in a series of publications (Presser *et al.*, 1997; Ross, 1993; Salter *et al.*, 1998):

$$\begin{aligned} \sqrt{k} = & c(T - T_{\min}) (1 - \exp(d(T - T_{\max}))) \sqrt{(a_w - a_{w\min})} \\ & \times (1 - \exp(g(a_w - a_{w\max}))) \sqrt{1 - 10^{pH_{\min} - pH}} \sqrt{1 - 10^{pH - pH_{\max}}} \\ & \times \sqrt{1 - \frac{LAC}{U_{\min}(1 + 10^{pH - pKa})}} \sqrt{1 - \frac{LAC}{D_{\min}(1 + 10^{pKa - pH})}} \end{aligned}$$

It has been applied to *Listeria monocytogenes* and *Escherichia coli* growth rates. It was presented in its most complete form in Ross *et al.* (2003); *c*, *d*, and *g* are fitted parameters, [LAC] is the lactic acid concentration (mM), *U*_{min} is the minimum concentration (mM) of undissociated lactic acid that prevents growth when all other factors are optimal, *D*_{min} is the minimum concentration (mM) of dissociated lactic acid that prevents growth when all other factors are optimal, and *pKa* is the pH for which concentrations of undissociated and dissociated lactic acid are equal. The gamma (*γ*) concept, or concept of dimensionless growth factors, was introduced by Zwietering *et al.* (1992). Later, minor changes and new

developments were added (Wijtzes *et al.*, 1998, 2001; Zwietering, 1999; Zwietering *et al.*, 1996). The gamma (*γ*) concept relies on the following factors:

- i. The observation is made that many factors that affect microbial growth rate act independently and that the effect of each measurable factor on the growth rate can be represented by a discrete term that is multiplied by terms for the effect of all other growth rates affecting factors, i.e.:

$$\begin{aligned} \mu = & f(T) \times f(a_w) \times f(pH) \times f(\text{organic acid}) \\ & \times f(\text{other factor}_1) \times f(\text{other factor}_2) \\ & \times \dots \langle \text{raise} \dots \rangle f(\text{other factor}_n) \end{aligned}$$

- ii. The effect on growth rate of any factor can be expressed as a fraction of the maximum growth rate (i.e., the rate when that environmental factor is at the optimum level). Under the gamma concept approach, the cumulative effect of many factors poised at suboptimal levels can be estimated from the product of the relative inhibition of the growth rate due to each factor:

$$\gamma = \frac{\mu_{\max}(T, a_w, pH, \dots)}{\mu_{\max opt}}$$

In the gamma model approach, the reference growth rate is $\mu_{\max}$, so that reference levels of temperature, water activity, etc., are those that are the optimum for growth rate, usually represented as T_{opt} , a_{wopt} , pH_{opt} , etc. The relative inhibitory effect of a specific environmental variable is described by a growth factor “gamma” (γ), a dimensionless measure that has a value between 0 and 1:

$$\gamma(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}} \right)^2$$

$$\gamma(pH) = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})}$$

$$\gamma(a_w) = \frac{(a_w - a_{w,\min})}{(1 - a_{w,\min})}$$

The relative inhibitory effect can be determined from the “distance” between the optimal levels of the factor and the minimum (or maximum) level that completely inhibits growth by recourse to a predictive model. The combined effect of several environmental factors is then determined by multiplication of their respective γ factors:

$$\mu_{\max} = \mu_{\max opt} \gamma(T) \gamma(pH) \gamma(a_w)$$

The effect of environmental parameters like carbon dioxide, sodium lactate, and nitrite has

also been included in square-root-type models. The absence of these inhibitory substances is optimal for growth. Therefore the calculation of γ factors requires information only about the lowest concentration of each substance that prevents growth. Cardinal parameter models (CPMs) were introduced to predictive microbiology in 1993 and have become an important group of empirical secondary models (Augustin and Carlier, 2000; Le Marc *et al.*, 2002; Messins *et al.*, 2002; Pouillot *et al.*, 2003; Rosso, 1999; Rosso *et al.*, 1993, 1995; Rosso and Robinson, 2001). The basic idea behind CPMs is to use model parameters that have a biological or graphical interpretation. When models are fitted to experimental data by non-linear regression, this has the obvious advantage that appropriate starting values are easy to determine. In addition, the models may be easily adjusted to account for different pathogen–food combinations by introducing the cardinal values and the maximum specific growth rate at optimum conditions (μ_{opt}) of the organisms in the target (e.g., new) food. General CPMs rely on the assumption that the inhibitory effect of environmental factors is multiplicative. CPMs consist of a discrete term for each environmental factor, with each term expressed as the growth rate relative to that when that factor is optimal. At optimal growth conditions all terms have a value of 1 and thus $\mu_{\max}$ is equal to μ_{opt} .

Augustin *et al.* (2005) proposed a new, mechanistically based, CP model. This model includes the effect of six fundamental variables (temperature, a_w , pH, phenol concentration, nitrites, and CO_2) and the interactions between these environmental parameters (Mejlholm *et al.*, 2010):

$$\mu_{\max} = \mu_{opt} CM_2(T) CM_1(pH) SR(a_w) SR(nit) SR(phe) SR(CO_2) \xi$$

where a_w is the water activity, *nit* is the concentration (mM) of undissociated sodium nitrite, *phe* is the concentration (ppm) of smoke components (phenol), CO_2 is the CO_2 proportion, and ξ is the effect of interactions between the environmental parameters, especially at combinations near the growth boundaries.

Abbreviations CM_n and SR were included as used by Augustin *et al.* (2005). With this model, both the growth rates and probability of growth of *Listeria monocytogenes* could be predicted accurately in dairy, meat, and seafood products

in a single model output. Augustin *et al.* (2005) estimated optimal specific growth rates for each of the product categories by fitting their model to growth data obtained from dairy ($n=340$), meat ($n=324$) and seafood ($n=80$) products:

$$CM_n = \begin{cases} 0, X \leq X_{\min} \\ \frac{(X - X_{\max})(X - X_{\min})^n}{(X_{opt} - X_{\min})^{n-1} \left[(X_{opt} - X_{\min})(X - X_{opt}) - (X_{opt} - X_{\max}) \right] \times ((n-1)X_{opt} + X_{\min} - nX)}, X_{\min} < X < X_{\max} \\ 0, X \geq X_{\max} \end{cases}$$

where X is the temperature or pH; $X_{\min}$, X_{opt} , and $X_{\max}$ are the theoretical minimal, optimal, and maximal values of X for growth of *Listeria monocytogenes*;

$$SR(a_w) = \begin{cases} 0, a_w \leq a_{w\min} \\ \left(\frac{a_w - a_{w\min}}{a_{wopt} - a_{w\min}} \right), a_{w\min} < a_w < a_{wopt} \\ 0, a_{wopt} \leq a_w \leq a_{w\max} \end{cases}$$

where $a_{w\min}$, a_{wopt} and $a_{w\max}$ are the theoretical minimal, optimal, and maximum a_w values allowing growth of *L. monocytogenes*;

$$SR(c) = \begin{cases} 1 - \frac{c}{MIC}, c < MIC \\ 0, c \geq MIC \end{cases}$$

where MIC is the minimum inhibitory concentration of undissociated sodium nitrite (mM), phenol (ppm), or CO_2 (proportion) against *L. monocytogenes* and c is the concentration of undissociated sodium nitrite (mM), the concentration of phenol (ppm), or the proportion of CO_2 . The modeling of the interactions between the environmental parameters (ξ) is based on the approach of Le Marc *et al.* (2002):

$$\xi = \begin{cases} 1, \psi < 0.5 \\ 2(1 - \psi), 0.5 < \psi < 1 \\ 0, \psi \geq 1 \end{cases}$$

The ψ -value is determined from sets of environmental parameters and describes how far specific combinations of product characteristics and storage conditions are from the predicted growth boundary (corresponding to a value of ψ equal to 1.0) (LeMarc *et al.*, 2002):

$$\psi = \sum_i \frac{\varphi_{e_i}}{2 \prod_{j \neq i} (1 - \varphi_{e_j})}$$

and

$$\varphi(T) = \left(\frac{T_{opt} - T}{T_{opt} - T_{\min}} \right)^3$$

$$\varphi(pH) = \left(\frac{pH_{opt} - pH}{pH_{opt} - pH_{\min}} \right)^3$$

$$\varphi(a_w) = \left(\frac{a_{wopt} - a_w}{a_{wopt} - a_{w\min}} \right)^3$$

$$\varphi(nit, phe, CO_2) = 1 - SR(nit)SR(phe)SR(CO_2)$$

The Augustin model has been expanded by introduction of the effect of different organic acids and their interactions, leading to the CPM of Zuliani *et al.* (2007). The new parameters added are acetic acid and lactic acid. The maximum growth rate is expressed by

$$\mu_{\max} = \mu_{opt} CM_2(T) CM_1(pH) SR_1(a_w) SR(OA) \xi$$

where OA represents the concentration (mM) of undissociated acetic or lactic acid. Zuliani *et al.* (2007) suggested that the antimicrobial

effect of acetic and lactic acid should be modeled as the effect of the dominating undissociated acid alone:

$$SR(AAC_U) = \begin{cases} 1 - \frac{[AAC_U]}{[MIC_{U\text{acetic acid}}]}, & AAC_U < MIC_{U\text{acetic acid}} \\ 0, & AAC_U > MIC_{U\text{acetic acid}} \end{cases}$$

$$SR(LAC_U) = \begin{cases} 1 - \frac{[LAC_U]}{[MIC_{U\text{lactic acid}}]}, & LAC_U < MIC_{U\text{lactic acid}} \\ 0, & LAC_U > MIC_{U\text{lactic acid}} \end{cases}$$

where $[AAC_U]$ and $[LAC_U]$ are the concentrations (mM) of undissociated acetic acid and lactic acid, respectively, and $[MIC_{U\text{acetic acid}}]$ and $[MIC_{U\text{lactic acid}}]$ are the MICs (mM) of acetic acid and lactic acid, respectively, that prevent growth of *Listeria monocytogenes*.

2.3 Thermal inactivation

Thermal processing is today one of the most important preservation methods in a wide variety of foods. Quantification of the microbial destruction during thermal treatment of a food product is one of the main issues tackled by predictive microbiology. Throughout the years, different mathematical models have been developed to describe microbial inactivation based either on empirical or biological insights to ensure food safety and quality. These models could further contribute to the microbiological aspect of HACCP by identifying certain microorganisms that could be potential hazards in the process and define steps at which critical control can be achieved (Valdramidis *et al.*, 2005). The efficacy of thermal processes has been calculated based on the assumption that microbial inactivation is a process following first-order kinetics (Stumbo, 1973). This model is based on the assumption that the inactivation of microbial cells takes place at a constant proportion in each successive time (Li *et al.*, 2007), and it has been proved effective for the last 90 years in the

canning industry for high-temperature treatments at sterilization level to control *Clostridium botulinum*. It was stipulated that for a given microorganism a thermal process should be expressed in terms of the number of log reductions that the process is expected to deliver (Heldman and Newsome, 2003). In most cases, 6D is the target for pasteurization and 12D for commercial sterility (ICMSE, 2005). First-order kinetic models with the derived D and z values have been extensively employed due to simplicity in the interpretation of kinetic parameters by the industry for a variety of pathogens (Van Asselt and Zwietering, 2006) including *Listeria monocytogenes* in chicken meats (Huang, 2013), traditional sausages (Felício *et al.*, 2011), kiwifruit puree (Benlloch-Tinoco *et al.*, 2014), *Salmonella* spp. in chicken shawirma (gyro) (Osaili *et al.*, 2013), peanut butter (Li *et al.*, 2014), catfish and tilapia (Rajkowski, 2012), *Yersinia enterocolitica* in liquid egg products (Favier *et al.*, 2008), *Escherichia coli* in buffalo Mozzarella curd (Trevisani *et al.*, 2014), and fish (Rajkowski, 2012). However, the concept of D value becomes ineffective in the case where deviation from linearity occurs. This deviation from first-order kinetics was tackled in various ways, the most important being to ignore the curvature of the survival curve and take into account only the linear segment, although such an approach would lead to over- or underestimation of the thermal treatment and could jeopardize the

safety of the product (Peleg and Penchina, 2000). From a practical point of view, microbial inactivation is often non-linear and a shoulder phase has been observed before initiation of a decline phase occurs, whereas in many cases a prolonged survival of a low population of the organism is observed (tailing), especially in the case of mild heat treatment. In an extensive survey for over 120 inactivation curves of non-spore-forming bacteria, it was demonstrated that only 5% of them followed a log-linear pattern (van Boekel, 2002). In view of these observations, a number of non-linear models has been developed, as detailed by other authors (Corradini and Peleg, 2007; Smelt and Brul, 2014). Among them, the most popular were the biphasic model (Cerf, 1977), modified Gompertz (Bhaduri *et al.*, 1991), log-logistic (Cole *et al.*, 1993), Whiting and Buchanan (1993), and Baranyi (Baranyi and Roberts, 1994). The modified Gompertz equation was one of the first models employed for the inactivation of *Listeria monocytogenes* during heat treatment and showed better performance than first-order kinetic models (Bhaduri *et al.*, 1991). However, Geeraerd *et al.* (2000) demonstrated that this model was not appropriate to model accurately the linear inactivation phase due to structural limitations and similar conclusions were reported for *Bacillus cereus* spores during heat treatment of *sous vide* foods (Li *et al.*, 2014). The variety of non-linear models in thermal inactivation of microorganisms has been extensively reviewed in the past (Xiong *et al.*, 1999; Li *et al.*, 2007) whereas GInaFit, an Excel add-in software, has been developed to include nine different model types covering eight different shapes of inactivation curves (Geeraerd *et al.*, 2005) in an attempt to facilitate curve fitting. All the above models are based on the assumption that survival curves can be treated in kinetic terms. Recently, a new category of inactivation models has been developed based on the alternative hypothesis that microbial inactivation is a cumulative form of a temporal distribution of lethal events that represent the spectrum of resistances of the treated microbial population

to the lethal agent (Peleg and Penchina, 2000). The Weibull model can be described by the following equation (Mafart *et al.*, 2002):

$$\log\left(\frac{N_t}{N_0}\right) = -\left(\frac{t}{\delta}\right)^p$$

in which N_0 and N_t are the population of the microorganism at time 0 and t , respectively, t is the heating time, and δ, p are the scale and shape parameters; a concave upward survival curve is related to $p < 1$, concave downward for $p > 1$, and linear if $p = 1$. The shape of the curve could provide biologically meaningful information not only as a reflection of the properties of an underlying distribution of lethal events but also as an indication of the cumulative effect of the lethal agent on the surviving microbial population, thus providing a link with physiological effects (van Boekel, 2002). Thus, a concave upward curve ($p < 1$) indicates that as the sensitive members of the population are destroyed, the remaining cells have the ability to adapt to the applied lethal agent and become more resistant. On the contrary, for concave downward curves ($p > 1$) the remaining cells become increasingly damaged by the lethal agent and it takes a shorter time to destroy them (Chen and Hoover, 2004). The majority of thermal inactivation models has been developed in buffer systems under controlled laboratory conditions that may be inappropriate when extrapolated to real food situations. The trend in the last few years is to design experiments on model systems or directly on foods that give reliable information on bacterial behavior. Thus, van Lieverloo *et al.* (2013) investigated the thermal inactivation of *Listeria monocytogenes* in liquid food products by means of multiple regression models, taking into account 51 different strains of the pathogen and 6 cocktails of strains. The food products assayed were dairy (milk, cream, butter), fruit and vegetable juices, liquid eggs, and meat gravy. The purpose of the work was to develop a model that could predict thermal inactivation of the pathogen while

accounting for effects of food composition (pH, sodium chloride, sugar) and processing conditions (storage temperature, heat shock). The authors demonstrated that multiple regression modeling can be used effectively to predict the inactivation of the pathogen with a probability of survival of less than 1 in a billion liters with a limited and realistic uncertainty level while retaining the variability of heat resistance due to the high number of strains assayed. Silva and Gibbs (2004) published a study for the design of pasteurization processes for high-acid shelf-stable fruit products using first-order kinetics modeling and reported the D and z values for a wide variety of spoilage microorganisms including ascospores of heat-resistant fungi and bacterial spores. Typical z values ranged from 7.7 to 12.9°C depending on the fruit product and spore strain. The authors concluded that the target microorganism in the design of the pasteurization process should be *Alicyclobacillus acidoterrestris*, a high heat-resistant bacterium that causes spoilage to fruit juices, despite the common assumption that microbial spores that can be found in pasteurized fruit products (pH < 4.6) do not grow because of the acidity of the product. The inactivation of *Salmonella* spp. in various food matrices under thermal and emerging treatments has been recently reviewed (Bermúdez-Aguirre and Corradini, 2012). It was reported that *Salmonella* inactivation does not follow first-order kinetics, emphasizing the need for models that adequately describe the survival curves and have predictive ability. Survival of the pathogen was explored with Weibullian and non-Weibullian models reporting the potential and limitations to characterize the survival curves. It was demonstrated that departure from linearity is frequently observed during thermal inactivation and thus model selection should be based on goodness of fit and simplicity rather than preselected kinetics. The thermal pasteurization requirements for *Salmonella* inactivation has also been reported in a variety of meat products (Silva and Gibbs, 2012) and minimum process times at various temperatures for 7D inactivation of the pathogen

have been indicated. Deviation from linearity was also confirmed and the authors proposed the application of an additional 1 or 2D inactivation in *Salmonella* numbers to overcome deviations from linearity and thus avoid underestimation of survivor numbers. Inactivation model fitting has been facilitated in the last few years by the development of specialized software tools such as Combase, GroPin, NIZO Premia, GInaFit, PMM-Lab, Sym'Previus, and FILTRES (Tenenhaus-Aziza and Ellouse, 2014) that estimate inactivation parameters on the user specific experimental data. Normally, the user can upload the dataset, choose the linear or non-linear model, select the temperature of treatment together with other parameters (e.g., pH, NaCl, etc.), and get the fitting estimates. It is expected that the wide use and acceptability of these tools by the industry will help in the design and optimization of thermal processes.

2.4 Non-thermal inactivation and modeling stress-adaptation strategies

Non-thermal inactivation is usually the result of the single or combined effect of low pH (<4.5) or a_w (<0.90) and moisture (<60%) at refrigeration or ambient temperatures in the presence or not of preservative agents close to their minimum inhibitory concentration (MIC). Although the lethality is attributed to heat-independent factors, temperature values within the biokinetic range of growth from the minimum (sub-optimal, 0–5°C) to the maximum (superoptimal: 45–47°C) value for growth remain the factor governing the non-thermal inactivation rate of bacteria (Shadbolt *et al.*, 1999; Ross *et al.*, 2008; McQuestin *et al.*, 2009; Zhang *et al.*, 2010). The latter studies sufficiently demonstrated this concept for non-thermal inactivation of *E. coli* and *L. monocytogenes* at pH (3.5 to 5.1) and a_w (0.76 to 0.94) combinations commonly applying to various dry and fermented meats. This concept has been known for a long time as the basis

for inactivation of *Salmonella*, *Clostridium perfringens* and *Staph. aureus* in homemade mayonnaise and related products (Radford and Board, 1993).

It needs to be noted that non-thermal inactivation may also be achieved by non-thermal pasteurization treatments, such as ultra-high pressure, cold atmospheric plasma, intense light pulses and pulsed-electric fields (Rajkovic *et al.*, 2010). However, the challenge in modeling these processes is rather associated with the potential shape of inactivation curves and less with the modeling principle behind that. Such technologies may alter the classical log-linear inactivation curves to concave downward curves in which the linear death is delayed (preceded) by an initial survival (shoulder) period, or concave upward curves with “tailing” due to the emergence of resistant subpopulations that either pre-existed or were induced by the treatment itself. In cases like that, the following non-linear Weibull- or logistic-based models accounting for the distinct inactivation of two subpopulations, one sensitive and one resistant, may be used (Whiting, 1993; Mafart *et al.*, 2002):

$$N(t) = N_0 \left(f \times 10^{-(t/\delta_1)p_1} + (1-f) \times 10^{-(t/\delta_2)p_2} \right)$$

where δ and p_1, p_2 are as in the classical Weibull model, and

$$N(t) = N_0 \left(\frac{f(1 + e^{-k_1 t_{lag}})}{1 + e^{k_1(t-t_{lag})}} + \frac{(1-f)(1 + e^{-k_2 t_{lag}})}{1 + e^{k_2(t-t_{lag})}} \right)$$

where k_1, k_2 are the inactivation rates for the sensitive (f) and resistant ($1-f$) fraction of the population, after an initial shoulder period represented by t_{lag} .

The non-thermal inactivation is a response worth modeling due to the demands of the consumers for more natural and nutritious foods, which have forced the food industry to confront the challenge of designing minimally processed foods with less preservatives, but without compromising their safety. Practically, this challenge

involves formulating foods at conditions approaching the microbial growth/no-growth boundaries, where either microbial inactivation or (slow) growth could occur. Therefore, it is of the utmost importance to understand and quantify the interaction effects of different hurdle technologies (Leistner, 2000) on microbial dynamics instead of empirically adopting the multiple hurdle technology. Few combined models describing changes in a microbial population subjected to conditions that vary from growth to inactivation have been reported (Corradini and Peleg, 2006; Ross *et al.*, 2005; Pin *et al.*, 2011). The work of Coroller *et al.* (2012) presents a modeling approach for non-thermal inactivation based on the Gamma hypothesis that predicts the global behaviour of *L. monocytogenes* in various media. The proposed model postulates that only two microbial responses can be observed: growth or inactivation. When the maximum growth rate (as estimated from the Gamma concept) is greater than zero, microbial growth is predicted. When the maximum growth rate is equal to zero, then the bacterial population is inactivated. The underlying principle is that growth, survival, or inactivation of microorganisms are time-dependent and it can be reasonably postulated that if the microbial behavior was observed in static conditions for an infinite time period, only growth or inactivation would be observed. Microbial survival would therefore be characterized by either slow growth or slow inactivation and the concept of infinite lag would have no meaning in this context. The environmental factors of interest are commonly temperature, pH, sodium chloride salt, a_w , and commonly encountered organic acids such as sorbic acid, lactic acid, and acetic acid. For further application in an industrial setup, the modeling approach of Coroller *et al.* (2012) had to meet the following requirements: (i) the biological parameters that are used in the model should be easily found in publications, or elicited by an expert, and (ii) the model should allow the prediction of change in the number of microbial populations (i.e., log increase or decrease) as a result of food

formulation and storage conditions and vice versa. More specifically, it should suggest specific food properties based on the targeted bacterial behavior or it should provide the storage condition for a given food formulation that leads to the targeted bacterial behavior (Coroller *et al.*, 2012).

A similar approach has been proposed by Pin *et al.* (2011) for various *Salmonella* serovars, based on literature reports that have been uploaded in the ComBase database. Three discrete models, namely a growth, a probabilistic, and an inactivation model, the first two having cardinal parameters, were fitted with non-linear regression to three corresponding datasets of 707 growth or inactivation rates of *Salmonella*, extracted from ComBase in response to temperature, pH, and a_w . Growth and inactivation responses were also translated into a consolidated binary dataset assigning values of 0 and 1 to no growth and growth responses, respectively, in order to fit the probabilistic (growth/no growth) logistic regression model for *Salmonella*. The aforementioned models were combined into a three-component unified model, which works as follows: the T, pH, and a_w for consecutive short time intervals (dt) are introduced into the probabilistic model and if the probability of growth corresponding to the momentary combination of the three independent variables is predicted as ≥ 0.5 then the change in numbers of *Salmonella* is predicted by the growth model, whereas if the expected growth probability is < 0.5 then the population of *Salmonella* is predicted by the inactivation model.

Apart from the potential risk by insufficient control of microbial growth, mild processing, including the contemporary non-thermal preservation technologies, may also increase the risk of emerging stress-hardened pathogens, as a result of the repeated exposure of microbial cells to sublethal conditions, which impart tolerance to lethal stresses (Rajkovic *et al.*, 2009). Therefore, there is an imperative need to encompass the microbial adaptive responses in the existing models predicting microbial

dynamics below, above, and across the growth boundaries. The next paragraphs provide a detailed overview of current advances in this area.

2.4.1 Modeling the adaptive responses of pathogens to inimical factors

Bacterial “stress-hardening” is a response resulting from exposure of cells to sublethal stresses for short (shock) or long (habituation) periods of time. Exposure to one stress induces resistance to the same (homologous) or different (heterologous) stress, the latter also termed cross-protection. The microbial response may be perceived either as increased survival against lethality treatments or as minimization of lag time and faster growth upon shift to growth-permitting conditions. Although numerous studies have investigated the adaptive response of pathogens to various stresses, especially low pH, few have systematically modeled this process by mathematically describing the shape of survival curves and estimating the inactivation kinetics of adapted pathogens (Greenacre *et al.*, 2003). By modeling the dependence of inactivation rate as a function of exposure to sublethal acidity, it was concluded that the acid tolerance response (ATR) is strongly dependent on the duration of previous exposure (e.g., 2–3 h) to sublethal acidity (e.g., pH 5–5.5) by different acids (e.g., HCl, lactic or acetic acid) before challenge against lethal pH (i.e., < 3.5 ; Greenacre *et al.*, 2003; Shadbolt *et al.*, 2001).

Another paradigm of active microbial adaptation strategy associated with self-adjustment (reduction) of the growth rate in order to combat stress is the “stringent response” and the emergence of persister cells within a population (McMeekin *et al.*, 2013; Lewis, 2007). A stringent response is the transition of cellular metabolism from the relaxed (growth oriented) state to the survival state, in response to nutrient (e.g., carbon, amino acid, and iron) starvation. It is usually perceived as entrance of cells into the viable but non-culturable (VBNC) state and is induced by the sudden accumulation of

intracellular signaling compounds, called alarmones, such as guanosine tetra- or pentaphosphate (ppG[p]pp). Persistence is the result of phenotypic switching between fast and slow growing cells, with the latter being less metabolically active and thus less susceptible to stresses, especially antibiotics (Lewis, 2007). Contrary to antibiotic resistance, which is commonly controlled by a genetic factor, persister cells are genetically identical to non-persisters and emerge, as a dormant subpopulation, from the same population forming a distinct stress-resistant phenotype (Ayrapetyan *et al.*, 2014). Fractionation of growth rates within homogeneous populations may resemble the persistence and enable modeling the spread of stress resistance within microcolonies, such as those of a biofilm on inert (e.g., stainless steel) or living (e.g., vegetable) surfaces (Nguyen *et al.*, 2011).

As far as growth modeling is concerned, bacterial adaptation to a new environment is manifested by the lag phase, representing the period during which the effect of the previous environment on microbial growth is gradually diminished and cells are prepared (in an “adjustment” process) to enter a new log phase. The original definition of a population lag time was “geometrical” and equaled the time when the tangent of the population growth curve with the maximum slope crosses the initial level of the population. Nowadays, mechanistic approaches have also been introduced, considering that: (i) lag time is dependent on the physiological state of cells (commonly represented by the parameter q_0 or p_0) and the work needed (h_0) for growth initiation, carried out at a constant rate ($h_0 = \text{lag} \times \mu_{\max}$), or (ii) at a certain time-point, a bacterial population consists of a subpopulation in the exponential phase and a no-growing fraction of cells in the lag phase either preparing for growth or incapable of initiating growth because the prevailing conditions are below their growth boundaries (Baranyi and Pin, 2004; Koutsoumanis, 2008; McKellar and Knight, 2000; McKellar, 2001; Robinson *et al.*, 1998; Standaert *et al.*, 2007).

In a dynamic environment, for example, under fluctuating temperatures, it is assumed that the growth rate of cells is instantaneously adapted to momentary temperature changes, whereas there is contrasting evidence for induction of short lag times before growth is resumed (Alavi *et al.*, 1999; Mitchell *et al.*, 1994, 1995). Following the initial lag phase, changes of environmental factors may induce a so-called “intermediate” lag phase (Swinnen *et al.*, 2005), the duration of which depends on the magnitude and direction of the environmental shift (Belessi *et al.*, 2011b; Le Marc *et al.*, 2010; Muñoz-Cuevas *et al.*, 2010). Quantification of the effect of temperature shifts on the lag time of bacteria has received more attention (Augustin *et al.*, 2000a, 2000b; Mellefont and Ross, 2003; Swinnen *et al.*, 2005, 2006; Whiting and Bagi, 2002; Zwietering *et al.*, 1994) than the corresponding effect of osmotic shifts (Mellefont *et al.*, 2003, 2004, 2005; Mellefont and Ross, 2003).

The most widely used approach for quantifying the effect of sudden changes in the environment on bacterial lag time is based on the amount of work that cells need to undertake and the rate at which this work is accomplished (work to be done, h_0 ; Mellefont *et al.*, 2003; Robinson *et al.*, 1998). The adaptation rate follows the environmental fluctuations, whereas the adaptation work may be reduced or increased if microorganisms adapt faster or become injured, respectively (Belessi *et al.*, 2011b; Le Marc *et al.*, 2010; Muñoz-Cuevas *et al.*, 2010). From a mathematical standpoint, if changes in the environment do not pose additional adaptation work, then the inverse of lag time (the so-called “lag rate”; Mellefont and Ross, 2003) and growth rate should follow the same trend as a function of the shift and they should be linearly correlated. This may be true for shifts of low magnitude (e.g., 3–5 °C) that are far from the growth boundaries. However, linearity is lost when the intensity of the stress imposed is so high that the bacteria cannot adapt to the new conditions and require additional effort to overcome the imposed stress.

This is the case with osmotic or acid shifts, which are considered more severe stresses than temperature (Robinson *et al.*, 1998; Shabala *et al.*, 2008) and are known to induce adaptation work (Mellefont and Ross, 2003; Mellefont *et al.*, 2003, 2004, 2005) or with shifts close to the growth boundaries. Mitchell *et al.* (1995) stressed that extended exposure to temperatures that do not support growth of *Salmonella* may affect subsequent growth of the bacterium at growth permitting temperatures. Existing reports also suggest that preincubation of *Listeria monocytogenes* at 4°C results in a faster growth rate at 7°C than the preculture at 7°C (Membré *et al.*, 1998). More recent papers have shown that the adaptation work and the physiological state parameter of cells under fluctuating conditions can be effectively modeled as a function of both the magnitude and the direction of the environmental changes. Such methodology may then be coupled with the differential form of primary models, such as the Baranyi model, and allow for simulation of the growth of pathogens in response to up- or down-shifts in pH, a_w , temperature, etc.

Following the classical viable count-based modeling, the state-of-the-art in adaptation modeling is the development of pure mechanistic models relating the effect of “cell history” on the potential of the pathogen to survive food-processing challenges and eventually cause illness. Detailed insights into the uncertainty of microbial responses from farm to fork can be provided by adopting systems biology principles and functional genomics (Brul and Westerhoff, 2007). These models are based on the hypothesis that the environment significantly influences the dynamic expression and assembly of all components encoded in the genome of an organism into functional biological networks. The ultimate goal is to integrate top-down approaches, which use “-omics” induction to identify underlying mechanisms, and bottom-up approaches, which explain or predict the overall cellular behavior (including “omics”) beginning with the molecular level (Bruggeman and Westerhoff, 2007).

This may be implemented through the development of an environmental and gene regulatory influence model (Mensonides *et al.*, 2002, 2005) for different stages of the food chain. Use of microarray data coupled with bioinformatic approaches may enable modeling of the global gene expression in response to environmental challenges and predict the lag time or even the virulence of pathogens based on their “history” (King *et al.*, 2014).

2.5 Fermentation: a dynamic environment for microbial growth and pathogen inactivation

Fermentation is one of the oldest food-processing technologies based on the biological activity of specific microbial groups that can produce a range of metabolic compounds that suppress the growth and survival of pathogenic and spoilage microbiota, thus ensuring the microbiological stability and extending the shelf life of foodstuffs (Caplice and Fitzgerald, 1999; Ross *et al.*, 2002; Bourdichon *et al.*, 2012). From the biochemical point of view, the main impact of fermentation is focused on food preservation through the development of inhibitory metabolic compounds (e.g., organic acids, ethanol, bacteriocins), often in combination with a decrease in water activity through drying or salting (Lee, 2004; Gaggia *et al.*, 2011; Peres *et al.*, 2012). The formation of these compounds results in the inhibition of food-borne pathogens, thus improving food safety (Adams and Nikolaides, 2008; Grounta *et al.*, 2013), and also in the improvement of the nutritional value and sensory attributes of the final product (van Boekel *et al.*, 2010; Lanza, 2013).

The use of mathematical models to describe the growth, survival, or death kinetics of bacteria has been extensively employed in a wide variety of food products and processes and the principles of predictive microbiology have been well established and elucidated in a series of articles (Buchanan, 1993; Ross and McMeekin, 1994;

Baranyi and Roberts, 1995; Whiting, 1995; McDonald and Sun, 1999). The most common approach to describe microbial growth and death under given ecological conditions is by means of a sigmoidal curve that approximates quite accurately the changes in bacteria cell population over time. However, on a real food matrix, bacteria do not grow independently from their environment but are influenced by the background microbiota (Malakar *et al.*, 2003) and the structure of the matrix (oxygen availability, water distribution, organic acids, food preservatives, etc.) (Noriega *et al.*, 2010). It is worth noting that single species growth can be differentiated in terms of a lag phase, an exponential phase, and stationary phase, a distinction that is not very clear for multiple species growth. Moreover, different kinds of interactions can be encountered on a food system, among which antagonistic interactions are most important from the food safety perspective, and, consequently, special models must be employed to describe the complex nature of interspecies interactions. Thus, dynamic models have been developed to describe the bacterial kinetics of two or more competitive species (Vereecken *et al.*, 2000; Pommier *et al.*, 2005; Mejlholm and Dalgaard, 2007a, 2007b) while other models take also into consideration substrate consumption and product inhibition of each culture (Sodini *et al.*, 2000).

Table olive fermentation is one of the most dynamic environments for microbial growth, where diverse microbial groups from the indigenous microbiota of olives compete for nutrients with a variety of contaminating microorganisms from fermentation vessels, pipelines, and pumps in contact with olives and brine. Those microorganisms best adapted to the food substrate and process parameters during fermentation dominate the process. It has been reported that in olive fermentation, despite the relatively low initial population of lactic acid bacteria and yeasts compared to Gram-negative bacteria, they eventually become the dominant groups driving fermenta-

tion (Sánchez Gómez *et al.*, 2006). The effect of environmental factors driving the fermentation process on the microbial association and the survival of pathogenic bacteria has been modeled through a variety of kinetic and probabilistic models, whereas other approaches include the application of artificial neural networks (ANNs) and susceptibility models for the determination of MIC and NIC values of antimicrobial compounds (for a review see Arroyo-López *et al.*, 2010). However, these models do not take into consideration the dynamic interactions prevailing in the process environment. In a recent work (Skandamis and Nychas, 2003), a dynamic model was developed based on six differential equations to describe the evolution of the fermenting microbiota and the survival of the pathogenic bacterium *Escherichia coli* O157:H7 during the inoculated fermentation of green olives with *Lactobacillus plantarum* in brines supplemented with different amounts of fermenting substrates. The dynamics of the fermentation process is described by the following equations:

$$\text{For lactic acid bacteria: } \frac{dN}{dt} = r_{s_{\max}} N \left(1 - \frac{Y}{Y_{\text{in}}} \right)$$

where N is the population of *L. plantarum* in the brine (cfu/ml), $r_{s_{\max}}$ is the maximum specific growth rate (days⁻¹) for the starter culture, Y is the population of yeasts (cfu/ml), and Y_{in} is the minimum population of yeasts (cfu/ml) that inhibits the growth of *L. plantarum*.

$$\text{For yeasts: } \frac{dY}{dt} = r_{y_{\max}} N \left(1 - \frac{Y}{Y_{\max}} \right) Y$$

where $r_{y_{\max}}$ is the maximum specific growth rate for yeasts (day⁻¹) and $Y_{\max}$ the maximum cell population of yeasts (cfu/ml). Apart from the above equations that describe the changes in microbial dynamics, two more equations were taken into consideration for fermentable substrate consumption and lactic acid formation.

For fermentable substrates: $\frac{dS}{dt} = \left(-m_s N - \frac{1}{Y_{Y/S}} \frac{dY}{dt} \right) S$

where S is the residual concentration (%) of fermentable substrate in the brine during the process, $Y_{Y/S}$ is the yeast yield coefficient (cfu/ml per mM of assimilated fermentable substrate), and m_s is the maintenance coefficients for *L. plantarum*.

For lactic acid formation: $\frac{dHA}{dt} = -Y_{HA/S} \frac{dS}{dt}$

In the above equation, lactic acid (in protonated form) is directly related to the consumption rate of fermentable substrates (dS/dt), with $Y_{HA/S}$ being a yield coefficient representing the conversion of fermentable material to lactic acid. Finally, as fermentation is a dynamic process and the value of pH changes over time, the changes in proton concentration were modeled as a function of HA as follows:

For proton concentration: $\frac{dP}{dt} = a \left(1 - \frac{P}{P_{\max}} \right) HA$

where a is a coefficient, P is the concentration of protons, and $P_{\max}$ is the maximum predicted concentration of protons corresponding to the predicted minimum value of pH in the fermentation process. Apart from the evolution of the indigenous microbiota in the process, it would be of great importance to investigate the fate and behavior of pathogenic bacteria in the case of contamination. It should be noticed that table olives are considered as a ready-to-eat food, thus making contamination with pathogens a potential public health concern, although no serious outbreaks have been reported so far due to the fact that the brine environment does not support the growth and survival of pathogenic bacteria (Medina *et al.*, 2013). To investigate the dynamics of pathogenic bacteria in the fermentation process, the brine was inoculated at the onset of fermentation with *E. coli* O157:H7 and

the changes in cell population were modeled by the following equation:

$$\frac{dN_e}{dt} = k \frac{dP}{dt} N_e - f \frac{HA}{S} N_e$$

where N_e is the population of the pathogen (cfu/ml) and k, f are coefficients. This equation is based on the assumption that the survival of the pathogen is directly proportional to the production rate of protons (P) and lactic acid (HA), and inversely proportional to the concentration of fermentable substrates. Yeasts were potential competitors of *L. plantarum* despite the fact that the latter species dominated the process (Figure 2.1). The growth of yeasts retarded the growth of the starter but not its metabolic activity, as evidenced by the increasing levels of lactic acid produced throughout the process. Finally, a progressive reduction in the population of the starter was observed at the end of fermentation that could be attributed to the reduction of available fermentable substrate due to antagonism by yeasts. Concerning the behavior of *E. coli* O157:H7, its inactivation pattern could be discriminated into three distinct stages. Specifically, the pathogen presented a rapid decrease within the first five days of the process, which coincides with a rapid drop in pH values in the same period. A short survival period was observed afterwards between 5 and 10 days, when the pH of the process reached a plateau and was close to the pK_a value of lactic acid, with the undissociated fraction of the acid to be apparently below lethal concentrations. Finally, a second death phase was observed from day 20 onwards due to the increased concentration of undissociated lactic acid, with a concurrent reduction in fermentable substrates. The results of the developed model proved the effectiveness of the modeling approach to describe quite accurately the fermentation process as a whole, taking into account the different biochemical mechanisms such as the production of microbial metabolites, consumption of fermentable substrates, as well as microbial effected changes in pH. Since fermentation remains one of the

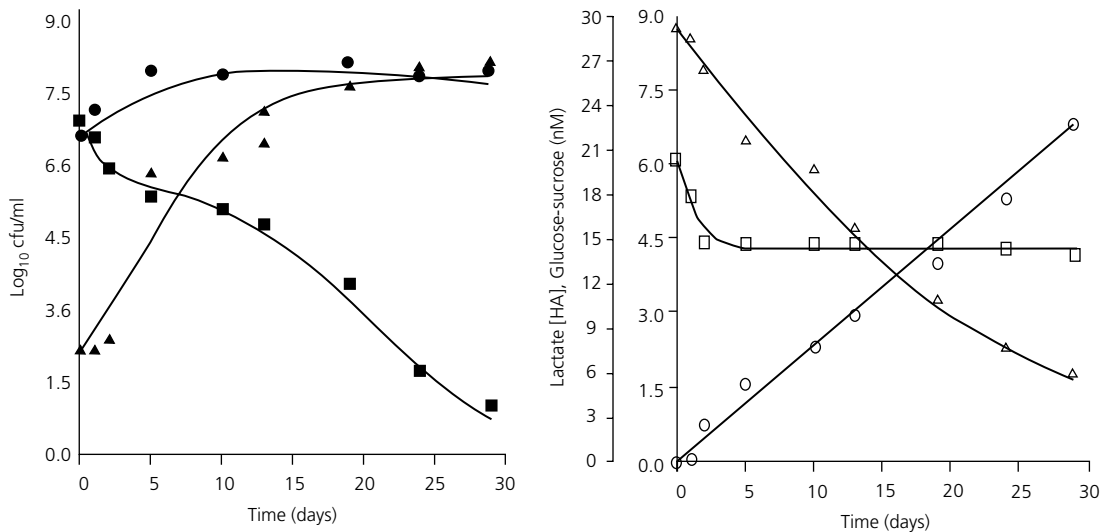


Figure 2.1 Changes in population of lactic acid bacteria (●), yeasts (▲), *E. coli* O157:H7 (■), pH (□), glucose concentration (Δ), and undissociated lactic acid (○) during the inoculated fermentation of green olives with *Lactobacillus plantarum*.

basic processes in food production, similar approaches could be extended to the survival of any food-borne pathogen on other fermented foods such as vegetables, meats, and dairy products.

2.6 Colonial versus planktonic type of growth: modes of microbial existence on surfaces and in liquid, semi-liquid, and solid foods

2.6.1 Biofilm formation on biotic and abiotic surfaces

During processing of contaminated raw materials and food preparation, microorganisms entrapped into the food residues may be transferred to the equipment surfaces (abiotic or inert surfaces) or cross-contaminate other foods (biotic surfaces). In the context of good hygienic and good manufacturing practice, processing plant surfaces are cleaned and disinfected after their use, in order to remove food soil and eliminate the transferred microorganisms. Inadequate disinfection may give rise to biofilm

formation. In addition, depending on the bacterial growth rate, the surrounding nutrient status, the attachment ability of the microorganisms, and the adaptive responses triggered by exposure to sublethal inimical factors, there may be an increase in microbial resistance to sanitizers and the establishment of persistent strains. Settlement of bacterial cells on solid surfaces leads to biofilm formation activity. Biofilms are referred to in the literature as biologically active matrices of cells and extracellular substances in association with a solid surface (Kumar and Anand, 1998). Placed in the solid–liquid interface, bacterial cells, irreversibly attached to the food surface, are organized into a glycocalyx (Kumar and Anand, 1998). Capillary water channels are part of that porous structure of biofilms, and distribute water and nutrients to the included microorganisms (Poulsen, 1999). Three critical steps are the content of the biofilm formation activity: (i) attachment, (ii) microcolony and EPS production, and (iii) maturation (Davey and O'Toole, 2000; Chmielewski and Frank, 2003). Bacterial cells may actively or passively adhere to the

surfaces, which depends on the bacterial cells surface properties, and the attachment can be reversible or irreversible. The attachment and biofilm-forming capabilities of bacteria depend on the interaction of multiple factors, including the surface characteristics and cell–surface interactions, the presence of other bacteria, the temperature, the availability of nutrients and pH, the production of extracellular polysaccharides, and cell-to-cell communication (Chmielewski and Frank, 2003; Van Houdt and Michiels, 2010).

2.6.2 Growth rate of microorganisms in different forms of growth

Microbial growth in foods occurs in the aqueous phase. The structural characteristics (also called “microarchitecture”) of this phase, in combination with the total concentration and dispersion of water compared to the fat phase, determine the form and rate of growth, i.e., the spatiotemporal microbial dynamics. Food may be characterized as liquid (e.g., juices, milk), gelled (e.g., jellies, cottage, marmalades), oil-in-water emulsions (e.g., mayonnaise) or water-in-oil emulsions (e.g., butter and margarine), and the composite form of gelled emulsions (i.e., an immobilized oil-in-water emulsion). Apparently, the type of emulsion determines the distribution of available water. The growth rate of microorganisms in response to food structure, for a given set of intrinsic and extrinsic parameters, is dependent on the motility of cells in the aqueous phase, the extent of resulting (micro-)colony immobilization, and the diffusion kinetics of nutrient, oxygen, and metabolites. Growth of microorganisms in a liquid aqueous phase typically is planktonic and the motility of microorganisms may enable access to certain nutrient-rich sites of the food (Wilson *et al.*, 2002). Access of cells to nutrients and transfer of metabolites away from cells contribute to the formation of a temporarily uniform environment, until the resources are depleted or the microbial metabolites are accumulated at self-toxic levels. If aqueous phase is structured, e.g., due to addition of thickeners or

gelling (structure-inducing) agents, such as gelatin, pectins, starch, gums, etc., microorganisms are immobilized in the gelled regions and constrained to grow as colonies, with their growth rates tending to be lower than that of planktonically growing cells (Theys *et al.*, 2008; Aspidou *et al.*, 2014; Wilson *et al.*, 2002; Boons *et al.*, 2013a, 2013b, 2014). This can be further enhanced by increasing the fat concentration on the expense of the water phase, thereby increasing the size of oil droplets with a concomitant trend of reversal of the oil-in-water emulsion. At low fat concentrations, the water phase is enough to allow cell motility that resembles planktonic growth. As the fat concentration increases and compresses water, growth is constrained and becomes colonial (actually spherical). A similar effect can be obtained by adding a structure-inducing agent, such as gelatin. On the surface of foods, such as meat and vegetables, growth is also colonial initially in two dimensions (mono-layer), whereas the center of the colony gradually develops in the third dimension, most likely upward, depending on aeration and nutrient availability. Replenishment of nutrients takes place only from the bottom or the perimeter of the colony and soon cells in the center of the colony experience starvation and self-toxication. This places growth constraints on the colony as a whole and causes suppression of the growth rate as compared to immobilized growth in the food matrix or planktonic growth. Thus, the growth rate of the aforementioned different forms of growth is known to follow the order: planktonic $\geq$ immobilized $>$ surface (Wilson *et al.*, 2002; Theys *et al.*, 2008).

These observations have also been explored in relation to the stochastic behavior of individual cells growing in liquid media or immobilized inside or on the surface of solid media. Starting from the single cell level and simulating the formation of a colony or the proliferation to high numbers in the planktonic state may assist in drawing useful conclusions on the expected behavior of large populations. These aspects are further discussed in the following paragraphs.

2.6.2.1 Individual-based modeling of floating or immobilized cells

The growth of a microbial population depends on the cumulative behavior of individual cells. It is well known that a great variability exists in the growth responses, i.e., lag time, generation time, and probability of growth among individual cells. This biological variability (also termed “*biovariability*”; Billon *et al.*, 1997) markedly impacts the dynamics, e.g., geometrical lag, germination time, and time to reach detectable levels of low populations, such as 1–50 cells and increases with the intensity of environmental stresses (Billon *et al.*, 1997; Dupont and Augustin, 2009; Francois *et al.*, 2005, 2006a; Guillier *et al.*, 2005; Guillier and Augustin, 2006; Smelt *et al.*, 2002, 2008). For instance, the distribution of germination times (lag) of individual *Cl. botulinum* spores became less peaky (i.e., with a lower kurtosis coefficient) and less positively skewed (i.e., a skewness factor close to 1) as the incubation temperature decreased from 37 to 20°C, suggesting that the variance in germination times increased with the intensity of temperature stress (Billon *et al.*, 1997). However, the variable behavior of single cells is masked by the massive behavior of large populations, e.g., >500 cells, or it is almost eliminated at optimal conditions (Llaudes *et al.*, 2001; Métris *et al.*, 2006; Smelt *et al.*, 2002). Most of the available predictive models quantify the response of high microbial populations at a given set of conditions, which may be constant or varying with time. In order to model the variability of single cells (or single spores), stochastic modelling may be applied, which is also the common approach in quantitative microbial risk assessment. Therefore, deterministic models apply to the population level, whereas stochastic models may describe the population dynamics taking into account the variability in the responses (e.g., lag time and generation time) of individual cells.

Although deterministic models average the behavior of individual cells, the characteristics of the latter cannot be deduced from population measurements (Kutalik *et al.*, 2005). Indeed, the

growth of a population may be simulated by superimposing the evolution of independent subpopulations derived from single cells, each receiving a lag time value, also termed “physiological lag” (Baranyi *et al.*, 2009; different from the geometrical population lag), from a specific probability distribution. The evolution of a microbial population can be modeled as a Poisson birth process with the constant birth intensity parameter μ (Baranyi, 1998; Baranyi and Pin, 2001). A cell is divided after an initial delay, which consists of the physiological lag and the generation time of the cell. Then each cell produces a subpopulation, which consists of cells growing independently in the same habitat with a constant growth rate (Baranyi, 1998; McKellar, 2001). To model this process, it is assumed that (Baranyi, 2002; Métris *et al.*, 2003): (i) after the first division of each cell, the daughter cells enter directly the exponential phase and (ii) daughter cells do not interact by any means, e.g., competition or quorum sensing. Both assumptions were reasonable when the experimental method used to describe the variability in lag times was the time to detect visible changes in the optical density of the liquid medium containing a single cell derived by a series of two- or tenfold dilutions of a standard concentrated microbial suspension, or even by sorting with flow cytometry (Baranyi *et al.*, 2009; Francois *et al.*, 2003; Smelt *et al.*, 2002, 2008; Standaert *et al.*, 2005). Individual lag times commonly follow Weibull, Gamma, exponential or normal distributions (Kutalik *et al.*, 2005; Francois, 2005, 2006a, 2007; Métris *et al.*, 2006; Standaert *et al.*, 2007). The development of sophisticated image analysis systems for real-time monitoring of single cell division (or spore germination) under the microscope, during continuous exposure of attached cells to flowing liquid media, allowed further insight into the variability assessment of single cells (Billon *et al.*, 1997; Elfving *et al.*, 2004). By targeting specific cells, it was observed that the generation time of daughter cells, which were removed after division, are not the same for all cells but they follow a distribution, the

variance of which decreases with the number of consecutive divisions (Pin and Baranyi, 2006; Métris *et al.*, 2005, 2006; Kutalik *et al.*, 2005).

Exposure of bacterial populations to sublethal stresses, such as chlorine, heat shock, pH, osmotic stresses, as well as suboptimal conditions in a new environment, shift the distribution of the time to the first division to higher levels (i.e., right) and the increase in its variance (Dupont and Augustin, 2009; Francois *et al.*, 2005, 2006a; Guillier *et al.*, 2005; Guillier and Augustin, 2006). Furthermore, stress may decrease the probability of a single cell to initiate growth and increase the number of cells needed for growth initiation (Dupont and Augustin, 2009; Koutsoumanis, 2008). As a result, both the extension of individual lag times and the reduction in single cell growth probability may lead to false negative detection, due to insufficient growth above the threshold level of enrichment or no growth at all during enrichment (Dupont and Augustin, 2009). Given that stress increases the biological variability, interactions between cells within colonies (e.g., due to competition for nutrients or the release of inhibitory metabolic products) may be an additional indigenous stress factor, which possibly increases cell lag variation, while retarding the growth of the total population (Guillier *et al.*, 2006).

Even though the variability of planktonic cells has been extensively characterized with OD or microscopic measurements, the variability in colonial growth associated with intracolony cell-to-cell interactions cannot be quantified in liquid cultures, nor by direct imaging of cells, when the daughter cell is removed after division. Alternatively, direct time-lapse imaging of microbial populations growing on agar surfaces of different intrinsic properties has enabled the characterization of population heterogeneity taking into account the interactions between adjacent cells (Guillier *et al.*, 2006; Koutsoumanis and Lianou, 2013). Experimental protocols for direct imaging of surface-growing cells include the gel-cassette system (Brocklehurst *et al.*, 1997), the systems

introduced by Billon *et al.* (1997), Guillier and Augustin (2006), and later on adopted by Koutsoumanis and Lianou (2013), consisting of an agar layer on top of a microscope slide, covered by a cover slip, sealed with paraffin wax, and placed under the microscope, and, more recently, the anopore strips (Ingham *et al.*, 2005). Applications of these methods at single cell or colony level may be found for *E. coli* O157:H7, for which a comparison between growth rates estimated from viable count data and changes in colony area (in pixels) is made (Skandamis *et al.*, 2007a), *Bacillus cereus* in response to salinity (den Besten *et al.*, 2007, 2010), and *Salmonella* showing the distribution of living and dead cells during the evolution of colonies in time and space (Theys *et al.*, 2009). These techniques may also allow for identification of injured or even dead subpopulations within bacterial microcolonies as a result of exposure to stresses or entrance into the stationary phase of growth. This is achievable through the use of well-established fluorophores, such as SYTO 9, which stains living cells, and propidium iodide, which stains dead cells or cells with impaired membranes. Experimental protocols involving microscopic observations have also been used to monitor the kinetics of single fungal spores, in response to temperature, a_w , pH, and ethanol stress (Dantigny *et al.*, 2005; Judet *et al.*, 2008; Gougouli and Koutsoumanis, 2013). Fungal spore kinetics include the germination time, which is the time until the length of the germ tube equals the diameter of the spore, the growth rate of the germination tube, and the percentage of germinated spores. Such data may serve as a basis in stochastic modeling for predicting the time until spoilage occurs, in the form of visible mycelium. Indeed, the variability in the germination time and rate of single fungal spores has been reflected on the lag time (i.e., the time to visible detection), the radial increase of *Aspergillus*, and *Fusarium* mycelia grown on corn solid media (Samapundo *et al.*, 2007). Advances in individual-based modelling (IbM) have suggested that, apart from the population

measurements, the complete characterization of lag time also requires the evolution of total biomass and thus the geometrical definition of lag time is not quite reliable (Prats *et al.*, 2008). Furthermore, since geometrical lag depends on the time required by the total viable population to exceed the detection limit of the enumeration method, a part of geometrical lag does not have practically biological meaning (also termed “pseudo-lag”; Koutsoumanis, 2008), because growth initiation of a fast-growing subpopulation, which will eventually give the detection signal, might have started quite earlier.

2.6.2.2 Individual-based modeling in foods

The well-established variability of single cells in laboratory media is expected to be even more pronounced in natural food ecosystems. This may be attributable to the combination of multiple stress factors in foods, such as limitations in nutrient diffusion, competition with natural flora, accumulation of inhibitory metabolic products, structural constraints, etc. Despite the low number of studies dealing with single cell variability in foods, a common conclusion is that the behavior of low inocula (e.g., <10 cfu/g) cannot be accurately approximated by models based on the responses of higher inocula on the same food nor by broth-based models (Manios *et al.*, 2013). For instance, the time that *L. monocytogenes* required for a hundredfold increase on vacuum packaged frankfurters stored at 4 and 8°C, starting from 0.007–0.1 cfu/g, was markedly higher than that expected based on the responses of 10–20 cfu/g on the safe food (Pal *et al.*, 2009). Likewise, the simulated variability in log-numbers of *L. monocytogenes* cells in liver pâté at 7°C or lettuce and cabbage fresh-cut salads, based on broth data, differed from the observed number (Francois *et al.*, 2006b; Manios *et al.*, 2013). Notably, Monte Carlo simulation based on stochastic description of lag times of individual *L. monocytogenes* cells from broth data slightly overpredicted the growth of single cells of *L. monocytogenes* after 12 days on lettuce. For instance, the model predicted that

there is a 60% likelihood for a single cell of the pathogen to reach 1.5 log cfu/g, while the observed growth under the same probability was 1 log cfu/g (Manios *et al.*, 2013). In contrast, remarked underestimation of the observed growth in cabbage was recorded, as the predictions showed that 60% of the individual cells could grow at 0.5 log cfu/g, whereas the observed growth was 2.6 log cfu/g. This deviation of the broth-based predictions from the observed growth suggests poor transferability of broth-based data to foods, because such models do not adequately encompass the effect of the epiphytic flora, the microstructure, or the scattered availability of nutrients. Thus, extrapolating broth-based predictions of microbial growth from single cells to foods may lead to either fail-safe or fail-dangerous predictions. As shown in Figure 2.2, the presence of antimicrobials and the competitive growth of indigenous microflora of ham slices increased the variability in a log-increase of *L. monocytogenes* single cells per slice at 10°C, as compared to higher initial populations, e.g., 100 cfu/slice. Furthermore, even competition among strains at the single cell level may inhibit growth initiation of each strain in the composite, especially when antimicrobials are added in the product formulation (Figure 2.3). In this case, a higher number of cells is needed so that one cell capable of overcoming strain competition and initiate growth is present in the population (Figure 2.3). Therefore, it is imperative that the evaluation of the response of single cells in foods should receive more focus in parallel to the optimization of laboratory media assays, which provide further theoretical aspects under controlled conditions.

2.7 Modeling microbial transfer between processing equipment and foods

The recent deeper insight into bacterial transfer phenomena, along with the pronounced necessity of incorporating them in risk

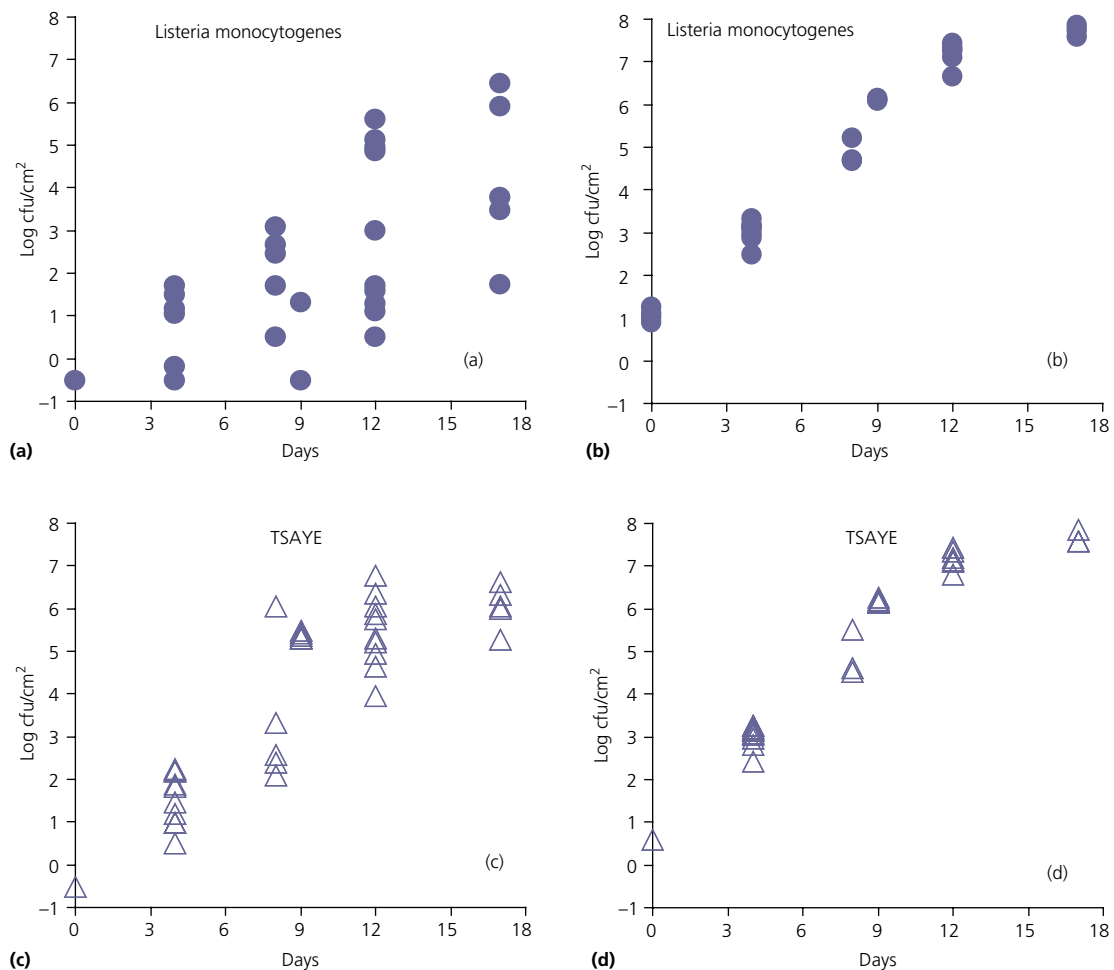


Figure 2.2 Growth of *L. monocytogenes* single cells (a), or 100 cells/slice (b), and the natural mesophilic flora (c, d) on the corresponding slices of ham formulated without antimicrobials, packaged under vacuum and stored at 10 °C (Skandamis and Sofos 2010; unpublished data).

assessment studies, has implemented predictive microbiology with a new type of model: bacterial transfer models. Modeling bacterial transfer can be considered as a challenge for predictive microbiologists. The significance of choosing the right model to describe a microbiological process is well known and the choice of the appropriate parameters as model inputs is decisive for the outcome of the prediction. This fact makes construction of bacterial transfer models a complex task. As Pérez-Rodríguez *et al.* (2008) state, not only do unknown factors govern

bacterial transfer but also the known factors (contact time, bacterial strain properties, pressure, etc.) that are involved in cross-contamination events cannot be easily controlled. The challenge in modeling bacterial transfer in food-processing environments, such as the common house-kitchen or a food industry, is the dynamic conditions that dominate those environments. At the very same time, a recipient surface can become the donor surface, in terms of contamination. Furthermore, this event can take place at every possible location. The

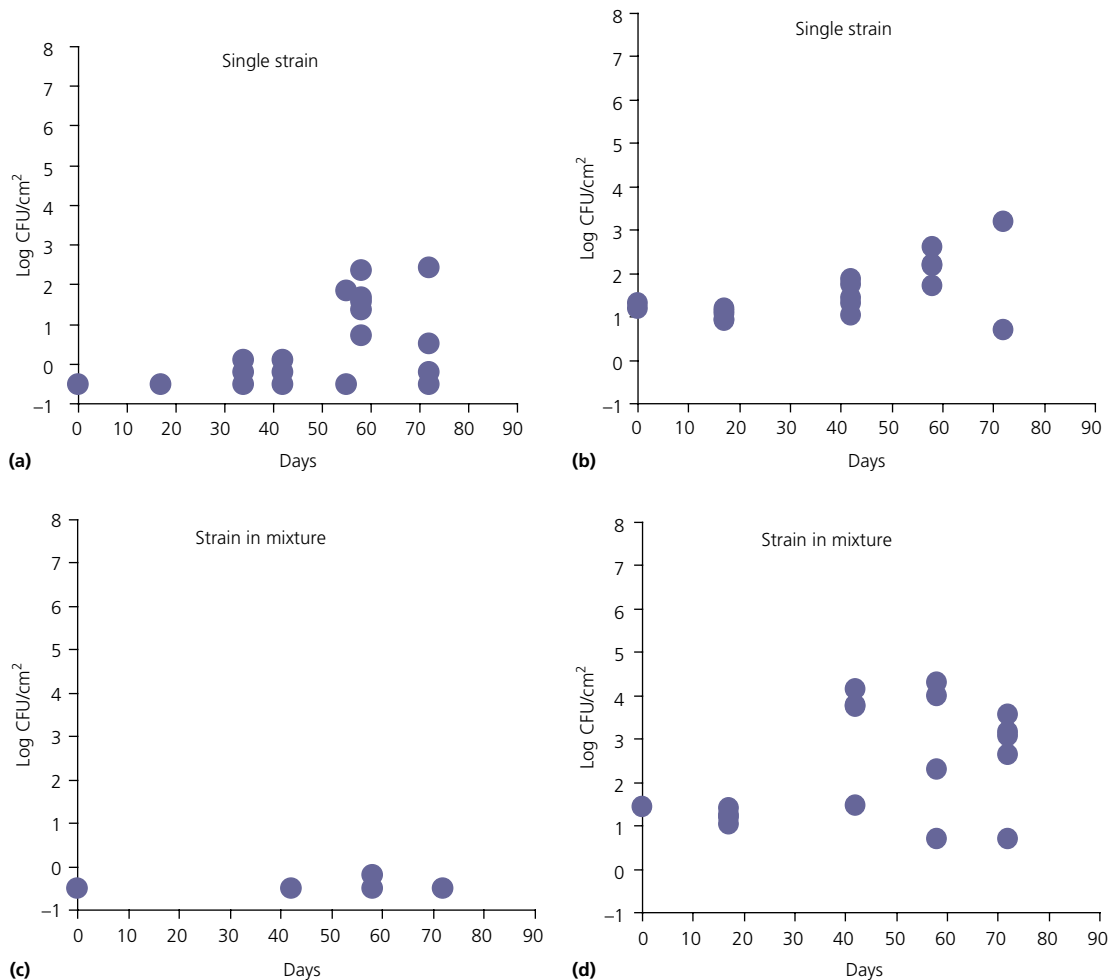


Figure 2.3 Growth of *L. monocytogenes* single cells/slice (a, c) or 100 cells/slice (b, d) as single strain (a, b) or in a mixture of three strains (c, d) on slices of ham formulated with antimicrobials, packaged under vacuum and stored at 10 °C (Skandamis and Sofos 2010; unpublished data).

contamination status changes constantly and each new generated pathway becomes the new starting point for more transfer scenarios.

As aforementioned, the first step to modeling transfer is the calculation of the transfer rates as the percentage of cells transferred from the source to the destination of contamination. It has also been mentioned that the multitude of factors that govern the bacterial transfer lead to a high variability regarding this phenomenon and subsequently regarding the data obtained from relevant studies. Therefore, this variability,

which is reflected in estimated transfer rates, can be depicted through probability distributions. For many authors, probability distributions can be constructed based on the log-transformed transfer rates instead of the transfer rates. In fact, normal distribution is considered as the most suitable to best fit the log-transformed transfer rates. Hoelzer *et al.* (2012) used available scientific data to produce probability distributions and models in order to describe the transfer of *L. monocytogenes* between foods and surfaces or during slicing, as well as the impact

of sanitation methods on subsequent cross-contamination events. Normal distributions were also chosen to fit the log-transformed transfer data. In a similar study carried out by Chen *et al.* (2001) to determine transfer rates of *E. aerogenes* between food and surfaces used for food preparation, normal distribution was chosen as the most suitable and statistically convenient way to represent the data. Similarly, Kusumaningrum *et al.* (2004) used the Anderson–Darling criteria and decided that the normal distribution gives the best fit to the log-transformed transfer rates. Alternatively, logistic distributions were ranked first, when normal ones did not give the best results.

Research advances include attempts to construct models that mathematically describe cross-contamination scenarios. Usually, the models are empirical due to the lack of concrete knowledge on bacterial transfer phenomena. The interest has mainly been focused on pathogen transfer that takes place between cutting, shredding, or slicing equipment and foods. The particular interest in those processes is apparently attributed to the employed equipment (e.g., knife or slicer blades) or the type of foods involved (e.g., sliced RTE meat products, fresh-cut salads, and salmon) and consequently the risk implied. The models developed to describe those experimental data represent transfer as a function of the cut/slice number or inoculation level. In respect to this, both Pérez-Rodríguez *et al.* (2007) and Vorst *et al.* (2006) agreed that, when different transfer scenarios were investigated, the logarithmic decrease that was observed in the numbers of the microorganisms under study could be efficiently described by the log-linear model. The goodness of the log-linear model to fit the experimental data was only comparable to that of the Weibull model as reported by Pérez-Rodríguez *et al.* (2007).

The empirical models used so far have been proven sufficiently accurate and promising for risk assessment studies (Aarnisalo *et al.*, 2007; Sheen, 2008; Sheen and Hwang, 2010). A more mechanistic approach was introduced by Møller *et al.* (2012) to treat their experimental data.

They developed a model simulating the transfer of *S. Typhimurium* during the grinding of pork. The proposed model was an improved version of the model presented by Nauta *et al.* (2005), taking into account the tailing phenomenon that is observed after sequential slices during a grinding process. The authors claim that this type of model, though similar to empirical models such as that of Sheen and Hwang (2010), can more efficiently describe all the events that take place in a grinder. It also introduces the idea of the two environments existing in the grinder that are responsible for two different transfer rates occurring during the process. This was successfully applied by Zilelidou *et al.* (2014) in modeling the transfer of *Salmonella* Typhimurium and *L. monocytogenes* between lettuce and knives and vice versa in a simulated process of fresh-cut salad preparation in the domestic or catering environment, which involved 50 consecutive cuts with a knife that was first contaminated by cutting a contaminated batch of lettuce leaves. Finally, a mechanistic approach was also chosen by Aziza *et al.* (2006) to explain cross-contamination during cheese-smearing industrial operations. In this study, a binomial distribution was applied and the constructed model reflected the potential risk associated with this type of cheese manufacturing.

The approach of Aziza *et al.* (2006) was based on certain assumptions, e.g., even bacterial distribution throughout the food matrix. Several authors (Hoelzer *et al.*, 2012; Kusumaningrum *et al.*, 2004; van Asselt *et al.*, 2008) have made some of the following assumptions for the setup of the experiment and the subsequent buildup of the model; no bacteria are lost during processes, no cleaning of the equipment takes place in between the use, no new contamination is introduced in the processing equipment for a certain number of operations, and the time after which the transfer event will take place does not have any impact on the event. Such assumptions seem to be necessary for the development of bacterial transfer models since the complexity

and inherent variability of the phenomenon demands a simplification and sometimes a more linear representation of transfer pathways (van Asselt *et al.*, 2008).

2.8 Alternative multivariate approaches: the use of bioinformatics for characterizing spoilage and product classification

Food quality changes are influenced by intrinsic factors such as pH, water activity, the food matrix, etc., and by extrinsic processing factors such as temperature, processing time, packaging conditions, etc. The interaction of these factors enables particular microorganisms to prevail in a food ecosystem having thus synergistic or antagonistic effects on the microbial activity of other species encountered on a food product (Nychas *et al.*, 2008). Proper selection and combination of intrinsic and extrinsic factors ensures that the safety and quality of a food commodity meets consumer expectations and complies with commercial trends. It is also acknowledged that food quality and safety is a multiresponse phenomenon based on the evolution of change of diverse food characteristics under a range of extrinsic processing variables linked to each other in a complex reaction network (Grauwet *et al.*, 2014).

In food science, metabolomics has been proved a valuable tool to investigate quality changes during processing, distribution, and storage of raw materials and final products (Cevallos-Cevallos *et al.*, 2009; Hall, 2011; Ammor *et al.*, 2009; Vervoort *et al.*, 2012). In the area of quality and safety of processed foods, metabolomics can contribute in (a) food classification, food adulteration, and authenticity assessment, (b) quality control in the food industry, (c) food spoilage, (d) association between sensory characteristics and chemical composition, and (e) impact of processing on food composition (Oms-Oliu *et al.*, 2013). Metabolomic analyses can be generally classified

as targeted and untargeted. In the first case, the focus is given on a specific compound or class of compounds requiring prior identification and quantification of the metabolites under study (Ramautar *et al.*, 2006). In contrast, untargeted metabolomics aims at the investigation of patterns of fingerprints of metabolites without the necessity of indentifying specific compounds (Cook and Rutan, 2014). In addition, based on the specific purpose of the analysis, the majority of metabolomic studies can be classified into three broad classes, namely, discriminative, informative, and predictive. The aim of discriminative analysis is to employ multivariate data analysis techniques to maximize classification of samples. In this direction, principal components analysis (PCA) has been extensively employed as a method of choice by many authors (Oms-Oliu *et al.*, 2013). Moreover, informative metabolomic analyses focus on the identification and quantification of metabolites in order to develop databases, identify biomarkers, discover bioactive compounds, etc. (Cevallos-Cevallos *et al.*, 2009). Finally, predictive metabolomic analyses employ statistical models based on a metabolite profile to estimate a property (e.g., microbial population, chemical property, etc.) that is difficult or time consuming to be quantified by conventional analyses. A key concept in metabolomic analysis is fingerprinting, an -omics approach that was adopted from the scientific discipline of metabolomics and employed in food systems (Grauwet *et al.*, 2014). The purpose of fingerprinting techniques is to perform comparative analysis in order to identify differences among food samples. Subsequently, using multivariate data analysis, metabolic fingerprints could result in the selection of specific compounds that differ when compared to other samples in different conditions. These compounds could be used as markers and linked to particular food characteristics for more insight (e.g., food spoilage). The development of rapid analytical technologies has contributed enormously to the expansion of metabolomics in food science. Metabolomic analytical platforms include mass

spectrometry (MS) instruments, high-resolution nuclear magnetic resonance (NMR) spectrometers, capillary electrophoresis (CE), as well as ultra-high pressure liquid chromatography systems for rapid compound separation (Oms-Oliu *et al.*, 2013). A major challenge in metabolomics involves the selection of the appropriate platform for data processing and analysis in order to turn raw metabolomic data into biologically meaningful information. To this end, multivariate statistical analysis is required to manipulate the huge amount of data generated in a metabolomic experiment. There are many multivariate techniques for data analysis based on principal components analysis (PCA), principal components regression (PCR), canonical discriminant analysis (CDA), cluster analysis (CA), and partial least squares (PLS) analysis (Jain *et al.*, 2000; Jurs *et al.*, 2000). Moreover, in the last few years, intelligent machine learning methodologies have been employed including artificial neural networks (ANNs), support vector machines (SVMs), and genetic algorithms (GAs) (Berrueta *et al.*, 2007).

Metabolomics have been proved very efficient in the detection of microbial metabolites that can be used as biomarkers in meat spoilage under different packaging conditions (air, vacuum, modified atmosphere packaging) using high-performance liquid chromatography (HPLC), gas chromatography (GC), and/or mass spectrometry (MS) (Casaburi *et al.*, 2015; Ercolini *et al.*, 2011; Perez *et al.*, 2008; Bhattacharjee *et al.*, 2011; Ferrocino *et al.*, 2013; Insausti *et al.*, 2002). In a recent work, Argyri *et al.* (2015) analyzed minced beef spoilage, packaged aerobically and under modified atmospheres, through the evolution of volatile compounds detected by headspace solid phase microextraction gas chromatography/mass spectroscopy (SPME-GC/MS) analysis. Many of the identified compounds were correlated with the sensory scores provided by a taste panel by means of principal components analysis (PCA), depicting possible spoilage indicators such as 2-pentanone, 2-nonanone, 2-methyl-1-butanol,

3-methyl-1-butanol, ethyl hexanoate, ethyl propanoate, ethyl lactate, ethyl acetate, ethanol, 2-heptanone, 3-octanone, diacetyl, and acetoin, whereas the compounds 2-butanone, 2,3-pentanedione, 2,5-octanedione, pentanal, hexanal, trans-2-heptanal and trans-2-octenal were positively correlated with acceptable samples, either fresh or semi-fresh as classified by the taste panel. The acquired GC/MS chromatograms were further associated with the changes of the microbial population enumerated on meat samples during storage at chill (0 and 5°C) and abuse temperatures (10 and 15°C) in an attempt to build models based on PLS regression (PLS-R) that could estimate the microbial population indirectly through the use of GC/MS data, thus avoiding time-consuming and expensive microbiological analysis. The PLS-R models were found to estimate sufficiently all the microbial groups based on the performance indices of root mean squared error (RMSE), bias, and accuracy factors. For other foods, spoilage of wine by *Botrytis cinerea*, leading to serious degradation of aroma compounds and off-odor development, has been studied with an NMR-based metabolomic approach in tandem with chemometrics (principal components analysis, PCA) and revealed several discriminant metabolites in low concentrations indicative of spoilage, such as glycerol, 2,3 butanediol, succinate, tyrosine, valine derivative, and phenylpropanoids (Hong *et al.*, 2011). Another serious issue for the fruit juice industry is spoilage due to the presence of *Alicyclobacillus acidoterrestris*, which causes off-flavor in different kinds of juice. A metabolomic approach based on GC-MS analysis has been investigated recently (Bianchi *et al.*, 2010) in an effort to develop an early detection system in orange juice. GC-MS acquired data were analyzed with principal components analysis (PCA) and linear discriminant analysis (LDA) and revealed that the presence of this microorganism could be associated with increased concentrations of certain terpenic alcohols, aldehydes, and ketones, whereas neither guaiacol nor 2,6-dibromophenol, usually regarded as contamination markers,

were detected in the analyzed samples. It must be noted that the advancement of metabolomics technology allows sensitive quantification of diverse compounds of microbial origin in food matrices that are related to food safety. Thus NMR technologies are effective in detecting microbial toxins in food, whereas MS-based methodologies could detect volatile metabolites related to a specific microbial contamination (Xu *et al.*, 2014). Thus, GC-MS has been successfully employed in the detection of mycotoxins in wheat and bee pollen (Rodriguez-Carrasco *et al.*, 2012, 2013), and patulin in apple juice (Xiao and Fu, 2012).

The contribution of metabolomics in food processing control is also significant. In the area of fermented foods, Bleve *et al.* (2015) used the solid-phase microextraction technique followed by gas chromatography/mass spectrometry (HS-SPME GC/MS) analysis in tandem with chemometrics (principal components analysis, PCA) to investigate the evolution of volatile compounds in table olive fermentation and define potential biomarkers that could be used as indicators of fermentation. They demonstrated that the early stage of fermentation (30 days) was associated with aldehydes that contribute to the herbaceous flavor of fruits and vegetables, the middle stage (90 days) by higher alcohols (isoamyl/alcohols) due to the presence of yeasts, and the final stage (180 days) by acetate esters and acetic acid due to the activity of bacteria.

Metabolomics have also a significant contribution in authentication and adulteration issues in a variety of food commodities, including edible oils, milk and dairy products, tea and coffee, honey, fruit juices, alcoholic beverages, meat, fish, and seafood (Cubero-leon *et al.*, 2014). In high-value products, such as meat, authenticity is a major issue, particularly in minced and homogenized meat, and there is a need for the development of fast, reliable, and on-line methods. Instruments based on vibrational spectroscopy (NIR, MIR, UV-VIS, and Raman) have been proposed to identify the authenticity of minced meat since they are

considered to be rapid, non-destructive, and easy to-use (Sun, 2009). Fourier transform infrared (FTIR) spectroscopy has been successfully employed to detect various types of adulteration (Rohman *et al.*, 2011; Alamprese *et al.*, 2013; Zhao *et al.*, 2014). Visible–near infrared (Vis–NIR) spectroscopy has been used for the differentiation of beef and kangaroo meat (Ding and Xu, 1999) and the adulteration of fresh and thawed beef burgers from pork, fat trimming, and offal (Morsy and Sun, 2013). On the other hand, Raman spectroscopy has been used to differentiate meat types or to distinguish purity of food products (Ellis *et al.*, 2005; Ebrahim *et al.*, 2013; Mohamadi Monavar *et al.*, 2013; Boyacı *et al.*, 2014).

Despite the considerable progress of metabolomics in food science and the continuously increasing number of publications in the area, the application of fingerprinting approaches is still in its infancy and certain challenges must be tackled successfully to draw robust conclusions in order to make metabolomics-based methods acceptable by regulatory and inspection authorities. At the moment, the majority of studies is based on a small sample size and thus the implementation of these techniques on a routine basis to replace currently employed analyses would require the establishment of larger datasets to account for the variability due to different batches, production practices, etc., to obtain models with global applicability. Another issue of interest that would contribute to the expansion of metabolomics is the development or improvement of existing food databases that would assist in the routine identification and quantification of metabolites encountered in different food matrices.

References

- Aarnisalo, K., Sheen, S., Raaska, L., and Tamplin, M. 2007. Modelling transfer of *Listeria monocytogenes* during slicing of “Gravad” salmon. *International Journal of Food Microbiology*, **118**, 69–78.

- Adams, M.R. and Nicolaides, I. 2008. Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control*, **8**, 227–239.
- Adams, M.R., Little, C.L., and Easter, M.C. 1991. Modelling the effect of pH, acidulant and temperature on the growth rate of *Yersinia enterocolitica*. *Journal of Applied Bacteriology*, **71**, 65–71.
- Alamprese, C., Casale, M., Sinelli, N., Lanteri, S., and Casiraghi, E. 2013. Detection of minced beef adulteration with turkey meat by UV–vis, NIR and MIR spectroscopy. *LWT - Food Science and Technology*, **53**, 225–232.
- Alavi, S.H., Puri, V.M., Knabel, S.J., Mohtar, R.H., and Whiting, R.H. 1999. Development and validation of a dynamic growth model for *Listeria monocytogenes* in fluid whole milk. *Journal of Food Protection*, **62**, 170–176.
- Ammor, M., Argyri, A., and Nychas, G.-J.E. 2009. Rapid monitoring of the spoilage of minced beef stored under conventionally and active packaging conditions using Fourier transform infrared spectroscopy in tandem with chemometrics. *Meat Science*, **81**, 507–515.
- Argyri, A.A., Mallouchos, A., Panagou, E.Z., and Nychas, G.-J.E. 2015. The dynamics of the HS/SPME-GC/MS as a tool to assess the spoilage of minced beef stored under different packaging and temperature conditions. *International Journal of Food Microbiology*, **193**, 51–58.
- Arroyo-López, F.N., Bautista-Gallego, J., Rodríguez-Gómez, F., and Garrido-Fernández, A. 2010. Predictive microbiology and table olives. In: *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology* (ed. Méndez-Vilas, A.), Microbiology Series No 2. Formatex, Spain.
- Aspidou, Z., Moschakis, T., Biliaderis, C.G., and Koutsoumanis, K.P. 2014. Effect of the substrate's microstructure on the growth of *Listeria monocytogenes*. *Food Research International*, **64**, 683–691.
- Augustin, J.-C. and Carlier, V. 2000. Modelling the growth of *Listeria monocytogenes* with a multiplicative type model including interactions between environmental factors. *International Journal of Food Microbiology*, **56**, 53–70.
- Augustin, J.C., Brouillaud-Delattre, A., Rosso, L., and Carlier, V. 2000a. Significance of inoculum size in the lag time of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, **66**, 1706–1710.
- Augustin, J.C., Rosso, L., Carlier, V. 2000b. A model describing the effect of temperature history on lag time for *Listeria monocytogenes*. *International Journal of Food Microbiology*, **57**, 169–181.
- Augustin, J.C., Zuliani, V., Cornu, M., and Guillier, L. 2005. Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions. *Journal of Applied Microbiology*, **99**, 1019–1042.
- Ayrapetyan, M., Williams, T.C., and Oliver, J.D. 2014. Bridging the gap between viable but non-culturable and antibiotic persistent bacteria. *Trends in Microbiology*, **23**, 7–13.
- Aziza, F., Mettler, E., Daudin, J.-J., and Sanaa, M. 2006. Stochastic, compartmental and dynamic modeling of cross-contamination during mechanical smearing of cheeses. *Risk Analysis*, **26**, 731–745.
- Baker, D.A. and Genigeorgis, C. 1990. Predicting the safe storage of fresh fish under modified atmospheres with respect to *Clostridium botulinum* toxigenesis by modelling length of the lag phase of growth. *Journal of Food Protection*, **53**, 131–140.
- Baranyi, J. 1998. Comparison of stochastic and deterministic concepts of bacterial lag. *Journal of Theoretical Biology*, **192**, 403–408.
- Baranyi, J. 2002. Stochastic modelling of bacterial lag phase. *International Journal of Food Microbiology*, **73**, 203–206.
- Baranyi, J. and Pin, C. 2001. A parallel study on bacterial growth and inactivation. *Journal of Theoretical Biology*, **210**, 327–336.
- Baranyi, J. and Pin, C. 2004. Modeling the history effect on microbial growth and survival: deterministic and stochastic approaches. In: *Modeling Microbial Responses in Foods* (eds McKellar, R.C. and Lu, X.), pp. 63–149, CRC Press, London.
- Baranyi, J. and Roberts, T.A. 1994. A dynamic approach to predicting bacterial growth in foods. *International Journal of Food Microbiology*, **23**, 277–294.

- Baranyi, J. and Roberts, T.A. 1995. Mathematics of predictive food microbiology. *International Journal of Food Microbiology*, **26**, 199–218.
- Baranyi, J., George, S.M., and Kutalik, Z. 2009. Parameter estimation for the distribution of single cell lag times. *Journal of Theoretical Biology*, **259**, 24–30.
- Belessi, C.E.A., Gounadaki, A.S., Schvartzman, S., Jordan, K. and Skandamis, P.N. 2011a. Evaluation of growth/no growth interface of *Listeria monocytogenes* growing on stainless steel surfaces, detached from biofilms or in suspension, in response to pH and NaCl. *International Journal of Food Microbiology*, **145**, S53–S60.
- Belessi, C.-I.A., Le Marc, Y., Merkouri, S.I., Gounadaki, A.S., Schvartzman, S., Jordan, K., Drosinos, E.H., and Skandamis, P.N. 2011b. Adaptive growth responses of *Listeria monocytogenes* to acid and osmotic shifts above and across the growth boundaries. *Journal of Food Protection*, **74**, 78–85.
- Benlloch-Tinoco, M., Pina-Pérez, M.C., Martínez-Navarrete, N., and Rodrigo, D. 2014. *Listeria monocytogenes* inactivation kinetics under microwave and conventional thermal processing in a kiwifruit puree. *Innovative Food Science and Emerging Technologies*, **22**, 131–136.
- Bermúdez-Aguirre, D. and Corradini, M.G. 2012. Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments. *Food Research International*, **45**, 700–712.
- Berrueta, L.A., Alonso-Salces, R.M., and Héberger, K. 2007. Supervised pattern recognition in food analysis. *Journal of Chromatography A*, **1158**, 196–214.
- Bhaduri, S., Smith, P.W., Palumbo, S.A., Turner-Jones, C.O., Smith, J.L., Marmer, B.S., Buchanan, R.L., Zaika, L.L., and Williams, A.C. 1991. Thermal destruction of *Listeria monocytogenes* in liver sausage slurry. *Food Microbiology*, **8**, 75–78.
- Bhattacharjee, P., Panigrahi, S., Lin, D., Logue, C.M., Sherwood, J.S., Doetkott, C., and Marchello, M.A. 2011. A comparative qualitative study of the profile of volatile organic compounds associated with *Salmonella* contamination of packaged aged and fresh beef by HS-SPME/GC-MS. *International Journal of Food Microbiology*, **48**, 1–13.
- Bianchi, F., Careri, M., Mangia, A., Mattarozzi, M., Musci, M., Concina, I., and Gobbi, E. 2010. Characterisation of the volatile profile of orange juice contaminated with *Alicyclobacillus acidoterrestris*. *Food Chemistry*, **123**, 653–658.
- Billon, C.M.-P., McKirgan, C.J., McClure, P.J., and Adair, C. 1997. The effect of temperature on the germination of single spores of *Clostridium botulinum* 62A. *Journal of Applied Microbiology*, **82**, 48–56.
- Blankenship, L.C., Craven, S.E., Leffler, R.G., and Custer, C. 1988. Growth of *Clostridium perfringens* in cooked chilli during cooling. *Applied and Environmental Microbiology*, **54**, 1104–1108.
- Bleve, G., Tufariello, M., Durante, M., Grieco, F., Ramires, F.A., Mita, G., Tasioula-Margari, M., and Logrieco, A.F. 2015. Physico-chemical characterization of natural fermentation process of Conservolea and Kalamata table olives and development of a protocol for the pre-selection of fermentation starters. *Food Microbiology*, **46**, 368–382.
- Boons, K., Mertens, L., Van Derlinden, E., David, C.C., Hofkens, J., and Van Impe, J.F. 2013a. Behavior of *Escherichia coli* in a heterogeneous gelatin-dextran mixture. *Applied and Environmental Microbiology*, **79**, 3126–3128.
- Boons, K., Van Derlinden, E., Mertens, L., Peeters, V., and Van Impe, J.F. 2013b. Effect of immobilization and salt concentration on the growth dynamics of *Escherichia coli* K12 and *Salmonella* Typhimurium. *Journal of Food Science*, **78**, 567–574.
- Boons, K., Noriega, E., Van den Broeck, R., David, C.C., Hofkens, J., and Van Impe, J.F. 2014. Effect of microstructure on population growth parameters of *Escherichia coli* in gelatin-dextran systems. *Applied and Environmental Microbiology*, **80**, 5330–5339.
- Booth, I.R. 2002. Stress and the single cell: intrapopulation diversity is a mechanism to ensure survival upon exposure to stress. *International Journal of Food Microbiology*, **78**, 19–30.
- Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., et al. 2012. Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology*, **154**, 87–97.

- Boyacı, İ.H., Temiz, H.T., Uysal, R.S., Velioglu, H.M., Yadegari, R.J., and Rishkan, M.M. 2014. A novel method for discrimination of beef and horsemeat using Raman spectroscopy. *Food Chemistry*, **148**, 37–41.
- Brocklehurst, T.F., Mitchell, G.A., and Smith, A.C. 1997. A model experimental gel surface for the growth of bacteria on foods. *Food Microbiology*, **14**, 303–311.
- Bruggeman, F.J. and Westerhoff, H.V. 2007. The nature of systems biology. *Trends in Microbiology*, **15**, 45–50.
- Brul, S., Westerhoff, H.V. 2007. Systems biology and food microbiology. In: *Modelling Microorganisms in Food* (ed. Brul, S., van Gerwen, S., and Zwietering, M.), pp. 250–288. Woodhead Publishing Limited and CRC Press LLC, England.
- Buchanan, R.L. 1993. Predictive food microbiology. *Trends in Food Science and Technology*, **4**, 6–11.
- Caplice, E. and Fitzgerald, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, **50**, 131–149.
- Casaburi, A., Piombino, P., Nychas, G.-J.E., Villani, F., and Ercolini, D. 2015. Bacterial population and volatile associated to meat spoilage. *Food Microbiology*, **45**, 83–102.
- Cerf, O. 1977. Tailing of survival curves of bacterial spores. *Journal of Applied Bacteriology*, **42**, 1–19.
- Cevallos-Cevallos, J.M., Reyes-De-Corcuera, J.I., Etcheberria, E., Danyluk, M.D.D., and Rodrick, G.E. 2009. Metabolomic analysis in food science: a review. *Trends in Food Science and Technology*, **20**, 557–566.
- Chandler, R.E., McMeekin, T.A. 1985a. Temperature function integration and the prediction of the shelf-life of milk. *Australian Journal of Dairy Technology*, **40**, 10–13.
- Chandler, R.E. and McMeekin, T.A. 1985b. Temperature function integration and its relationship to the spoilage of pasteurised, homogenised milk. *Australian Journal of Dairy Technology*, **40**, 37–41.
- Chandler, R.E. and McMeekin, T.A. 1989a. Modelling the growth response of *Staphylococcus xylosus* to changes in temperature and glycerol concentration/water activity. *Journal of Applied Bacteriology*, **66**, 543–548.
- Chandler, R.E. and McMeekin, T.A. 1989b. Combined effect of temperature and salt concentration/water activity on the growth rate of *Halobacterium* spp. *Journal of Applied Bacteriology*, **67**, 71–76.
- Chen, H. and Hoover, D.G. 2004. Use of Weibull model to describe and predict pressure inactivation of *Listeria monocytogenes* Scott A in whole milk. *Innovative Food Science and Emerging Technologies*, **5**, 269–276.
- Chen, Y., Jackson, K.M., Chea, F.P., and Schaffner, D.W. 2001. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. *Journal of Food Protection*, **64**, 72–80.
- Chmielewski, R.N. and Frank, J.F. 2003. Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 22–32.
- Cole, M.B., Davies, K.W., Munro, G., Holyoak, C.D., and Kilsby, D.C. 1993. A vitalistic model to describe thermal inactivation of *Listeria monocytogenes*. *Journal of Industrial Microbiology*, **12**, 232–237.
- Cook, W.D. and Rutan, S.C. 2014. Chemometrics for the analysis of chromatographic data in metabolomics investigations. *Journal of Chemometrics*, **28**, ii (doi: 10.1002/cem.2631).
- Corkrey, R., Olley, J., Ratkowsky, D., McMeekin, T., and Ross, T. 2012. Universality of thermodynamic constants governing biological growth rates. *PLoS One*, **7**, e32003.
- Corkrey, R., McMeekin, T.A., Bowman, J.P., Ratkowsky, D.A., Olley, J., and Ross, T. 2014. Protein thermodynamics can be predicted directly from biological growth rates. *PLoS One*, **9**, e96100.
- Coroller, L., Kan-King-Yu, D., Leguerinel, I., Mafart, P., and Membre, J.-M. 2012. Modelling of growth, growth/no-growth interface and non-thermal inactivation areas of *Listeria* in foods. *International Journal of Food Microbiology*, **152**, 139–152.
- Corradini, M.G. and Peleg, M. 2006. On modeling and simulating transitions between microbial

- growth and inactivation or vice versa. *International Journal of Food Microbiology*, **108**, 22–35.
- Corradini, M.G. and Peleg, M. 2007. The non-linear kinetics of microbial inactivation and growth of foods. In: *Modelling Microorganisms in Food* (eds Brul, S., van Gerwen, S., and Zwietering, M.). Woodhead Publishing Ltd, Cambridge, UK.
- Cubero-Leon, E., Peñalver, R., Maquet, A. 2014. Review on metabolomics for food authentication. *Food Research International*, **60**, 95–107.
- Dalgaard, P. 1995. Modelling of microbial activity and prediction of shelf life for packed fresh fish. *International Journal of Food Microbiology*, **26**, 305–317.
- Dalgaard, P., Mejlholm, O., and Huss, H.H. 1997. Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. *International Journal of Food Microbiology*, **38**, 169–179.
- Dang, T.D.T., Mertens, L., Vermeulen, A., Geeraerd, A.H., Van Impe, J.F., Debevere, J., and Devlieghere, F. 2010. Modelling the growth/no growth boundary of *Zygosaccharomyces bailii* in acidic conditions: a contribution to the alternative method to preserve foods without using chemical preservatives. *International Journal of Food Microbiology*, **137**, 1–12.
- Dantigny, P., Tchobanov, I., Bensoussan, M., and Zwietering, M.H. 2005. Modeling the effect of ethanol vapor on the germination time of *Penicillium chrysogenum*. *Journal of Food Protection*, **68**, 1203–1207.
- Davey, K.R. 1989. A predictive model for combined temperature and water activity on microbial growth during the growth phase. *Journal of Applied Bacteriology*, **67**, 483–488.
- Davey, M.E. and O' Toole, G.A. 2000. Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular*, **64**, 847–867.
- den Besten, H.M.W., Ingham, C.J., van Hylckama Vlieg, J.E.T., Beerthuyzen, M.M., Zwietering, M.H., and Abee, T. 2007. Quantitative analysis of population heterogeneity of the adaptive salt stress response and growth capacity of *Bacillus cereus* ATCC 14579. *Applied and Environmental Microbiology*, **73**, 4797–4804.
- den Besten, H.M.W., Garcia, D., Moezelaar, R., Zwietering, M.H., and Abee, T. 2010. Direct-imaging-based quantification of *Bacillus cereus* ATCC 14579 population heterogeneity at a low incubation temperature. *Applied and Environmental Microbiology*, **76**, 927–930.
- Devlieghere, F., Debevere, J., and van Impe, J. 1998. Concentration of carbon dioxide in the water-phase as a parameter to model the effect of a modified atmosphere on microorganisms. *International Journal of Food Microbiology*, **43**, 105–113.
- Devlieghere, F., Geeraerd, A.H., Versyck, K.J., Bernaert, H., Van Impe, J.F., and Debevere, J. 2000a. Shelf life of modified atmosphere packed cooked meat products: addition of Na-lactate as a fourth shelf life determinative factor in a model and product validation. *International Journal of Food Microbiology*, **58**, 93–106.
- Devlieghere, F., Lefevre, I., Magnin, A., and Debevere, J. 2000b. Growth of *Aeromonas hydrophila* in modified-atmosphere-packed cooked meat products. *Food Microbiology*, **17**, 185–196.
- Ding, H.B. and Xu, R.J. 1999. Differentiation of beef and kangaroo meat by visible/near-infrared reflectance spectroscopy. *Journal of Food Science*, **64**, 814–817.
- Dodds, K.L. 1993. An introduction to predictive microbiology and the development and use of probability models with *Clostridium botulinum*. *Journal of Industrial Microbiology*, **12**, 139–143.
- Dupont, C. and Augustin, J.-C. 2009. Influence of stress on single-cell lag time and growth probability for *Listeria monocytogenes* in Half Fraser broth. *Applied and Environmental Microbiology*, **75**, 3069–3076.
- Ebrahim, H.A., Sowoidnich, K., and Kronfeldt, H.D. 2013. Raman spectroscopic differentiation of beef and horse meat using a 671 nm micro-system diode laser. *Applied Physics B*, **113**, 159–163.
- Elfving, A., Le Marc, Y., Baranyi, J., and Ballagi, A. 2004. Observing growth and division of large numbers of individual bacteria by image analysis. *Applied and Environmental Microbiology*, **70**, 675–678.

- Ellis, D.I., Broadhurst, D., Clarke, S.J., and Goodacre, R. 2005. Rapid identification of closely related muscle foods by vibrational spectroscopy and machine learning. *The Analyst*, **130**, 1648–1654.
- Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., La Stora, A., Laghi, L., Gianluigi, M., Guerzoni, E., and Villani, F. 2011. Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. *Applied and Environmental Microbiology*, **77**, 7372–7381.
- Favier, G.I., Escudero, M.E., and de Guzman, A.M.S. 2008. Thermal inactivation of *Yersinia enterocolitica* in liquid egg products. *Journal of Food Safety*, **28**, 157–169.
- Felício, M.T.S., Ramalheira, R., Ferreira, V., Brandão, T., Silva, J., Hogg, T., and Teixeira, P. 2011. Thermal inactivation of *Listeria monocytogenes* from alheiras, traditional Portuguese sausage during cooking. *Food Control*, **22**, 1960–1964.
- Fernandez, P.S., Baranyi, P.S., and Peck, M.W. 2001. A predictive model of growth from spores of non-proteolytic *Clostridium botulinum* in the presence of different CO₂ concentrations as influenced by chill temperature, pH and NaCl. *Food Microbiology*, **18**, 453–461.
- Ferrocino, I., La Stora, A., Torrieri, E., Musso, S.S., Mauriello, G., Villani, F., and Ercolini, D. 2013. Antimicrobial packaging to retard the growth of spoilage bacteria and to reduce the release of volatile metabolites in meat stored under vacuum at 1°C. *Journal of Food Protection*, **76**, 52–58.
- Foegeding, P.M. and Busta, F.F. 1983. Effect of carbon dioxide, nitrogen and hydrogen gases on germination of *Clostridium botulinum* spores. *Journal of Food Protection*, **46**, 987–989.
- Francois, K., Devlieghere, F., Standaert, A.R., Geeraerd, A.H., Van Impe, J.F., and Debevere, J. 2003. Modelling the individual cell lag phase. Isolating single cells: protocol development. *Letters in Applied Microbiology*, **37**, 26–30.
- Francois, K., Devlieghere, F., Smet, K., Standaert, A.R., Geeraerd, A.H., Van Impe, J.F., and Debevere, J. 2005. Modelling the individual cell lag phase: effect of temperature and pH on the individual cell lag distribution of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **100**, 41–53.
- Francois, K., Devlieghere, F., Standaert, A.R., Geeraerd, A.H., Van Impe, J.F., and Debevere, J. 2006a. Effect of environmental parameters (temperature, pH and a_w) on the individual cell lag phase and generation time of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **108**, 326–335.
- Francois, K., Devlieghere, F., Uyttendaele, M., Standaert, A.R., Geeraerd, A.H., Nadal, P., Van Impe, J.F., and Debevere, J. 2006b. Single cell variability of *L. monocytogenes* grown on liver pâté and cooked ham at 7°C: comparing challenge test data to predictive simulations. *Journal of Applied Microbiology*, **100**, 800–812.
- Francois, K., Valero, A., Geeraerd, A.H., Van Impe, J.F., Debevere, J., Garcia-Gimeno, R.M., Zurera, G., and Devlieghere, F. 2007. Effect of preincubation temperature and pH on the individual cell lag phase of *Listeria monocytogenes*, cultured at refrigeration temperatures. *Food Microbiology*, **24**, 32–43.
- Fu, B., Taoukis, P.S., and Labuza, T.P. 1991. Predictive microbiology for monitoring spoilage of dairy products with time-temperature integrators. *Journal of Food Science*, **56**, 1209–1215.
- Gaggia, F., Di Gioia, D., Baffoni, L., and Biavati, B. 2011. The role of protective and probiotic cultures in food and feed and their impact on food safety. *Trends in Food Science and Technology*, **22**, S58–S66.
- Garcia, G. and Genigeorgis, C. 1987. Quantitative evaluation of *Clostridium botulinum* nonproteolytic types B, E, and F growth risk in fresh salmon tissue homogenates stored under modified atmospheres. *Journal of Food Protection*, **50**, 390–397.
- Garcia, D., Ramos, A.J., Sanchis, V., and Marín, S. 2011. Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus ochraceus* and *Aspergillus parasiticus*. *Food Microbiology*, **28**, 406–417.

- Geeraerd, A.H., Herremans, C.H., and Van Impe, J.F. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, **59**, 185–209.
- Geeraerd, A.H., Valdramidis, V.P., and Van Impe, J.F. 2005. GInaFit, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102**, 95–105.
- Gougouli, M. and Koutsoumanis, K.P. 2013. Relation between germination and mycelium growth of individual fungal spores. *International Journal of Food Microbiology*, **161**, 231–239.
- Grauwet, T., Vervoort, L., Colle, I., Loey, A.V., and Hendrickx, M. 2014. From fingerprinting to kinetics in evaluating food quality changes. *Trends in Biotechnology*, **32**, 125–131.
- Greenacre, E.J., Brocklehurst, T.F., Waspe, C.R., Wilson, D.R., and Wilson, P.D.G. 2003. *Salmonella enterica* Serovar Typhimurium and *Listeria monocytogenes* acid tolerance response induced by organic acids at 20°C: optimization and modeling. *Applied and Environmental Microbiology*, **69**, 3945–3951.
- Grounta, A., Nychas, G.-J.E., and Panagou, E.Z. 2013. Survival of foodborne pathogens on natural black table olives after post-processing contamination. *International Journal of Food Microbiology*, **161**, 197–202.
- Guillier, L. and Augustin, J.-C. 2006. Modelling the individual cell lag time distributions of *Listeria monocytogenes* as a function of the physiological state and the growth conditions. *International Journal of Food Microbiology*, **111**, 241–251.
- Guillier, L., Pardon, P., and Augustin, J.-C. 2005. Influence of stress on individual lag time distributions of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, **71**, 2940–2948.
- Guillier, L., Pardon, P., and Augustin, J.-C. 2006. Automated image analysis of bacterial colony growth as a tool to study individual lag time distributions of immobilized cells. *Journal of Microbiological Methods*, **65**, 324–334.
- Gyseman, K.P.M., Bernaerts, K., Vermeulen, A., Geeraerd, A.H., Debevere, J., Devlieghere, F., and Van Impe, J.F. 2007. Exploring the performance of logistic regression model types on growth/no growth data of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **114**, 316–331.
- Hall, R.D. 2011. Plant metabolomics in a nutshell: potential and future challenges. *Annual Plant Reviews*, **43**, 1–24.
- Hauschild, A.H.W. 1982. Assessment of botulism hazards from cured meat products. *Food Technology*, **36**, 95–104.
- Heldman, D.R. and Newsome, R.L. 2003. Kinetic models for microbial survival during processing. *Food Technology*, **57**, 40–46.
- Hoelzer, K., Pouillot, R., Gallagher, D., Silverman, M.B., Kause, J., and Dennis, S. 2012. Estimation of *Listeria monocytogenes* transfer coefficients and efficacy of bacterial removal through cleaning and sanitation. *International Journal of Food Microbiology*, **157**, 267–277.
- Hong, Y.S., Cilindre, C., Belair, G.L., Jeandet, P., Hertkorn, N., and Schmitt-Kopplin, P. 2011. Metabolic influence of *Botrytis cinerea* infection in champagne base wine. *Journal of Agricultural and Food Chemistry*, **59**, 7237–7245.
- Huang, L. 2013. Determination of thermal inactivation kinetics of *Listeria monocytogenes* in chicken meats by isothermal and dynamic methods. *Food Control*, **33**, 484–488.
- ICMSF. 2005. *Microorganisms in Foods 6: Microbial Ecology of Food Commodities*, International Commission on Microbial Specifications of Foods (ICMSF). Kluwer Academic Publishers, New York.
- Ingham, C.J., van den Ende, M., Pijnenburg, D., Wever, P.C., and Schneeberger, P.M. 2005. Growth and multiplexed analysis of microorganisms on a subdivided, highly porous, inorganic chip manufactured from Anopore. *Applied and Environmental Microbiology*, **71**, 8978–8981.
- Insausti, K., Beriain, M.J., Gorraiz, C., and Purroy, A. 2002. Volatile compounds of raw beef from 5 local Spanish cattle breeds stored under modified atmosphere. *Journal of Food Science*, **67**, 1580–1589.
- Jain, A.K., Duin, R.P.W., and Mao, J. 2000. Statistical pattern recognition: a review. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, **22**, 1–19.

- Jenkins, P., Poulos, P.G., Cole, M.B., Vandeven, M.H., and Legan, J.D. 2000. The boundary for growth of *Zygosaccharomyces bailii* in acidified products described by models for time to growth and probability of growth. *Journal of Food Protection*, **63**, 222–230.
- Judet, D., Bensoussan, M., Perrier-Cornet, J.-M., Dantigny, P. 2008. Distribution of the growth rate of the germ tubes and germination time of *Penicillium chrysogenum* conidia depend on water activity. *Food Microbiology*, **25**, 902–907.
- Jurs, P.C., Bakken, G.A., and McClelland, H.E. 2000. Computational methods for the analysis of chemical sensor array data from volatile analytes. *Chemical Reviews*, **100**, 2649–2678.
- King, T., Kocharunchitt, C., Gobius, K., Bowman, J.P., and Ross, T. 2014. Global genome response of *Escherichia coli* O157:H7 Sakai during dynamic changes in growth kinetics induced by an abrupt temperature downshift. *PLoS One*, **9**, e99627.
- Koseki, S. 2009. Microbial Responses Viewer (MRV): A new ComBase-derived database of microbial responses to food environments. *International Journal of Food Microbiology*, **134**, 75–82.
- Koutsoumanis, K., 2008. A study on the variability in the growth limits of individual cells and its effect on the behavior of microbial populations. *International Journal of Food Microbiology*, **128**, 116–121.
- Koutsoumanis, K.P. and Lianou, A. 2013. Stochasticity in colonial growth dynamics of individual bacterial cells. *Applied and Environmental Microbiology*, **79**, 2294–2301.
- Koutsoumanis, K.P. and Sofos, J.N. 2005. Effect of inoculum size on the combined temperature, pH and a_w limits for growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **104**, 83–91.
- Koutsoumanis, K.P., Kendall, P.A., Sofos, J.N., 2004a. A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and a_w when grown in suspension or on a solid surface. *Food Microbiology*, **21**, 415–422.
- Koutsoumanis, K.P., Kendall, P.A., Sofos, J.N. 2004b. Modeling the boundaries of growth of *Salmonella* Typhimurium in broth as a function of temperature, water activity, and pH. *Journal of Food Protection*, **67**, 53–59.
- Kumar, C.G. and Anand, S.K. 1998. Significance of microbial biofilms in food industry: a review. *International Journal of Food Microbiology*, **42**, 9–27.
- Kusumaningrum, H.D., van Asselt, E.D., Beumer, R.R., and Zwietering, M.H. 2004. A quantitative analysis of cross-contamination of *Salmonella* and *Campylobacter* spp. via domestic kitchen surfaces. *Journal of Food Protection*, **67**, 1892–1903.
- Kutalik, Z., Razaz, M., and Baranyi, J. 2005. Connection between stochastic and deterministic modelling of microbial growth. *Journal of Theoretical Biology*, **232**, 285–299.
- Labuza, T.P. 1980. The effect of water activity on reaction kinetics of food deterioration. *Food Technology*, **34**, 36.
- Labuza, T.P. 1982. *Shelf-life Dating of Foods*. Food & Nutrition Press, Westport, CT, USA.
- Labuza, T.P. and Fu, B. 1993. Growth kinetics for shelf-life prediction: theory and practice. *Journal of Industrial Microbiology*, **12**, 309–323.
- Labuza, T.P. and Riboh, D. 1982. Theory and application of Arrhenius kinetics to the prediction of nutrient losses in foods. *Food Technology*, **36**, 66–74.
- Labuza, T.P., Fu, B., and Taoukis, P.S. 1992. Prediction of shelf life and safety of minimally processed CAP/MAP chilled foods: a review. *Journal of Food Protection*, **55**, 743–750.
- Lanza, B. 2013. Abnormal fermentations in table olive processing: microbial origin and sensory evaluation. *Frontiers in Microbiology*, **4**, 1–7.
- Lee, S.Y. 2004. Microbial safety of pickled fruits and vegetables and hurdle technology. *Internet Journal of Food Safety*, **4**, 21–32.
- Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology*, **55**, 181–186.
- Le Marc, Y., Huchet, V., Bourgeois, C.M., Guyonnet, J.P., Mafart, P., and Thuault, D. 2002. Modelling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. *International Journal of Food Microbiology*, **73**, 219–237.

- Le Marc, Y., Pin, C., and Baranyi, J. 2005. Methods to determine the growth domain in a multidimensional environmental space. *International Journal of Food Microbiology*, **100**, 3–12.
- Le Marc, Y., Skandamis, P.N., Belessi, C.I.A., Merkouri, S.I., George, S.M., Gounadaki, A.S. Schwartzman, S., Jordan, K., Drosinos, E.H., and Baranyi, J. 2010. Modeling the effect of abrupt acid and osmotic shifts within the growth region and across the growth boundaries on adaptation and growth of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, **76**, 6555–6563.
- Lewis, K. 2007. Persister cells, dormancy and infectious disease. *Nature Reviews*, **5**, 48–56.
- Li, C.C., Huang, L., and Chen, J. 2014. Comparative study of thermal inactivation kinetics of *Salmonella* spp. in peanut butter and peanut butter spread. *Food Control*, **45**, 143–149.
- Li, H., Xie, G., and Edmondson, A. 2007. Evolution and limitations of primary mathematical models in predictive microbiology. *British Food Journal*, **109**, 608–625.
- Lindblad, M. and Lindqvist, R. 2010. Modelling the time to growth of *Escherichia coli* as a function of water activity and undissociated lactic acid. *Letters in Applied Microbiology*, **50**, 308–313.
- Llaudes, M.K., Zhao, L., Duffy, S., and Schaffner, D.W. 2001. Simulation and modelling of the effect of small inoculum size on time-to-spoilage by *Bacillus stearothermophilus*. *Food Microbiology*, **18**, 395–405.
- Mafart, P., Couvert, O., Gaillard, S., and Leguerinel, I. 2002. On calculating sterility in thermal preservation methods: application of Weibull frequency distribution model. *International Journal of Food Microbiology*, **72**, 107–113.
- Malakar, P.K., Barker, G.C., Zwietering, M.H., and van't Riet, K. 2003. Relevance of microbial interactions to predictive microbiology. *International Journal of Food Microbiology*, **84**, 263–272.
- Manios, S.G., Konstantinidis, N., Gounadaki, A.S., Skandamis, and P.N. 2013. Dynamics of low (1–4 cells) vs high populations of *Listeria monocytogenes* and *Salmonella* Typhimurium in fresh-cut salads and their sterile liquid or solidified extracts. *Food Control*, **29**, 318–327.
- Marín, S., Hodžić, I., Ramos, A.J., Sanchis, V. 2008. Predicting the growth/no-growth boundary and ochratoxin A production by *Aspergillus carbonarius* in pistachio nuts. *Food Microbiology*, **25**, 683–689.
- Marín, S., Colom, C., Sanchis, V., and Ramos, A.J. 2009. Modelling of growth of aflatoxin *A. flavus* isolates from red chilli powder as a function of water availability. *International Journal of Food Microbiology*, **128**, 491–496.
- Mataragas, M., Drosinos, E.H., Siana, P., Skandamis, P., and Metaxopoulos, I. 2006. Growth limits and kinetic behaviour of *Listeria monocytogenes* in a sliced cooked cured meat product. *Journal of Food Protection*, **69**, 1312–1321.
- McDonald, K. and Sun, D.-W. 1999. Predictive food microbiology for the meat industry: a review. *International Journal of Food Microbiology*, **52**, 1–27.
- McKellar, R.C. 2001. Development of a dynamic continuous-discrete-continuous model describing the lag phase of individual bacterial cells. *Journal of Applied Microbiology*, **90**, 407–413.
- McKellar, R.C. and Knight, K. 2000. A combined discrete-continuous model describing the lag phase of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **54**, 171–180.
- McKellar, R.C. and Lu, X., 2001. A probability of growth model for *Escherichia coli* O157:H7 as a function of temperature, pH, acetic acid, and salt. *Journal of Food Protection*, **64**, 1922–1928.
- McKellar, R.C., Lu, X., and Delaquis, P.J. 2002. A probability model describing the interface between survival and death of *Escherichia coli* O157:H7 in a mayonnaise model system. *Food Microbiology*, **19**, 235–247.
- McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., Olley, J., Putro, S., and Ratkowsky, D.A. 1987. Model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosus*. *Journal of Applied Bacteriology*, **62**, 543–550.
- McMeekin, T.A., Ross, T., and Olley, J. 1992. Application of predictive microbiology to assure the quality and safety of fish and fish products. *International Journal of Food Microbiology*, **15**, 13–32.

- McMeekin, T.A., Olley, J.N., Ross, T., and Ratkowsky, D.A. 1993. *Predictive Microbiology*. John Wiley & Sons Ltd, Chichester, UK.
- McMeekin, T., Olley, J., Raktowsky, D., Corkrey, R., and Ross, T. 2013. Predictive microbiology theory and application: is it all about rates? *Food Control*, **29**, 290–299.
- McQuestin, O.J., Shadbolt, C.T., and Ross, T. 2009. Quantification of the relative effects of temperature, pH, and water activity on inactivation of *Escherichia coli* in fermented meat by meta-analysis. *Applied and Environmental Microbiology*, **75**, 6963–6972.
- Medina, E., Brenes, M., Romero, C., Ramírez, E., and de Castro, A. 2013. Survival of foodborne pathogenic bacteria in table olive brines. *Food Control*, **34**, 719–724.
- Mejlholm, O. and Dalgaard, P. 2007a. Modeling and predicting the growth of lactic acid bacteria in lightly preserved seafood and their inhibiting effect on *Listeria monocytogenes*. *Journal of Food Protection*, **70**, 2485–2497.
- Mejlholm, O. and Dalgaard, P. 2007b. Modeling and predicting the growth boundary of *Listeria monocytogenes* in lightly preserved seafood. *Journal of Food Protection*, **70**, 70–84.
- Mejlholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D., and Dalgaard, P. 2010. Predicting growth rates and growth boundary of *Listeria monocytogenes* — An international validation study with focus on processed and ready-to-eat meat and seafood. *International Journal of Food Microbiology*, **141**, 137–150.
- Mellefont, L.A. and Ross, T. 2003. The effect of abrupt shifts in temperature on the lag phase duration of *Escherichia coli* and *Klebsiella oxytoca*. *International Journal of Food Microbiology*, **83**, 295–305.
- Mellefont L.A., McMeekin, T.A., and Ross, T. 2003. The effect of abrupt osmotic shifts on the lag phase duration of foodborne bacteria. *International Journal of Food Microbiology*, **83**, 281–293.
- Mellefont, L.A., McMeekin, T.A., and Ross, T. 2004. The effect of abrupt osmotic shifts on the lag phase duration of physiologically distinct populations of *Salmonella* Typhimurium. *International Journal of Food Microbiology*, **99**, 111–120.
- Mellefont, L.A., McMeekin, T.A., and Ross, T. 2005. Viable count estimates of lag time responses for *Salmonella* Typhimurium M48 subjected to abrupt osmotic shifts. *International Journal of Food Microbiology*, **105**, 399–410.
- Membré, J.-M., Ross, T., and McMeekin, T.A. 1998. Behaviour of *Listeria monocytogenes* under combined chilling processes. *Letters in Applied Microbiology*, **28**, 216–220.
- Membré, J.-M., Kubaczka, M., and Chéné, C. 2001. Growth rate and growth–no-growth interface of *Penicillium brevicompactum* as functions of pH and preservative acids. *Food Microbiology*, **18**, 531–538.
- Meng, J. and Genigeorgis, C.A. 1993. Modeling lag phase of nonproteolytic *Clostridium botulinum* toxigenesis in cooked turkey and chicken breast as affected by temperature, sodium lactate, sodium chloride and spore inoculum. *International Journal of Food Microbiology*, **19**, 109–122.
- Mensonides, F.I.C., Schuurmans, J.M., De Mattos, M.J.T., Hellingwerf, K.J., and Brul, S. 2002. The metabolic response of *Saccharomyces cerevisiae* to continuous heat stress. *Molecular Biology Reports*, **29**, 103–106.
- Mensonides, F.I.C., Brul, S., Klis, F.M., Hellingwerf, K.J., and De Mattos, M.J.T. 2005. Activation of the protein kinase C1 pathway upon continuous heat stress in *Saccharomyces cerevisiae* is triggered by an intracellular increase in osmolarity due to trehalose accumulation. *Applied and Environmental Microbiology*, **71**, 4531–4538.
- Messins, W., Verluyten, J., Leroy, F., and De Vurst, L. 2002. Modelling growth and bacteriocin production by *Lactobacillus curvatus* LTH 1174 in response to temperature and pH values used for European sausage fermentation processes. *International Journal of Food Microbiology*, **81**, 41–52.
- Métris, A., George, S.M., Peck, M.W., and Baranyi, J. 2003. Distribution of turbidity detection times produced by single cell-generated bacterial populations. *Journal of Microbiological Methods*, **55**, 821–827.

- Métris, A., Le Marc, Y., Elfwing, A., Ballagi, A., and Baranyi, J. 2005. Modelling the variability of lag times and the first generation times of single cells of *Escherichia coli*. *International Journal of Food Microbiology*, **100**, 13–19.
- Métris, A., George, S.M., and Baranyi, J. 2006. Use of optical density detection times to assess the effect of acetic acid on single-cell kinetics. *Applied and Environmental Microbiology*, **72**, 6674–6679.
- Mitchell, G.A., Brocklehurst, T.F., Parker, R., and Smith, A.C. 1994. The effect of transient temperatures on the growth of *Salmonella* Typhimurium LT2. I: Cycling within the growth region. *Journal of Applied Microbiology*, **77**, 113–119.
- Mitchell, G.A., Brocklehurst, T.F., Parker, R., and Smith, A.C. 1995. The effect of transient temperatures on the growth of *Salmonella* Typhimurium LT2. II: Excursions outside the growth region. *Journal of Applied Microbiology*, **79**, 128–134.
- Mohamadi Monavar, H., Afseth, N.K., Lozano, J., Alimardani, R., Omid, M., and Wold, J.P. 2013. Determining quality of caviar from Caspian Sea based on Raman spectroscopy and using artificial neural networks. *Talanta*, **111**, 98–104.
- Møller, C.O.A., Nauta, M.J., Christensen, B.B., Dalgaard, P., and Hansen, T.B. 2012. Modelling transfer of *Salmonella* Typhimurium DT104 during simulation of grinding of pork. *Journal of Applied Microbiology*, **112**, 90–98.
- Morsy, N. and Sun, D.W. 2013. Robust linear and non-linear models of NIR spectroscopy for detection and quantification of adulterants in fresh and frozen-thawed minced beef. *Meat Science*, **93**, 292–302.
- Muñoz-Cuevas, M., Fernández, P.S., George, S., and Pin, C. 2010. Modeling the lag period and exponential growth of *Listeria monocytogenes* under conditions of fluctuating temperature and water activity values. *Applied and Environmental Microbiology*, **76**, 2908–2915.
- Nauta, M., I. van der Fels-Klerx, I., and A. Havelaar, A. 2005. A poultry-processing model for quantitative microbiological risk assessment. *Risk Analysis: An Official Publication of the Society for Risk Analysis*, **25**, 85–98.
- Nguyen, D., Joshi-Datar, A., Lepine, F., Bauerle, E., Olakanmi, O., Beer, K., McKay, G., Siehnel, R., Schafhauser, J., Wang, Y., Britigan, B.E., and Singh, P.K. 2011. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science*, **334**, 982–986.
- Noriega, E., Laca, A., and Díaz, M. 2010. Decisive role of structure in food microbial colonization and implications for predictive microbiology. *Journal of Food Protection*, **73**, 938–951.
- Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., and Koutsoumanis, K.P. 2008. Meat spoilage during distribution. *Meat Science*, **78**, 77–89.
- Oms-Oliu, G., Odriozola-Serrano, I., and Martín-Belloso, O. 2013. Metabolomics in assessing safety and quality of plant-derived food. *Food Research International*, **54**, 1172–1183.
- Osaili, T.M., Al-Nabulsi, A.A., Shaker, R.R., Olaimat, A.N., Jaradat, Z.W., and Holley, R.A. 2013. Thermal inactivation of *Salmonella* Typhimurium in chicken shawirma (gyro). *International Journal of Food Microbiology*, **166**, 15–20.
- Pal, A., Labuza, T.P., and Diez-Gonzalez, F. 2009. Safety-based shelf life model for frankfurters based on time to detect *Listeria monocytogenes* with initial inoculum below detection limit. *Journal of Food Protection*, **72**, 1878–1884.
- Panagou, E.Z., Chelonas, S., Chatzipavlidis, I., and Nychas, G.-J.E. 2010. Modelling the effect of temperature and water activity on the growth rate and growth/no growth interface of *Byssoschlamys fulva* and *Byssoschlamys nivea*. *Food Microbiology*, **27**, 618–627.
- Peleg, M. and Penchina, C.M. 2000. Modeling microbial survival during exposure to a lethal agent with varying intensity. *Critical Reviews in Food Science and Nutrition*, **40**, 159–172.
- Peres, C.M., Peres, C., Hernandez-Mendoza, A., and Xavier Malcata, F. 2012. Review of fermented plant materials as carriers and sources of potentially probiotic lactic acid bacteria – With an emphasis on table olives. *Trends in Food Science and Technology*, **26**, 31–42.

- Perez, A.R., Rojo, D.M., Gonzalez, G., and De Lorenzo, C. 2008. Solid-phase microextraction for the determination of volatile compounds in the spoilage of raw ground beef. *Journal of AOAC International*, **91**, 1409–1415.
- Pérez-Rodríguez, F., Valero, A., Todd, E.C.D., Carrasco, E., García-Gimeno, R.M., and Zurera, G. 2007. Modeling transfer of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during slicing of a cooked meat product. *Meat Science*, **76**, 692–699.
- Pérez-Rodríguez, F., Valero, A., Carrasco, E., García, R.M., and Zurera, G. 2008. Understanding and modelling bacterial transfer to foods: a review. *Trends in Food Science and Technology*, **19**, 131–144.
- Phillips, J.D. and Griffiths, M.W. 1987. The relation between temperature and growth of bacteria in dairy products. *Food Microbiology*, **4**, 173–185.
- Pin, C. and Baranyi, J. 2006. Kinetics of single cells: observation and modelling of a stochastic process. *Applied and Environmental Microbiology*, **72**, 2163–2169.
- Pin, C., Avendaño-Perez, G., Cosciani-Cunico, E., Gómez, N., Gounadaki, A., Nychas, G.-J., Skandamis, P., and Barker, G. 2011. Modelling *Salmonella* concentration throughout the pork supply chain by considering growth and survival in fluctuating conditions of temperature, pH and a_w . *International Journal of Food Microbiology*, **145**, S96–S102.
- Pommier, S., Strehaiano, P., and Délia, M.L. 2005. Modeling the growth dynamics of interacting mixed cultures: a case of amensalism. *International Journal of Food Microbiology*, **100**, 131–139.
- Pooni, G.S. and Mead, G.C. 1984. Prospective use of temperature function integration for predicting the shelf life of non-frozen poultry meat products. *Food Microbiology*, **1**, 67–78.
- Pouillot, R., Albert, I., Cornu, M., and Denis, J.-B. 2003. Estimation of uncertainty and variability in bacterial growth using Bayesian inference. *Application to Listeria monocytogenes*. *International Journal of Food Microbiology*, **81**, 87–104.
- Poulsen, L.V. 1999. Review: article on microbial biofilm in food processing. *Lebensmittel-Wissenschaft und-Technologie*, **32**, 321–226.
- Prats, C., Giró, A., Ferrer, J., López, D., and Vives-Rego, J. 2008. Analysis and IbM simulation of the stages in bacterial lag phase: basis for an updated definition. *Journal of Theoretical Biology*, **252**, 56–68.
- Presser, K.A., Ratkowsky, D.A., and Ross, T. 1997. Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. *Applied and Environmental Microbiology*, **63**, 2355–2360.
- Presser, K.A., Ross, T., and Ratkowsky, D.A. 1998. Modelling the growth limits (growth/no growth interface) of *Escherichia coli* as a function of temperature, pH, lactic acid concentration, and water activity. *Applied and Environmental Microbiology*, **64**, 1773–1779.
- Radford, S.A. and Board, R.G. 1993. Review: fate of pathogens in home-made mayonnaise and related products. *Food Microbiology*, **10**, 269–278.
- Rajkovic, A., Smigic, N., Uyttendaele, M., Medic, H., de Zutter, L., and Devlieghere, F. 2009. Resistance of *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Campylobacter jejuni* after exposure to repetitive cycles of mild bactericidal treatments. *Food Microbiology*, **26**, 889–895.
- Rajkovic, A., Smigic, N., and Devlieghere, F. 2010. Contemporary strategies in combating microbial contamination in food chain. *International Journal of Food Microbiology*, **141**, S29–S42.
- Rajkowski, K.T. 2012. Thermal inactivation of *Escherichia coli* O157:H7 and *Salmonella* on catfish and tilapia. *Food Microbiology*, **30**, 427–431.
- Ramautar, R., Demirci, A., and Jong, G.J. 2006. Capillary electrophoresis in metabolomics. *Trends in Analytical Chemistry*, **25**, 455–466.
- Ratkowsky, D.A., Olley, J., McMeekin, T.A., and Ball, A. 1982. Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*, **149**, 1–5.
- Razavilar, V. and Genigeorgis, C. 1998. Prediction of *Listeria* spp. growth as affected by various levels of chemicals, pH, temperature and storage time in a model broth. *International Journal of Food Microbiology*, **40**, 149–157.

- Roberts, T.A. and Gibson, A.M. 1986. Chemical methods for controlling *Clostridium botulinum* in processed meats. *Food Technology*, **40**, 163–171.
- Robinson T.P., Ocio, M.J., Kaloti, A., and Mackey, B.M. 1998. The effect of the growth environment on the lag phase of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **44**, 83–92.
- Rodriguez-Carrasco, Y., Berrada, H., Font, G., and Manes, G. 2012. Multi-mycotoxin analysis in wheat semolina using an acetonitrile-based extraction procedure and gas chromatography–tandem mass spectrometry. *Journal of Chromatography A*, **1270**, 28–40.
- Rodriguez-Carrasco, Y., Font, G., Manes, G., and Berrada, H. 2013. Determination of mycotoxins in bee pollen by gas chromatography–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, **61**, 1999–2005.
- Rohman, A., Sisindari, Erwanto, Y., and Che Man, Y.B. 2011. Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. *Meat Science*, **88**, 91–5.
- Ross, T. 1993. Bełhradec-type models. *Journal of Industrial Microbiology*, **12**, 180–189.
- Ross, T. and Dalgaard, P. 2004. Secondary models. In: *Modeling Microbial Responses in Foods* (eds McKellar, R.C. and Lu, X.), pp. 63–149. CRC Press, London, UK.
- Ross, T. and McMeekin, T.A. 1994. Predictive microbiology. *International Journal of Food Microbiology*, **23**, 241–264.
- Ross, R.P., Morgan, S., and Hill, C. 2002. Preservation and fermentation: past, present and future. *International Journal of Food Microbiology*, **79**, 3–16.
- Ross, T., Ratkowsky, D.A., Mellefont, L.A., and McMeekin, T.A. 2003. Modelling the effects of temperature, water activity, pH and lactic acid concentration on the growth rate of *Escherichia coli*. *International Journal of Food Microbiology*, **82**, 33–43.
- Ross, E.W., Taub, I.A., Doona, C.J., Feeherly, F.E., and Kustin, K. 2005. The mathematical properties of the quasi-chemical model for microorganism growth–death kinetics in foods. *International Journal of Food Microbiology*, **99**, 157–171.
- Ross, T., Zhang, D., and McQuestin, O.J. 2008. Temperature governs the inactivation rate of vegetative bacteria under growth-preventing conditions. *International Journal of Food Microbiology*, **128**, 129–135.
- Rosso, L. 1999. Models using cardinal values. *Refrigeration Science and Technology Procedures*, EUR **18816**, 48–55.
- Rosso, L. and Robinson, T.P. 2001. A cardinal model to describe the effect of water activity on the growth of moulds. *International Journal of Food Microbiology*, **63**, 265–273.
- Rosso, L., Lobry, R., and Flandrois, J.P. 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *Journal of Theoretical Biology*, **162**, 447–463.
- Rosso, L., Lobry, J.R., Bajard, S., and Flandrois, J.P. 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. *Applied and Environmental Microbiology*, **61**, 610–616.
- Salter, M.A., Ross, T., and McMeekin, T.A. 1998. Applicability of a model for non-pathogenic *Escherichia coli* for predicting the growth of pathogenic *Escherichia coli*. *Journal of Applied Microbiology*, **85**, 357–364.
- Salter, M.A., Ratkowsky, D.A., Ross, T., and McMeekin, T.A. 2000. Modelling the combined temperature and salt (NaCl) limits for growth of a pathogenic *Escherichia coli* strain using non-linear logistic regression. *International Journal of Food Microbiology*, **61**, 159–167.
- Samapundo, S., Devlieghere, F., De Meulenaer, B., and Debevere, J. 2007. Growth kinetics of cultures from single spores of *Aspergillus flavus* and *Fusarium verticillioides* on yellow dent corn meal. *Food Microbiology*, **24**, 336–345.
- Sánchez Gómez, A.H., García García, P., and Rejano Navarro, L. 2006. Elaboration of table olives. *Grasas y Aceites*, **57**, 86–94.
- Schoolfield, R.M., Sharpe, P.J.H., and Magnuson, C.E. 1981. Non-linear regression of biological temperature-dependent rate models based on absolute reaction rate theory. *Journal of Theoretical Biology*, **88**, 719–731.

- Schvartzman, M.S., Belessi, X., Butler, F., Skandamis, P., and Jordan, K. 2010. Comparison of growth limits of *Listeria monocytogenes* in milk, broth and cheese. *Journal of Applied Microbiology*, **109**, 1790–1799.
- Shabala, L., Lee, S.H., Cannesson, P., and Ross, T. 2008. Acid and NaCl limits of growth of *Listeria monocytogenes* and influence of sequence of inimical acid and NaCl levels on inactivation kinetics. *Journal of Food Protection*, **71**, 1169–1177.
- Shadbolt, C.T., Ross, T., and McMeekin, T.A. 1999. Nonthermal death of *Escherichia coli*. *International Journal of Food Microbiology*, **49**, 129–138.
- Shadbolt, C., Ross, T., and McMeekin, T.A. 2001. Differentiation of the effects of lethal pH and water activity: food safety implications. *Letters in Applied Microbiology*, **32**, 99–102.
- Sharpe, P.J.H. and De Michele, D.W. 1977. Reaction kinetics and poikilotherm development. *Journal of Theoretical Biology*, **64**, 649–670.
- Sheen, S. 2008. Modeling surface transfer of *Listeria monocytogenes* on salami during slicing. *Journal of Food Science*, **73**, 304–311.
- Sheen, S. and Hwang, C.-A. 2010. Mathematical modeling the cross-contamination of *Escherichia coli* O157:H7 on the surface of ready-to-eat meat product while slicing. *Food Microbiology*, **27**, 37–43.
- Silva, F.V.M., and Gibbs, P. 2004. Target selection in designing pasteurization processes for shelf-stable high-acid fruit products. *Critical Reviews in Food Science and Nutrition*, **44**, 353–360.
- Silva, F.V.M. and Gibbs, P. 2012. Thermal pasteurization requirements for the inactivation of *Salmonella* in foods. *Food Research International*, **45**, 695–699.
- Skandamis, P.N. and Nychas, G.-J.E. 2003. Modeling the microbial inactivation and the death of *Escherichia coli* O157:H7 during the fermentation of Spanish-style green table olives. *Journal of Food Protection*, **66**, 1166–1175.
- Skandamis, P.N., Brocklehurst, T.F., Panagou, E.Z., and Nychas, G.-J.E. 2007a. Image analysis as a means to model growth of *Escherichia coli* O157:H7 in gel cassettes. *Journal of Applied Microbiology*, **103**, 937–947.
- Skandamis, P.N., Stopforth, J.D., Kendall, P.A., Belk, K.E., Scanga, J.A., Smith, G.C., and Sofos, J.N. 2007b. Modeling the effect of inoculum size and acid adaptation on growth/no growth interface of *Escherichia coli* O157:H7. *International Journal of Food Microbiology*, **120**, 237–249.
- Skandamis, P.N., Stopforth, J.D., Yoon, Y., Kendall, P.A., and Sofos, J.N. 2007c. Modeling the effect of storage atmosphere on growth-no growth interface of *Listeria monocytogenes* as a function of temperature, sodium lactate, sodium diacetate, and NaCl. *Journal of Food Protection*, **70**, 2329–2338.
- Smelt, J.P.P.M. and Brul, S. 2014. Thermal inactivation of microorganisms. *Critical Reviews in Food Science and Nutrition*, **54**, 1371–1385.
- Smelt, J.P.P.M., Otten, G.D., and Bos, A.P. 2002. Modelling the effect of sublethal injury on the distribution of the lag times of individual cells of *Lactobacillus plantarum*. *International Journal of Food Microbiology*, **73**, 207–212.
- Smelt, J.P.P.M., Bos, A.P., Kort, R., and Brul, S. 2008. Modelling the effect of sub(lethal) heat treatment of *Bacillus subtilis* spores on germination rate and outgrowth to exponentially growing vegetative cells. *International Journal of Food Microbiology*, **128**, 34–40.
- Sodini, I., Latrille, E., and Corrieu, G. 2000. Identification of interacting mixed cultures of lactic acid bacteria by their exclusion from a model predicting the acidifying activity of non-interacting mixed cultures. *Applied Microbiology and Biotechnology*, **54**, 715–718.
- Standaert, A.R., Geeraerd, A.H., Bernaerts, K., Francois, K., Devlieghere, F., Debevere, J., and Van Impe, J.F. 2005. Obtaining single cells: analysis and evaluation of an experimental protocol by means of a simulation model. *International Journal of Food Microbiology*, **100**, 55–66.
- Standaert, A.R., Francois, K., Devlieghere, F., Debevere, J., Van Impe, J.F., and Geeraerd, A.H. 2007. Modeling individual cell lag time distributions for *Listeria monocytogenes*. *Risk Analysis*, **27**, 241–254.
- Stumbo, C.R. 1973. *Thermobacteriology in Food Processing*. Academic Press, New York, USA.

- Sun, D.W. 2009. *Infrared Spectroscopy for Food Quality Analysis and Control*. Academic Press, Burlington, MA, USA.
- Swinnen, I.A.M., Bernaerts, K., Gysemans, K., and Van Impe, J.F. 2005. Quantifying microbial lag phenomena due to a sudden rise in temperature: a systematic macroscopic study. *International Journal of Food Microbiology*, **100**, 85–96.
- Swinnen, I.A.M., Bernaerts, K., and Van Impe, J.F. 2006. Modelling the work to be done by *Escherichia coli* to adapt to sudden temperature upshifts. *Letters in Applied Microbiology*, **42**, 507–513.
- Taoukis, P.S. and Giannakourou, M.C. 2004. Temperature and food stability: analysis and control. In: *Understanding and Measuring the Shelf-Life of Food* (ed. Steel, R.), pp. 42–68. Elsevier.
- Taoukis, P.S., Labuza T.P., and Saguy I.S., 1997. Kinetics of food deterioration and shelf-life prediction. In: *Handbook of Food Engineering Practice* (eds Valentas, K.J., Rotstein, E., and Singh, R.P.), pp. 361–403. CRC Press, New York, USA.
- Tassou, C.C., Natskoulis, P.I., Magan, N., and Panagou, E.Z. 2009. Effect of temperature and water activity on growth and ochratoxin A production boundaries of two *Aspergillus carbonarius* isolates on a simulated grape juice medium. *Journal of Applied Microbiology*, **107**, 257–268.
- Tenenhaus-Aziza, F. and Ellouze, M. 2014. Software for predictive microbiology and risk assessment: qa description and comparison of tools presented at the ICPMF8 Software Fair. *Food Microbiology*, **45**, 290–299.
- Theys, T.E., Geeraerd, A.H., Verhulst, A., Poot, K., Van Bree, I., Devlieghere, F., Moldenaers, P., Wilson, D., Brocklehurst, T., and Van Impe, J.F. 2008. Effect of pH, water activity and gel micro-structure, including oxygen profiles and rheological characterization, on the growth kinetics of *Salmonella* Typhimurium. *International Journal of Food Microbiology*, **128**, 67–77.
- Theys, T.E., Geeraerd, A.H., Devlieghere, F., and Van Impe, J.F. 2009. Extracting information on the evolution of living- and dead-cell fractions of *Salmonella* Typhimurium colonies in gelatine gels based on microscopic images and plate-count data. *Letters in Applied Microbiology*, **49**, 39–45.
- Tienungoon, S., Ratkowsky, D.A., McMeekin, T.A., and Ross, T. 2000. Growth limits of *Listeria monocytogenes* as a function of temperature, pH, NaCl, and lactic acid. *Applied and Environmental Microbiology*, **66**, 4979–4987.
- Tompkin, R.B. 1986. Microbiological safety of processed meat: new products and processes – new problems and solutions. *Food Technology*, **40**, 172–176.
- Tompkin, R.B., Christiansen, L.N., and Shaparis, A.B. 1978. Enhancing nitrite inhibition of *Clostridium botulinum* with isoascorbate in perishable canned cured meat. *Applied and Environmental Microbiology*, **35**, 59–61.
- Trevisani, M., Mancusi, R., and Valero, A. 2014. Thermal inactivation kinetics of Shiga toxin-producing *Escherichia coli* in buffalo Mozzarella curd. *Journal of Dairy Science*, **97**, 642–650.
- Valdramidis, V.P., Bernaerts, K., Van Impe, J.F., and Geeraerd, A.H. 2005. An alternative approach to non-log-linear thermal microbial inactivation: modeling the number of log cycles reduction with respect to temperature. *Food Technology and Biotechnology*, **43**, 321–327.
- Valero, A., Carrasco, E., Pérez-Rodríguez, F., García-Gimeno, R.M., and Zurera, G. 2006. Growth/no growth model of *Listeria monocytogenes* as a function of temperature, pH, citric acid and ascorbic acid. *European Food Research and Technology*, **224**, 91–100.
- Valero, A., Pérez-Rodríguez, F., Carrasco, E., Fuentes-Alventosa, J.M., García-Gimeno, R.M., and Zurera, G. 2009. Modelling the growth boundaries of *Staphylococcus aureus*: effect of temperature, pH and water activity. *International Journal of Food Microbiology*, **133**, 186–194.
- Valero, A., Rodríguez, M., Carrasco, E., Pérez-Rodríguez, F., García-Gimeno, R.M., and Zurera, G. 2010. Studying the growth boundary and subsequent time to growth of pathogenic *Escherichia coli* serotypes by turbidity measurements. *Food Microbiology*, **27**, 819–828.
- Van Asselt, E.D. and Zwietering, M.H. 2006. A systematic approach to determine global thermal inactivation parameters for various food pathogens. *International Journal of Food Microbiology*, **107**, 73–82.

- Van Asselt, E.D., de Jong, A.E.I., de Jonge, R., and Nauta, M.J. 2008. Cross-contamination in the kitchen: estimation of transfer rates for cutting boards, hands and knives. *Journal of Applied Microbiology*, **105**, 1392–1401.
- Van Boekel, M.A.J.S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, **74**, 139–159.
- Van Boekel, M., Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., *et al.* 2010. A review on the beneficial aspects of food processing. *Molecular Nutrition and Food Research*, **54**, 1215–1247.
- Van Gerwen, S.J.C. and Zwietering, M.H. 1998. Growth in activation models to be used in quantitative risk assessments. *Journal of Food Protection*, **61**, 1541–1549.
- Van Houdt, R. and Michiels, C.W. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology*, **109**, 1117–1131.
- Van Lieverloo, J.H.M., de Roode, M., Fox, M.B., and Zwietering, M.H. 2013. Multiple regression model for thermal inactivation of *Listeria monocytogenes* in liquid food products. *Food Control*, **29**, 394–400.
- van Schothorst, M., Zwietering, M.H., Ross, T., Buchanan, R.L., Cole, M.B., and International Commission on Microbiological Specifications for Foods (ICMSF). 2009. Relating microbiological criteria to food safety objectives and performance objectives. *Food Control*, **20**, 967–979.
- Vereecken, K.M., Dens, E.J., and Van Impe, J.F. 2000. Predictive modeling of mixed microbial populations in food products: evaluation of two-species models. *Journal of Theoretical Biology*, **205**, 53–72.
- Vermeulen, A., Devlieghere, F., Bernaerts, K., Van Impe, J., and Debevere J., 2007a. Growth/no growth models describing the influence of pH, lactic and acetic acid on lactic acid bacteria developed to determine the stability of acidified sauces. *International Journal of Food Microbiology*, **119**, 258–269.
- Vermeulen, A., Gysemans, K.P.M., Bernaerts, K., Geeraerd, A.H., Van Impe, J.F., Debevere, J., and Devlieghere, F. 2007b. Influence of pH, water activity and acetic acid concentration on *Listeria monocytogenes* at 7°C: data collection for the development of a growth/no growth model. *International Journal of Food Microbiology*, **114**, 332–341.
- Vermeulen, A., Smigic, N., Rajkovic, A., Gyseman, K., Beranerts, K., Geeraerd, A., Van Impe, J., Debevere, J., and Devlieghere, F. 2007c. Performance of a growth-no growth model for *Listeria monocytogenes* developed for mayonnaise-based salads: influence of strain variability, food matrix, inoculation level, and presence of sorbic acid and benzoic acid. *Journal of Food Protection*, **70**, 2118–2126.
- Vermeulen, A., Dang, T.D.T., Geeraerd, A.H., Bernaerts, K., Debevere, J., Van Impe, J., and Devlieghere, F. 2008. Modelling the unexpected effect of acetic and lactic acid in combination with pH and a_w on the growth/no growth interface of *Zygosaccharomyces bailii*. *International Journal of Food Microbiology*, **124**, 79–90.
- Vermeulen, A., Gysemans, K.P.M., Bernaerts, K., Geeraerd, A.H., Debevere, J., Devlieghere, F., and Van Impe, J.F. 2009. Modelling the influence of the inoculation level on the growth/no growth interface of *Listeria monocytogenes* as a function of pH, a_w and acetic acid. *International Journal of Food Microbiology*, **135**, 83–89.
- Vervoort, L., Grauwet, T., Kebede, B.T., Van der Plancken, I., Timmermans, R., Hendrickx, M., and Van Loey, A. 2012. Headspace fingerprinting as an untargeted approach to compare novel and traditional processing approaches: a case study on orange juice pasteurization. *Food Chemistry*, **134**, 2303–2312.
- Vorst, K.L., Todd, E.C.D., and Ryser, E.T. 2006. Transfer of *Listeria monocytogenes* during mechanical slicing of turkey breast, bologna, and salami. *Journal of Food Protection*, **69**, 619–626.
- Whiting, R.C. 1993. Modeling bacterial survival in unfavorable environments. *Journal of Industrial Microbiology*, **12**, 240–246.
- Whiting, R.C. 1995. Microbial modeling in foods. *Critical Reviews in Food Science and Nutrition*, **35**, 467–494.
- Whiting, R.C. and Bagi, L.G. 2002. Modelling the lag phase of *Listeria monocytogenes*.

- International Journal of Food Microbiology*, **73**, 291–295.
- Whiting, R.C. and Buchanan, R.L. 1993. A classification of models for predictive microbiology. *Food Microbiology*, **10**, 175–177.
- Whiting, R.C. and Oriente, J.C. 1997. Time-to-turbidity model for non-proteolytic type B *Clostridium botulinum*. *International Journal of Food Microbiology*, **35**, 49–60.
- Whiting, R.C. and Strobaugh, T.P. 1998. Expansion of the time-to-turbidity model for proteolytic *Clostridium botulinum* to include spore numbers. *Food Microbiology*, **15**, 449–453.
- Wijtzes, T., McClure, P.J., Zwietering, M.H., and Roberts, T.A. 1993. Modelling bacterial growth of *Listeria monocytogenes* as a function of water activity, pH and temperature. *International Journal of Food Microbiology*, **18**, 139–149.
- Wijtzes, T., van't Riet, K., Huis in't Veld, J.H., and Zwietering, M.H. 1998. A decision support system for the prediction of microbial food safety and food quality. *International Journal of Food Microbiology*, **42**, 79–90.
- Wijtzes, T., Rombouts, F.M., Kant-Muermans, M.L.T., Van't Riet, K., and Zwietering, M.H. 2001. Development and validation of a combined temperature, water activity, pH model for bacterial growth rate of *Lactobacillus curvatus*. *International Journal of Food Microbiology*, **63**, 57–64.
- Wilson, P.D.G., Brocklehurst, T.F., Arino, S., Thuault, D., Jakobsen, M., Lange, M., Farkas, J., Wimpenny, J.W.T., and Van Impe, J.F. 2002. Modelling microbial growth in structured foods: towards a unified approach. *International Journal of Food Microbiology*, **73**, 275–289.
- Xiao, H. and Fu, S. 2012. A sensitive gas chromatography–mass spectrometry method for the determination of patulin in apple juice. *Journal of AOAC International*, **95**, 1709–1712.
- Xiong, R., Xie, G., Edmondson, A.E., and Sheard, M.A. 1999. A mathematical model for bacterial inactivation. *International Journal of Food Microbiology*, **46**, 45–55.
- Xu, Y.J., Wang, C., Ho, W.E., and Ong, C.N. 2014. Recent developments and applications of metabolomics in microbiological investigations. *Trends in Analytical Chemistry*, **56**, 37–48.
- Zhang, D., McQuestin, O.J., Mellefont, L.A., and Ross, T. 2010. The influence of non-lethal temperature on the rate of inactivation of vegetative bacteria in inimical environments may be independent of bacterial species. *Food Microbiology*, **27**, 453–459.
- Zhao, M., Downey, G., and O'Donnell, C.P. 2014. Detection of adulteration in fresh and frozen beefburger products by beef offal using mid-infrared ATR spectroscopy and multivariate data analysis. *Meat Science*, **96**, 1003–1011.
- Zilelidou, E.A., Tsourou, V., Poimenidou, S., Loukou, A., Skandamis, P.N. 2014. Modeling transfer of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during preparation of fresh-cut salads: impact of cutting and shredding practices. *Food Microbiology*, **45**, 254–265.
- Zuliani, V., Lebert, I., Augustin, J.-C., Garry, P., Vendeuvre, J.-L., and Lebert, A. 2007. Modelling the behaviour of *Listeria monocytogenes* in ground pork as a function of pH, water activity, nature and concentration of organic acid salts. *Journal of Applied Microbiology*, **103**, 536–550.
- Zwietering, M. 1999. Microbial and engineering characteristic numbers for process evaluation. *Refrigeration Science and Technology Proceedings, EUR 18816*, 272–279.
- Zwietering, M.H., De Koos, J.T., Hasenack, B.E., De Wit, J.C., and Van't Riet, K. 1991. Modelling of bacterial growth as a function of temperature. *Applied and Environmental Microbiology*, **57**, 1094–1101.
- Zwietering, M.H., Wijtzes, T., De Wit, J.C., and Van't Riet, K. 1992. A decision support system for prediction of the microbial spoilage in foods. *Journal of Food Protection*, **55**, 973–979.
- Zwietering, M.H., De Wit, J.C., Cuppers, H.G.A.M., and Van't Riet, K. 1994. Modeling of bacterial growth with shifts in temperature. *Applied and Environmental Microbiology*, **60**, 204–213.
- Zwietering, M.H., De Wit, J.C., and Notermans, S. 1996. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the time of consumption. *International Journal of Food Microbiology*, **30**, 55–70.

CHAPTER 3

Principles of unit operations in food processing

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3.1 Introduction

When evaluating food processes, it can be observed that they are composed of a series of specific and basic operations such as mixing, pumping, heating, cooling, concentration, drying, filtration, evaporation, distillation, extraction, and many others. Each one of those operations is called a “unit operation”, being common in many industrial processes.

The individual operations have common techniques and are based on the same scientific principles. They can be evaluated using the same approaches and described with the same equations. Consequently, the industrial processes can be simply studied and designed.

This chapter describes the principles of unit operations in food processing, providing basic information and covering the most important knowledge in order to support the following chapters.

3.2 Principles of transport phenomena

Although being widely different and specific, all unit operations can be simplified into three kinds of phenomena, involving momentum, heat, and mass transfer. The unit operations are, thus, composed of fundamental transport phenomena.

It is interesting to note that transport phenomena are correlated and can be described by a simple relation:

$$\text{Rate} = \frac{\text{driving potential}}{\text{resistance}} \quad (3.1)$$

where the rate (quantity per time) of transfer is proportional to the driving potential divided by the resistance to the transportation. It can be also written as

$$\text{Rate} = -\lambda \cdot \nabla Y \quad (3.2)$$

where λ is the transport coefficient and ∇Y is the gradient of the transported quantity (the difference in the quantity in relation to the position inside the system).

In momentum transfer phenomena, such as pumping, mixing, flowing through pipes, and others, the driving potential is the difference in the fluid velocity (v) and the viscosity (η) is the transport coefficient. Therefore, the rate of momentum transfer per area, defined as the shear stress (σ), is described by the following equation (Newton's equation):

$$\frac{d(\text{momentum})}{dt} \frac{1}{A} = \sigma = -\eta \cdot \nabla v \quad (3.3)$$

In heat transfer phenomena, such as heating and cooling, the driving potential is the difference in the product temperature (T) and

the thermal conductivity (k) is the transport coefficient. Therefore, the heat flux, i.e., the heat flow ($\dot{q}$) per area, is described by the following equation (Fourier's equation):

$$\frac{d(\text{heat})}{dt} \frac{1}{A} = \frac{\dot{q}}{A} = -k \cdot \nabla T \quad (3.4)$$

In mass transfer phenomena, such as extraction, hydration, acidification, and others, the driving potential is the difference in the transported molecule concentration (C) and the diffusivity (D) is the transport coefficient. The mass flux, i.e., its flow ($\dot{m}$) per area, is described by the following equation (Fick's equation):

$$\frac{d(\text{mass})}{dt} \frac{1}{A} = \frac{\dot{m}}{A} = -D \cdot \nabla C \quad (3.5)$$

Although some unit operations are composed preferably by only one of the transport phenomena, such as the thermal process of conductive (solid) foods, which can be considered only as a heat transfer phenomenon, most of the unit operations contain two or three transport phenomena, such as distillation (heat and mass transfer), solid–liquid extraction (momentum, heat and mass transfer), and others.

3.3 Principles and unit operations of momentum transfer

The momentum associated with a system is defined as the product of its mass and velocity. Similar to energy, momentum is a conserved quantity, i.e., the total momentum of a closed system, without external forces, is constant.

The way that a fluid flows is related to the momentum transfer through it, resulting in a shear stress profile. Consequently, momentum conservation and transfer are important for any unit operation involving fluid flow. Mixing and pumping are typical examples of unit operations in food processing, preferably defined by momentum transfer phenomena.

3.3.1 Food rheology

Rheology is the science that studies the flow and deformations of solids and fluids under the influence of mechanical forces. The rheological characterization of food is important for the design of unit operations, process optimization, and high-quality product assurance. The momentum transfer phenomena are defined by the rheological properties of the product, combined with the process conditions.

The rheological properties related to the fluid flow (steady-state shear rheological properties) can be understood by an imaginary experiment, where a fluid is placed between two parallel plates (Figure 3.1a) with a contact area A separated by a distance dy . If the upper plate is moved by a force F applied in the z direction (Figure 3.1b), after a short transient period the upper plate will keep moving with velocity v while the force F still operates on it, while the lower plate will stay at rest. In this scenario, the fluid layer just below the plate will follow it, moving to the z direction with the same velocity v . On the other hand, the fluid layer just above the lower plate will stay at rest. Therefore, the fluid between these two layers will move in the z direction with a velocity profile as shown in Figure 3.1c, as each fluid layer will drag the one immediately below it.

The shear stress (σ) is thus defined by

$$\sigma = \frac{F}{A} \quad (3.6)$$

which relates the force F with the applied area A , tangent to the F direction (in the experiment, the area A is on the xy plane, while the force F is in the z direction; see Figure 3.1). Further, the shear rate ($\dot{\gamma}$) is defined by

$$\dot{\gamma} = \frac{dv}{dy} \quad (3.7)$$

relating the fluid velocity gradient across the plates.

Based on the behavior relating the shear stress (σ) and the shear rate ($\dot{\gamma}$), and more specifically

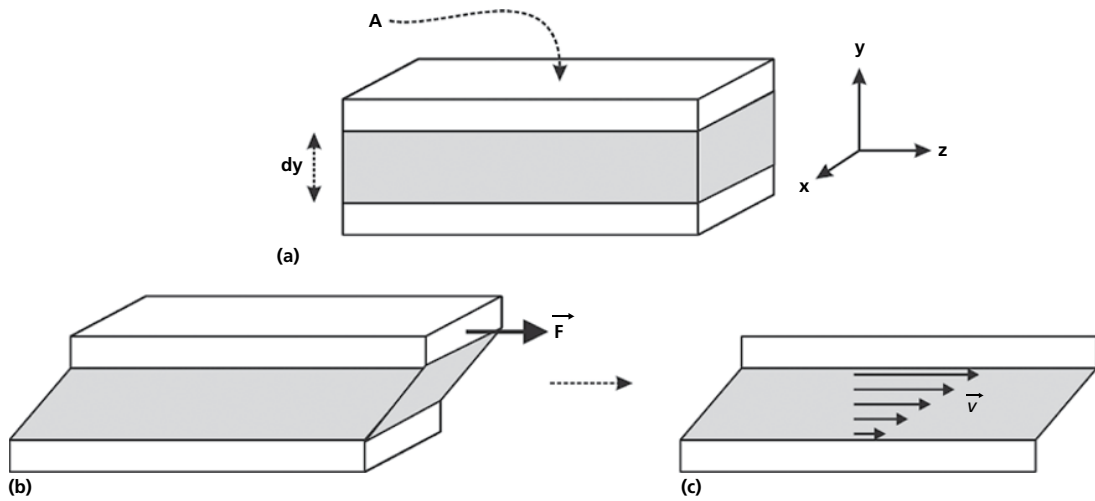


Figure 3.1 A fluid (in gray) between two parallel plates (in white) at rest. Each plate has an area A in contact with the fluid, and the distance between the plates is dy (a); a force f is applied to the upper plate, which is moved in the z direction (b); a cross-section in the yz plane, showing the velocity vectors v on the fluid (c).

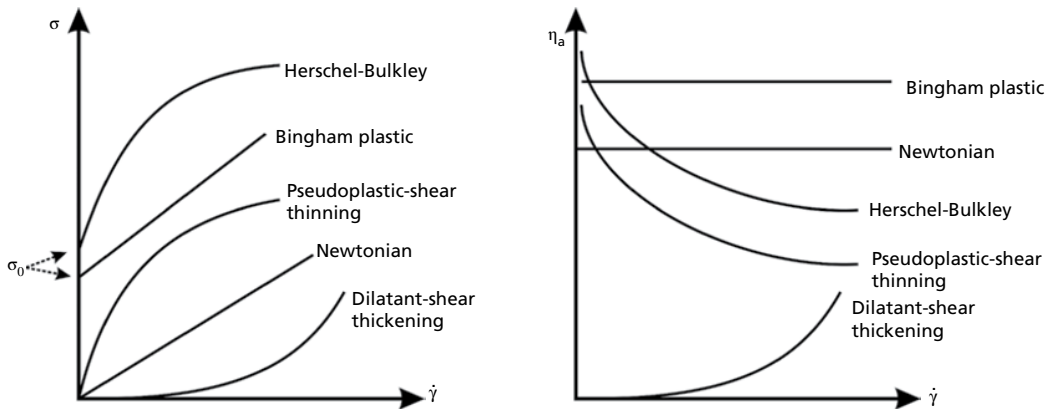


Figure 3.2 Flow behavior of fluids. Shear stress versus shear rate (left) and the variation on the apparent viscosity as a function of the shear rate (right).

the mathematical model that describes this relation, fluids are called Newtonian (Equation (3.8), describing the behavior of perfect fluids, with some real fluid such as gases, water, oils, milk and diluted juices), pseudo-plastic or shear-thinning (Equation (3.9), the Ostwald-de-Waele model, also called the power law, with $n < 1$, as some fruit juices and pulps), dilatant or shear-thicker (Equation (3.9), with $n > 1$, as some starch suspensions), Bingham or Bingham plastic (Equation (3.10), as some emulsions such as mayonnaise,

and Herschel–Bulkley fluid or HB (Equation (3.11), as some fruit juices and pulps). It can be seen that the Herschel–Bulkley model describes all these behaviors using three parameters, σ_0 (the yield stress, which relates the minimum value for the shear stress to initiate the fluid flow), k (the consistency index, which relates the product consistency, the effort to keep flowing), and n (the flow behavior index, which relates the fluid behavior during flowing). Figure 3.2 shows the different fluid behaviors during flowing, by both

the shear stress (σ) and the shear rate ($\dot{\gamma}$) relation, and apparent viscosity ($\eta_a = \sigma/\dot{\gamma}$) and the shear rate ($\dot{\gamma}$) relation.

$$\sigma = \eta \dot{\gamma} \quad (3.8)$$

$$\sigma = k \dot{\gamma}^n \quad (3.9)$$

$$\sigma = \sigma_0 + \eta_p \dot{\gamma} \quad (3.10)$$

$$\sigma = \sigma_0 + k \dot{\gamma}^n \quad (3.11)$$

Therefore, it can be observed that the fluids show different behaviors, which define their process conditions.

3.3.2 Fluid flow

The fluid flow through a pipe is a similar process to that described in Figure 3.1, where the internal pipe surface behaves as the solid plate in the described experiment. Consequently, the existing friction results in a velocity distribution inside the fluid, whose profile is a function of the product rheological properties and the process conditions.

Figure 3.3 shows a typical velocity profile of a Newtonian fluid flowing inside a pipe. After an initial period of accommodation, the fluid reaches a stationary profile where its velocity is not constant, but is a function of its position inside the pipe.

For Newtonian and incompressible fluids under isothermal flow, the velocity profile and flow regime is described by the Reynolds number (Re):

$$Re = \frac{\rho \bar{v} D}{\eta} \quad (3.12)$$

defined by the product properties (density (ρ) and viscosity (η)) and the process conditions (average fluid velocity ($\bar{v}$) and pipe internal diameter (D)). For Newtonian fluids, if $Re < 2100$, the flow regime is called laminar, as the fluid flows in a multilaminae condition, with specific regions without macroscopic mixing of laminae and with a velocity profile with a parabolic

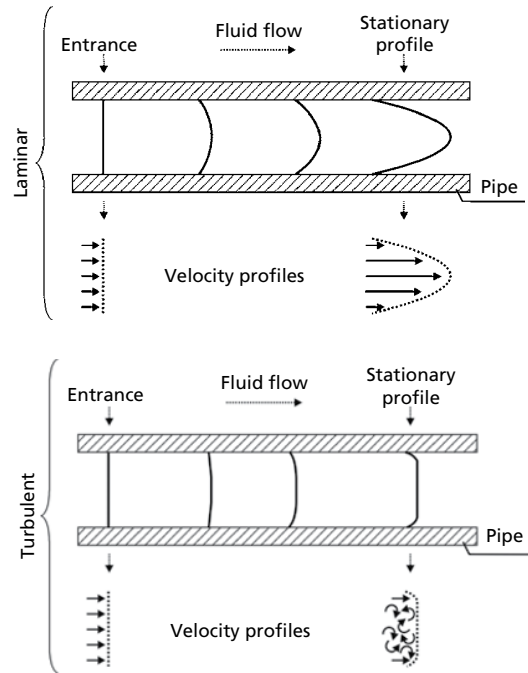


Figure 3.3 Typical fluid velocity profiles during flow inside a pipe (Newtonian fluid at laminar and turbulent flow regimes).

pattern. On the other hand, when $Re > 10^4$, the flow regime is called turbulent, as the fluid flow is agitated, with continuous mixing of its different regions. In this regime, the velocity profile is more uniform. For Re values between 2100 and 10^4 , the flow regime is called transient, with intermediate properties.

For non-Newtonian fluids, the generalized Reynolds number must be evaluated, while taking consideration of the other rheological parameters (σ_0 , k , and n). In addition, the velocity profiles during flow inside a pipe are different (Figure 3.4).

The flow regime definition and properties are thus extremely important for the correct unit operations design. For example, the residence time inside a pipe is defined by the fluid velocity profile, which is not constant but a function of Re . It directly defines the microbial inactivation in the retention tube during the thermal process of liquid foods. Further, the flow regime inside heat exchangers defines the fluid mixture and, consequently, the heat transfer efficiency.

3.3.3 Pumping and mixing

The flow regime also affects the power requirement during pumping and mixing, and the product of rheological properties defines the shear stress profile during processing.

The friction between the fluid and the internal pipe surface dissipates the associated kinetic energy, resulting in “energy losses”. This amount of energy must be provided by a pump in order to allow the desired process, being the power requirement proportional to a friction factor (f), also called a Fanning factor. Figure 3.5

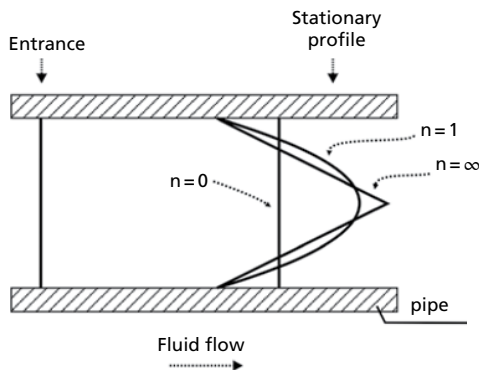


Figure 3.4 Velocity profiles during laminar flow inside a pipe for fluids described by the Ostwald-de-Waele model: Newtonian ($n = 1$), infinite shear thinning ($n = 0$), and infinite shear thicker ($n = \infty$).

shows the friction factor (f) for Newtonian fluids under different flow regimes and relative roughness of the pipes, called the Moody diagram. Similar charts, with other properties, are available for non-Newtonian fluids.

Further, the power requirement ($\dot{W}$) for mixing and agitating fluids can be obtained using the associated power number (Po),

$$Po = \frac{\dot{W}}{\rho \omega^3 Di^5} \quad (3.13)$$

defined by the fluid properties (density (ρ)) and process conditions (angular velocity (ω) and impeller dimension (Di)). The power number (Po), and consequently its requirement ($\dot{W}$), decreases with the Reynolds Number (Re), which must be calculated in this situation using the following equation:

$$Re = \frac{\rho \omega Di^2}{\eta} \quad (3.14)$$

Figure 3.5 shows the power requirement during mixing of Newtonian fluids with different impellers, by correlating the power number (Po) and the Reynolds number (Re). Similar charts, with other properties, are available for non-Newtonian fluids.

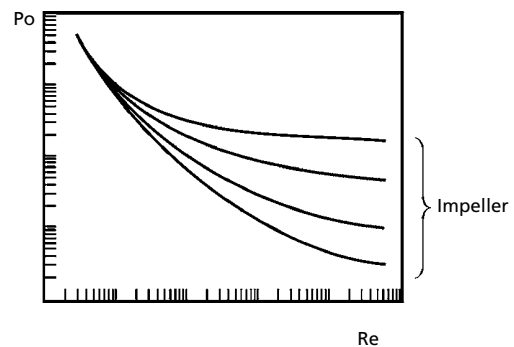
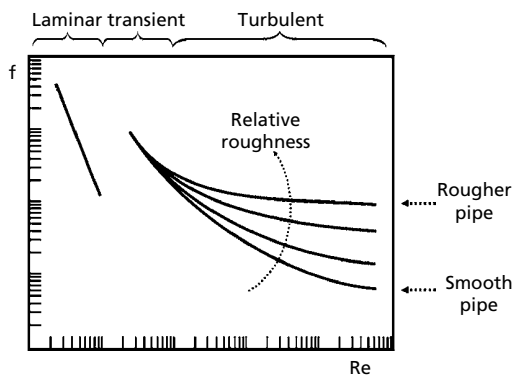


Figure 3.5 Friction factor chart for Newtonian fluid flow in pipes (left); power requirement during mixing of Newtonian fluids with different impellers (right).

3.4 Principles and unit operations of heat transfer

Heat transfer phenomena are widely applied in food processing for promoting both temperature (heating and cooling) and physical state (mainly melting, freezing, evaporation, condensation, and sublimation) changes. Consequently, many unit operations in food processing are based on, or at least contain, heat transfer mechanisms, such as thermal processing, freezing, refrigerating, boiling, drying, baking, frying, distillation, lyophilization, and many others.

The driving potential of heat transfer is a difference in temperature (ΔT) between bodies or regions, which results in a heat flow ($\dot{q}$) from the hottest to the coldest region until thermal equilibrium is reached (i.e., after sufficient time the energy transferred produces a state of temperature equilibrium in the system). There are different mechanisms of heat transfer, as there are also different effects produced when heat is introduced or removed from matter.

3.4.1 Absorbing or removing thermal energy

The received energy can increase body temperature and/or promote a change in its physical state, and the transferred energy can also decrease the body temperature and/or promote a change in its physical state.

When the thermal energy promotes a change in the system temperature, it is called sensible heat. The energy necessary to promote the heating or cooling (q_{sensible}) is defined by the mass of product (m), its specific heat (Cp) and the difference in temperature (ΔT), as described by

$$q_{\text{sensible}} = m Cp \Delta T \quad (3.15)$$

When the thermal energy promotes a change in the system physical state, it is called latent heat. The energy necessary to promote the change (q_{latent}) is defined by the mass of product (m) and the specific latent heat at the phase change (L), which can be of melting/

solidification, evaporation/condensation, sublimation/desublimation, as described by

$$q_{\text{latent}} = mL \quad (3.16)$$

However, although the phase transition is a well-defined phenomenon for pure substances, it is not for solutions, suspensions, dispersions, and complex products, such as food products. For example, the freezing process of pure water and a food product is highlighted in Figure 3.6.

For pure water at atmospheric pressure, a decrease in its temperature is observed when the heat is removed, until 0°C at atmospheric pressure (i.e., sensible heat). At this point, any amount of energy removed will change the physical state of the corresponding water molecules amount, which will pass from liquid to solid, without changing the temperature (i.e., latent heat). If further energy is removed after completely freezing the water, the ice is then cooled (i.e., sensible heat).

On the other hand, the freezing process of food products is not defined by a demarcated change of the water physical state from liquid to solid. Instead, due to the colligative properties between the solutes and water, the temperature at the beginning of freezing is slightly below 0°C , and when further energy is removed, the product behavior is described by a continuous cooling–freezing progression. As is the case for water that is frozen in the process, the solution concentration is increased when a water molecule passes from liquid to solid, reducing the freezing point. Consequently, it is almost impossible to dissociate the phase change and the cooling in the freezing curve, as clearly shown in Figure 3.6. As a result, both Equations (3.15) and (3.16) cannot be used, and the heat amount must be determined by the associated specific enthalpy ($\Delta\hat{H}$), as described by

$$q = m \Delta\hat{H} \quad (3.17)$$

Figure 3.6 also shows other interesting issues. Firstly, it is highlighted how the latent

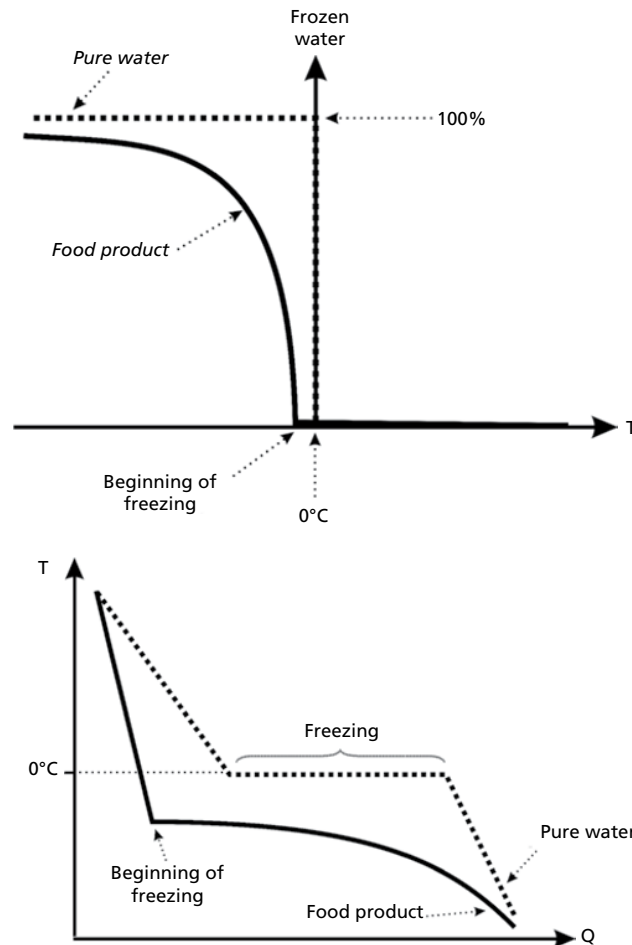


Figure 3.6 Pure water and food products behavior during cooling and freezing (atmospheric pressure): frozen water content at the equilibrium as a function of temperature; total thermal energy removed during the process (q).

heat of a substance is proportionally higher than the sensible heat, with important consequences in food processing. Secondly, the specific heat (C_p) of foods is smaller than that of water, due to other components, as can be seen by the slope of the graphic in the cooling region. Further, the specific heat of ice is smaller than that of the liquid water. Finally, due to the unfrozen fraction concentration effect when the water is frozen in foods, it is interesting to notice that the water is not totally frozen in normal processing of foods.

3.4.2 Heat transfer mechanisms and process conditions

The heat transfer driving potential is the difference of temperature (ΔT) between bodies or regions, resulting in a heat flow ($\dot{q}$). The heat is transferred from the hot to the cold place, and the rate of heat transfer is directly proportional to ΔT and inversely proportional to the thermal resistance (R_T), as described by

$$\dot{q} = \frac{\Delta T}{R_T} \quad (3.18)$$

There are three basic mechanisms of heat transfer, called conduction, convection, and radiation.

The conduction heat transfer mechanism is defined by the energy transfer at the molecular level, from the hot molecule to the neighbor cold molecule. It is the typical heat transfer mechanism of solids and is characterized by being relatively slow.

The convection heat transfer mechanism takes place in fluids (liquids and gases), as there is a macroscopic flow of matter. When a region of fluid is heated, its volume is increased (thermal dilatation), although its mass does not change, which results in a reduction in its density. With a smaller density than the neighbor regions, the hot region buoyancy force is increased, becoming higher than its weight force and promoting an upper movement. A similar behavior, although in the opposite sense, occurs for cooling. Consequently, when a fluid is heated or cooled, there is a resultant movement, which increases the rate of heat transfer.

The radiation heat transfer mechanism does not need matter to occur, being the energy transferred through electromagnetic radiation from a hot to a cold body. The heat transfer, being independent of the mechanism, can be carried out at a steady state or an unsteady state condition.

The steady state condition is defined as one when all the physical variables remain constant and invariable with time throughout the system. Consequently, a steady state process is one whose conditions are constant, and the process conditions and properties are not a function of time. Some examples of steady state heat transfer processes are heat exchangers, refrigerated chambers, and continuously operating ovens.

The unsteady state condition, on the other hand, is defined as one when at least one physical variable changes with time. In this case, the process conditions and properties are thus a function of time. Some examples of unsteady

state heat transfer processes are the thermal process of packaged foods and any discontinuous process.

3.4.3 Steady state heat transfer

The conduction heat transfer at a steady state condition is described by the Fourier equation:

$$\dot{q}_{conduction} = \frac{kA \Delta T}{x} \quad (3.19)$$

where the heat flow (rate of heat transfer) is a function of the product thermal conductivity (k), the area of heat transfer (A), the difference of temperature (ΔT) between the hot and cold regions, and the length crossed by the heat flow (x). Therefore, it is highlighted that the heat flow is reduced when an insulating material is used (i.e., with a small k value) and when its thickness is increased. It is interesting to notice that the thermal conductivity (k) of food products is, in general, close or slightly below that of the water (~ 0.6 W/m K). Further, it is found that this property for expanded polystyrene (EPS), one of the most used insulating materials, is 0.03 W/m K and that the k of stainless steel (~ 14 W/m K), the most used material in the food industry, is relatively small when compared with other metals, such as carbon steel (~ 60 W/m K), aluminum (~ 240 W/m K), and copper (~ 400 W/m K).

The convective heat transfer at the steady state condition is described by the Newton equation:

$$\dot{q}_{convection} = hA \Delta T \quad (3.20)$$

where the heat flow is a function of the area of heat transfer (A), the difference in temperature (ΔT) between the hot and cold regions, and a property of the system called the convective heat transfer coefficient (h). The convective heat transfer coefficient (h) is not a property of the fluid, but of the system, i.e., both fluid and process conditions and properties. Its value varies while involved in food processing,

considering both natural and forced convection, internal and external flow, different fluids, temperatures, velocities, and further processes. In general, the h value for food processes is between 10^{-1} and $10^4 \text{ W/m}^2\text{K}$ and may reach $10^5 \text{ W/m}^2\text{K}$ when there are phase changes (boiling, condensing). The h value can be obtained experimentally for the specific system using dimensionless numbers such as the numbers of Nusselt (Nu), Reynolds (Re), Prandtl (Pr), Grashof (Gr), and Rayleigh (Ra).

The radiation heat transfer at steady state condition is described by the Stefan-Boltzmann Equation (Equation 3.21), where the heat flow is function of the area of heat transfer (A_1), the difference of the hot (T_1) and cold (T_2) bodies surface temperatures, the Stefan-Boltzmann Constant ($\sigma = 5.67 \cdot 10^{-8} \text{ W/m}^2 \cdot \text{K}^4$), the emissivity of both bodies ($\varepsilon_1, \varepsilon_2$), the emission factor of one body intercepted by the other (F_{12}) and the absorption coefficient of the bodies (a_1, a_2). Although less studied in food processing than the conductive and convective heat transfer, the radiation heat transfer is important in some specific processes, such as ovens and infrared heating.

$$\dot{q}_{\text{radiação}} = F_{12} \cdot A_1 \cdot \sigma \cdot (a_2 \cdot \varepsilon_1 \cdot T_1^4 - a_1 \cdot \varepsilon_2 \cdot T_2^4) \quad (3.21)$$

A typical application of steady state heat transfer in food processing is heat exchangers. In that equipment, the heat is transferred both by conduction (metal body) and convection (fluids), and the difference in temperature (ΔT) between the hot and cold fluids changes with the position inside the equipment. Consequently, in spite of evaluating the heat transfer phenomenon by considering each small region, the process is evaluated for the whole equipment by considering the overall heat transfer coefficient (U) and the following equation:

$$\dot{q}_{\text{heat\_exchanger}} = UA \Delta T_{\text{ln}} \quad (3.22)$$

The overall heat transfer coefficient (U) is defined for each equipment and process

conditions (fluids, flows, temperatures), being in general between 10 and $10^4 \text{ W/m}^2\text{K}$ for food processes. Also, it is important to notice that, as the difference in temperature (ΔT) between the hot and cold fluids is a function of the position inside the heat exchanger, the property used in Equation (3.22) is the logarithmic mean temperature difference (ΔT_{ln}), also called LMTD, which is calculated using the hot and cold fluids temperature difference on both sides of the heat exchanger ($\Delta T_1, \Delta T_2$):

$$\Delta T_{\text{ln}} = \frac{\Delta T_1 - \Delta T_2}{\ln(\Delta T_1 / \Delta T_2)} \quad (3.23)$$

3.4.4 Unsteady state heat transfer

Unlike the steady state condition, the driving potential is not constant in the unsteady state heat transfer, where the temperature difference changes along the processing time ($\Delta T = f(t)$). Consequently, the unsteady state heat transfer is a complex phenomenon, whose modeling and analytical solution is difficult. As a result, this evaluation can be carried out using numerical solutions (using computers) or charts and predictive equations for simplified conditions and geometries.

The Fourier equation describes the heating (or cooling) rate of a conductive body at the unsteady state condition and is described by the tendency of heat transfer (defined by the product thermal conductivity (k)) to absorb the energy, increasing its temperature (defined by the product density (ρ) and specific heat (Cp)), but the thermal diffusivity (α) is the property that describes it:

$$\frac{\partial T}{\partial t} = \frac{k}{\rho Cp} \nabla^2 T = \alpha \nabla^2 T \quad (3.24)$$

As the direct resolution of Equation (3.24) is quite difficult, a series of methods were developed in order to study the conductive heat transfer at the unsteady state. Figure 3.7 shows one of the most popular methods, using charts

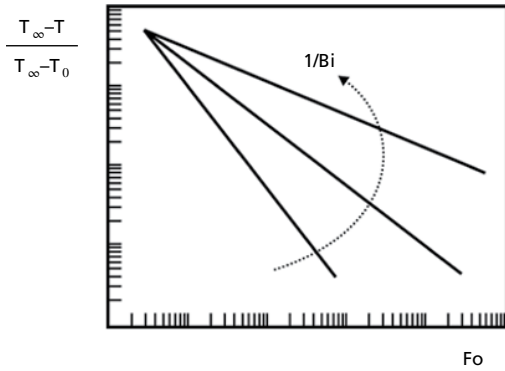


Figure 3.7 Dimensionless temperature at a specific position and geometry (slab, cylinder, sphere) as a function of time (as the Fourier number), the product thermal properties, and the system conditions (as the Biot number).

and dimensionless numbers. There, the dimensionless temperature (θ) is

$$\theta = \frac{T_{\infty} - T}{T_{\infty} - T_0} \quad (3.25)$$

where T_{∞} is the temperature at the equilibrium and T_0 is the initial temperature, at a specific position (there are charts for different positions inside the geometry) and geometry (there are charts for perfect slab, infinite cylinder and sphere), is a function of the dimensionless time, the product of thermal properties, and the system conditions.

The dimensionless time is defined by the Fourier number (Fo),

$$Fo = \frac{\alpha t}{(L^*)^2} \quad (3.26)$$

where α is the product thermal diffusivity, t is the processing time, and L^* is the product characteristic length (the radius for spheres and long cylinders, half of the slab thickness).

The Biot number (Bi) describes the relative importance of the conductive heat transfer, inside the solid body, and the convective heat transfer, at the body surface. It is the ratio of the heat transfer resistances inside the body and at its surface.

$$Bi = \frac{hL^*}{k} \quad (3.27)$$

By using the appropriate charts, similar to those in Figure 3.7, the heating (or cooling) rate of a conductive body can be evaluated.

The convective unsteady state heat transfer, however, is much more complex, as it involves the fluid flow. Consequently, more equations must be solved, in a coupled procedure, describing the conservation of mass, momentum, and energy.

The conservation of mass is described as the continuity equation, where v_x , v_y , and v_z are the fluid velocities in the x , y , and z directions:

$$\frac{\partial v_x}{\partial x} + \frac{\partial v_y}{\partial y} + \frac{\partial v_z}{\partial z} = 0 \quad (3.28)$$

The conservation of momentum is described by Newton's second law of motion,

$$\rho \left(\frac{\partial v_x}{\partial t} + v_x \frac{\partial v_x}{\partial x} + v_y \frac{\partial v_x}{\partial y} + v_z \frac{\partial v_x}{\partial z} \right) = \rho a_x - \frac{\partial p}{\partial x} + \eta \left(\frac{\partial^2 v_x}{\partial x^2} + \frac{\partial^2 v_x}{\partial y^2} + \frac{\partial^2 v_x}{\partial z^2} \right) \quad (3.29)$$

$$\rho \left(\frac{\partial v_y}{\partial t} + v_x \frac{\partial v_y}{\partial x} + v_y \frac{\partial v_y}{\partial y} + v_z \frac{\partial v_y}{\partial z} \right) = \rho a_y - \frac{\partial p}{\partial y} + \eta \left(\frac{\partial^2 v_y}{\partial x^2} + \frac{\partial^2 v_y}{\partial y^2} + \frac{\partial^2 v_y}{\partial z^2} \right) \quad (3.30)$$

$$\rho \left(\frac{\partial v_z}{\partial t} + v_x \frac{\partial v_z}{\partial x} + v_y \frac{\partial v_z}{\partial y} + v_z \frac{\partial v_z}{\partial z} \right) = \rho a_z - \frac{\partial p}{\partial z} + \eta \left(\frac{\partial^2 v_z}{\partial x^2} + \frac{\partial^2 v_z}{\partial y^2} + \frac{\partial^2 v_z}{\partial z^2} \right) \quad (3.31)$$

where a_x , a_y , and a_z are the acceleration in the x , y , and z directions, p is the product momentum, and η is its viscosity (or for non-Newtonian fluids, the value of apparent viscosity for that condition). Note that the momentum must be conserved independently for each direction.

Finally, the conservation of energy is described by the first law of thermodynamics, where the convective terms (described by the fluid flowing) were introduced in Equation (3.24):

$$\frac{\partial T}{\partial t} + v_x \frac{\partial T}{\partial x} + v_y \frac{\partial T}{\partial y} + v_z \frac{\partial T}{\partial z} = \frac{1}{\rho C_p} \left[\frac{\partial}{\partial x} \left(k \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left(k \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z} \left(k \frac{\partial T}{\partial z} \right) \right] \quad (3.32)$$

3.4.5 Heat transfer in the thermal process

Food thermal processes can be carried out with the product being heated before packaging (in heat exchangers, frequently called aseptic processing) or after packaging (in-packaged thermal process, also called Appertization in honor of Nicholas Appert, who developed the methodology).

The thermal process in heat exchangers can be carried out for fluid foods and, in some cases, particulates (small solid pieces dispersed in a fluid). It is a typical steady state heat transfer process.

The in-packaged thermal process can be carried out for any kind of food, being a typical unsteady state heat transfer process. As the heating is not instantaneous and it is not uniform throughout the product, it is important to understand how it is carried out.

In this process, the system (food + package) is firstly put in contact with a hot fluid (steam, water, air, and their mixtures) to be heated to the process temperature. The system is thus retained at the process temperature during a pre-established time, in order to achieve the process target. This time is calculated to guarantee the product safety and stability at the storage conditions, and can be optimized with

the aim to provide better nutritional and sensory attribute retention. Finally, the system is put in contact with a cold fluid (water) to be cooled and to stop the thermal effects. Thus, heat flux is transferred from the fluids to the food package surface, through the package, to the food surface and finally through the food.

The heat transfer inside the product is not instantaneous and can occur by convection or conduction. Consequently, there is a region of slower heating inside the product, called the cold spot.

During the heat transfer by conduction, the thermal energy is slowly transmitted, molecule by molecule. It is characteristic for solid, semi-solid, and liquid food with high consistency. In these cases, the thermal diffusivity (α) is the food property that regulates the product behavior during the thermal processing. The heat flux in homogeneous conductive food is uniform and their cold spot is located in the geometric center of the package (Figure 3.8). However, the cold spot location can change due to non-homogeneity of the food composition or due to non-homogeneity of the heat influx on the packaging surface.

For example, Figure 3.8b shows the effect of the headspace in the location of the conductive food cold spot when food is packaged in cylindrical cans. The vacuum in the headspace strongly reduces the heat flux, being insulation. Therefore, the heat influx by the can lid is lower than that at the other walls. Consequently, the cold spot is moved up from the geometric center. Similar behavior is observed when the food structure is not uniform, containing regions with different thermal resistances. For example, Figure 3.9 shows the effect of a fat layer on the cold spot location of cooked ham, where the product cold spot is relocated close to the insulated region.

Also, if the heating flux is not uniform at all can surfaces, the cold spot will also move from the geometric center. This is the case when foods are heated by water aspersion or when the package is incompletely immersed in the hot fluid. When the packaged food travels through

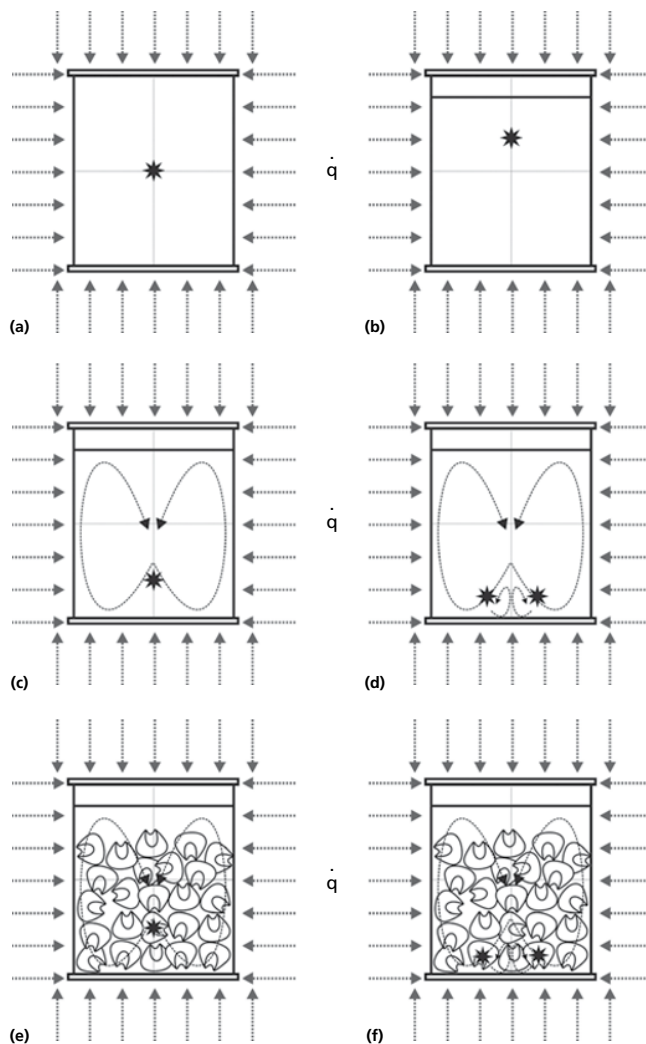


Figure 3.8 Representation of the cold spot location (black stars) during in-package food thermal processing using cylindrical cans (longitudinal section): (a) conductive food without headspace, (b) conductive food with headspace, (c) high consistency convective food, (d) low consistency convective food, (e) particulates food with high consistency fluid, and (f) particulates food with low consistency fluid.

the pasteurization tunnel, hot water is sprayed only on the can lid and body. Thus, the heating influx through the can base is less efficient and the cold spot is pushed towards the bottom of the package. Figure 3.9 shows the effect of heating a canned product using water sprinklers (non-homogeneity of the heat influx).

During heat transfer by convection, the thermal energy is transmitted through the fluid

flow due to natural convection currents. It is characteristic for liquid foods, although it is important to highlight that liquids with a high consistency may not form the convective currents and, thus, only transfer heat by conduction.

When the liquid food is heated inside the package, the food portion near the heating source (in this case, the interface with the packaging) heats first and, consequently, expands,

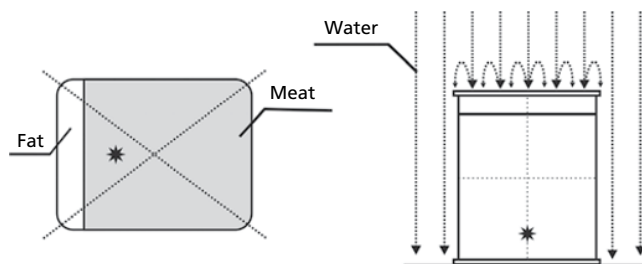


Figure 3.9 Representation of the cold spot location (black stars) during the thermal process of conductive foods: cooked ham containing a fat layer (left) and a conductive food packaged on a cylindrical can, heated by water sprinklers (right).

thus becoming of a lower density. For fluids, the density differences among its portions result in a buoyancy force, with a magnitude higher than the weight force. Therefore, the resulting force sense is against the gravitational acceleration and, consequently, the fluid forms an upward flow. Upon reaching the top of the package, the fluid is conducted to the central axis (i.e., displaced away from the heating source). As a result of the continuous food upward flow, the fluid in the center of the package is forced down through the central axis, generating characteristics circular currents, called natural convection currents (Figure 3.8c).

In this case, the location of the cold spot is complex once it is transient (i.e., it changes during the heating time) and is dependent on the process conditions, the food properties, and the package. Traditionally, the cold spot of convective food is considered to be located in the symmetrical axis of the package, at one-third of the package height. However, the real cold spot location is in the lower portion of the package, which is, in general, between 10 and 20% of the height of the cylindrical package (Figure 3.8c).

On the other hand, when the heating is uniform throughout the package walls, there are different fluid flows, as a result of heat transfer with the package walls. These flows meet at certain locations, forming small circular currents, which can change the location of the cold spot (then called the slowest heating zone). Figure 3.8d shows a package with secondary currents formation, with opposite directions, caused by the encounter of the heating currents

from the package base and downward flow through the central axis. In this case, the cold region is moved away from the central axis, forming a toroid that surrounds the central axis. This is the typical behavior of heating low consistency foods (beer, for example) in packages.

The heating rate of liquid foods is a function of their thermal properties (determined by thermal diffusivity (α)) and of the magnitude of the convection currents, which is related to its coefficient of thermal expansion (β) and viscosity (η) or, for non-Newtonian fluids, other properties that define its apparent viscosity in the process (η_a). For higher consistent or viscous fluids, the resistance to flow is higher and the convection currents become negligible. In this case, the food is heated only by conduction.

Considering that the heat transfer by conduction is less effective than the heat transfer by convection, many solid foods are thermally processed with a liquid portion, aiming to guarantee a faster and more uniform heating profile. Consequently, the final products are more homogeneous and have a lower gradient of undesirable reactions. These products are called particulates food, and the main examples are vegetables in brine (corn, peas, pickles, heart of palm, beans), tuna and sardine in oil or brine, and meat products in sauces. In these cases, the liquid fraction is heated by convection and flows through the spaces between the solid fraction, resulting in more uniform heating of the product. However, it is important to remember that the velocity of the convection currents are slow (some millimeters per second) and lower than

the terminal velocity of the solid fraction. Consequently, in general, the fluid movement is not strong enough to move the solid fraction. Therefore, the cold spot of the product is established for the worst case, i.e., at the geometrical center of the solid located at the region of slower fluid heating (Figures 3.8e and 3.8f).

3.5 Principles and unit operations of mass transfer

The mass transfer phenomena describe the transference of matter, being regulated by the conservation of mass (matter) law (also called the Lavoisier law) and the thermodynamic equilibrium. Typical unit operations of mass transfer are the extractions (solid–liquid, liquid–liquid, supercritical), osmotic dehydration, acidification, hydration, salting, drying, and others. Other unit operations contain both heat and mass transfer mechanisms, such as drying, baking, frying, distillation, lyophilization, evaporation, smoking, and many others.

Matter is transferred from a region of high concentration (or activity) to another of smaller concentration (or activity), trying to reach an equilibrium (i.e., after enough time, the matter transferred produces a state of equilibrium in the system concentration). Consequently, the driving potential to the mass transfer is a difference in concentration (ΔC) between regions, which results in a mass flow ($\dot{m}$). There are different mechanisms of mass transfer that have been very well studied and understood, but there are other mechanisms that are still not completely understood, such as that on food processing.

3.5.1 Mass transfer mechanisms

There are two main mechanisms of mass transfer, called diffusion and convection, which can occur at a steady or an unsteady state condition. However, there are other mechanisms of mass transfer (such as capillarity) whose importance is dependent on the process.

Some mass transfer evaluations are carried out using analogies with heat transfer phenomena, where mass diffusion is compared with conductive heat transfer and convection is compared with both heat and mass transfers.

The diffusion process is described by the Fick equation (Equation (3.5)), where the flux of mass is proportional to the gradient of concentration (∇C) of the transported matter and its diffusivity (D) across the stationary phase. It is the main transport mechanism of liquids and solids in solids, such as the water influx into grains (hydration), H^+ ions into vegetables (acidification), Na^+ and Cl^- into meat (salting), solvent into grains (extraction), and others.

At the steady state condition, the Fick equation can be written as

$$\frac{\dot{m}_S}{A} = \frac{D_{SB}(C_{S_1} - C_{S_2})}{x_2 - x_1} \quad (3.33)$$

where a substance S is transported through another substance B . Therefore, the flux of mass is a function of the S diffusivity across B (D_{SB}), the difference in S concentration between the points 1 and 2 and the space between those points. In fact, there are few examples of steady state mass transfer in food processing.

The variation of the evaluated substance along the process time at the unsteady state condition is described by

$$\frac{\partial C}{\partial t} = D \nabla^2 C \quad (3.34)$$

Although the direct resolution of Equation (3.34) is quite difficult, its solution is available as a series of equations for some specific geometries, such as the sphere, infinite cylinder, and perfect slabs. Further, the charts method, based on dimensionless numbers, is also available, as shown in Figure 3.10. There the dimensionless mean concentration (C^*),

$$C^* = \frac{C_m - C_\infty}{C_0 - C_\infty} \quad (3.35)$$

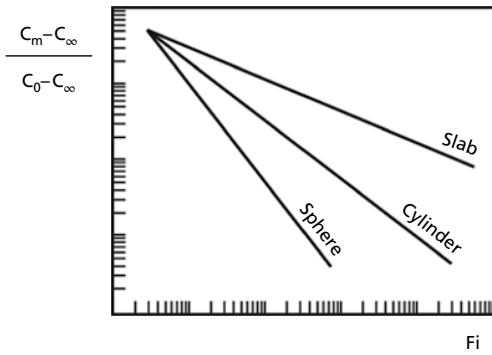


Figure 3.10 Dimensionless mean concentration at specific geometries (slab, cylinder, sphere) as a function of time (as the Fick number) in a diffusion mass transfer process.

where C_m is the mean concentration across the product, C_∞ is the concentration at the equilibrium, and C_0 is the initial concentration, is given at a specific geometry (there are charts for the perfect slab, cylinder, and sphere) as a function of dimensionless time.

The dimensionless time is defined by the Fick number (Fi),

$$Fi = \frac{D \times t}{(L^*)^2} \quad (3.36)$$

$$\frac{\partial C}{\partial t} + v_x \frac{\partial C}{\partial x} + v_y \frac{\partial C}{\partial y} + v_z \frac{\partial C}{\partial z} = \frac{\partial}{\partial x} \left(D \frac{\partial C}{\partial x} \right) + \frac{\partial}{\partial y} \left(D \frac{\partial C}{\partial y} \right) + \frac{\partial}{\partial z} \left(D \frac{\partial C}{\partial z} \right) \quad (3.38)$$

Even so, it can be evaluated by coupling solving Equation (3.38) with the general conservation of mass (Equation (3.28)), the conservation of momentum (Equations (3.29), (3.30), and (3.31)), and, if it is the case, the conservation of energy (Equation (3.32)). It is, in general, carried out using numerical solutions and computers.

3.6 Conclusions

The studying and evaluation of unit operations is essential for guaranteeing adequate and optimized food processing. Each unit operation has

where D is the substance diffusivity, t is the processing time, and L^* is the product characteristic length. By using the appropriate charts, the mass transfer phenomena can be evaluated.

When a fluid concentration is changed, its density is increased or decreased, and its buoyance force become different from the weight force. Consequently, natural convective currents form and the fluid flows. The convective mass transfer is defined by the macroscopic transfer of matter due to the fluid flow, which is similar to the heat transfer phenomenon. At the steady state condition, this is described by

$$\dot{m}_{convection} = h_m A \Delta C \quad (3.37)$$

where the mass flow is a function of the area of transference (A), the difference of concentration (ΔC) between the regions, and the convective mass transfer coefficient (h_m). The h_m value can be obtained experimentally for the specific system using dimensionless numbers such as the numbers of Sherwood (Sh), Schmidt (Sc), Reynolds (Re), and Lewis (Le).

The convective unsteady state mass transfer, however, is much more complex as it involves the fluid flow

its own impact on the microbial load and physiological state. Therefore, it is essential to understand the physical principles involved in each unit operation in food processing.

This chapter described the principles of transport phenomena and unit operations in food processing. The main characteristics and equations of momentum, heat, and mass transfer operations were shown, providing the basic information needed for a better understanding of the next chapters. Consequently, it is a suggestion to the reader to consult this chapter to aid the discussion of the specific chapters.

References

- Augusto, P.E.D., Tribst, A.A.L., and Cristianini, M. 2014. Thermal processes, commercial sterility (report, Chapter 405). In: *Encyclopedia of Food Microbiology* (eds Batt, C. and Tortorello, M.L., 2nd edn). Elsevier.
- Augusto, P.E.D. and Vitali, A.A. 2014. Assessing juices quality: advances in the determination of rheological properties of fruit juices and derivatives. In: *Juice Processing: Quality, Safety and Value-Added Opportunities* (eds Falguera, V. and Ibarz, A.). CRC Press, Boca Raton, FL, USA.
- Campbell-Platt, G. (ed.). 2009. *Food Science and Technology*. Wiley-Blackwell.
- Earle, R.L. and Earle, M.D. 1983. *Unit Operations in Food Processing*. Pergamon Press, Oxford. Available at: <http://www.nzifst.org.nz/unitoperations/>.
- Fellows, P. 2000. *Food Processing Technology: Principles and Practice*, 2nd edn. Woodhead Publishing Limited, Cambridge, UK.
- Ibarz, A. and Barbosa-Cánovas, G.V. 2003. *Unit Operations in Food Engineering*. CRC Press, Boca Raton, FL, USA.
- Ibarz, A. and Barbosa-Cánovas, G.V. 2014. *Introduction to Food Process Engineering*. CRC Press, Boca Raton, FL, USA.
- Incropera, F.P. and DeWitt, D.P. 2003. *Fundamentos de Transferência de Calor e de Massa*. Livros Técnicos e Científicos, Rio de Janeiro, Brazil.
- Ordóñez, J.A. (ed.). 2005. *Tecnologia de Alimentos. Volume 1: Componentes dos Alimentos e Processos*. Porto Alegre: Artmed, Porto Alegre, Brazil.
- Rao, M.A. 1999. *Rheology of Fluid and Semisolid Foods: Principles and Applications*. Aspen Publishers, Gaithersburg, MD, USA.
- Saravacos, G.D. and Maroulis, Z.B. 2011. *Food Process Engineering Operations*. CRC Press, Boca Raton, FL, USA.
- Singh, R.P. and Heldman, D.R. 2009. *Introduction to Food Engineering*. Academic Press, San Diego, CA, USA.
- Steffe, J.F. 1996. *Rheological Methods in Food Process Engineering*, 2nd edn. Freeman Press, East Lansing, MI, USA.

PART II

Impact of unit operations on microorganisms of relevance in foods

CHAPTER 4

Impact of materials handling at pre- and post-harvest operations on the microbial ecology of foods of vegetable origin

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4.1 Introduction

Fresh fruits and vegetables contribute significantly to vitamin, mineral, and fibre requirements for a healthy human diet. The World Health Organization (WHO, 2000) currently recommends a daily intake of 400 g to improve overall health and reduce the risk of chronic disease. Public health agencies in several countries including Canada, the United States of America, the Netherlands, Spain, Norway, Belgium, and Brazil recommend the consumption of at least five daily servings to reduce the risk of cardiovascular disease and cancer (Abadias *et al.*, 2008); consequently, the consumption of fresh fruits and vegetables has increased in many countries over the last three decades. For example, statistics from the United States of America indicate that per capita consumption rose from 91.6 to 121.1 kg between 1982 and 1997 (FDA, 2013).

Unfortunately, concerns about potential food safety threats due to the latent risk of contamination with hazardous microorganisms have risen in parallel with increasing consumption and demand for fresh fruits and vegetables (Lynch *et al.*, 2009; Olaimat and Holley, 2012; Gil *et al.*, 2015). Reports of fresh-produce-associated infections and outbreaks caused by a range of protozoan, bacterial, or viral human pathogens

have increased in number and frequency since the early 1990s. Fresh produce accounted for an estimated 46% of food-borne illnesses, 38% of hospitalizations, and 23% of deaths due to contaminated food in the US between 1998 and 2008 (Painter *et al.*, 2013), with produce-associated costs of \$39 billion annually (Scharff, 2010). In Canada, there were 27 reported outbreaks (1,549 illnesses) linked to fresh produce during 2001–2009 (Kozak *et al.*, 2013). In England and Wales 122 outbreaks from 1992 to 2008 were linked to fresh produce (Soon *et al.*, 2012). Changes in production systems, processing methods, and distribution chains to satisfy growing demand have undoubtedly contributed to this significant shift in food-borne disease association (Olaimat and Holley, 2012).

Table 4.1 lists food-borne illness outbreaks reported internationally since 2005 where produce was the implicated vehicle of transmission. Of 1100 reported outbreaks in which an etiological agent was identified, 53% were caused by bacteria, 42.5% by viruses, and 4.5% by parasites (Ramos *et al.*, 2013). Notable bacterial outbreaks have been linked to *E. coli* O157:H7 on lettuce and spinach; *Salmonella* on lettuce, melons, spinach, tomatoes, seed sprouts, and spices; *L. monocytogenes* on cantaloupes; non-O157 Shiga-toxicogenic *E. coli* (STEC) on sprouts; and

Table 4.1 Outbreaks linked to fresh produce from 2005 to 2014.

Location	Year	Pathogen	Produce	Cases (deaths)	Reference
Canada	2005	<i>Salmonella</i>	Mung bean sprouts	592	Rohekar <i>et al.</i> , 2008
USA	2005	<i>Salmonella</i>	Tomatoes	459	CDC, 2007
Sweden	2005	<i>E. coli</i> O157:H7	Lettuce	135	Söderström <i>et al.</i> , 2008
Finland	2005	<i>Salmonella</i>	Lettuce	60	Takkinen <i>et al.</i> , 2005)
USA	2006	<i>E. coli</i> O157:H7	Spinach	199 (3)	CDC, 2006b
Australia	2006	<i>Salmonella</i>	Alfalfa sprouts	125	Compton <i>et al.</i> , 2008
USA, Canada	2006	<i>Salmonella</i>	Fruit salad	41	Landry <i>et al.</i> , 2007
USA	2006	<i>Salmonella</i>	Tomatoes	183	CDC 2006a
Sweden	2006	<i>Salmonella</i>	Mung beans	115	De Jong <i>et al.</i> , 2007
USA	2006	<i>E. coli</i> O157:H7	Lettuce	81	FDA, 2007
Australia	2006	<i>Salmonella</i>	Cantaloupe	115	Munnoch <i>et al.</i> , 2008
USA	2006	<i>E. coli</i> O157:H7	Spinach	22	Grant <i>et al.</i> , 2008
Europe	2007	<i>Salmonella</i>	Baby spinach	354	Denny <i>et al.</i> , 2007
Netherlands and Iceland	2007	<i>E. coli</i> O157:H7	Lettuce	50	Friesema <i>et al.</i> , 2008
North America, Europe	2007	<i>Salmonella</i>	Basil	51	Pezzoli <i>et al.</i> , 2007
Australia, Europe	2007	<i>Shigella</i>	Baby carrots	230	Lewis <i>et al.</i> , 2007
Europe	2007	<i>Salmonella</i>	Alfalfa sprouts	45	Emberland <i>et al.</i> , 2007
Sweden	2007	<i>Salmonella</i>	Alfalfa sprouts	44	Werner <i>et al.</i> , 2007
USA, Canada	2008	<i>Salmonella</i>	Peppers	1442 (2)	CDC, 2008b; Mody <i>et al.</i> , 2011
USA, Canada	2008	<i>E. coli</i> O157:H7	Lettuce	134	Warriner and Namvar, 2010
UK	2008	<i>Salmonella</i>	Basil	32	Elviss <i>et al.</i> , 2009
USA	2008	<i>Salmonella</i>	Cantaloupe	51	CDC, 2008a
Canada	2008	<i>E. coli</i> O157:H7	Onion	235	Kozak <i>et al.</i> , 2013
USA, Canada	2008	<i>Salmonella</i>	Peanut butter	714 (9)	CDC, 2009b
Netherlands	2008	<i>Salmonella</i>	Raspberries	33	EFSA Panel on Biological Hazards, 2013
USA	2009	<i>Salmonella</i>	Alfalfa sprouts	235	CDC, 2009a
Finland	2009	<i>Salmonella</i>	Alfalfa sprouts	42	Rimhanen-Finne <i>et al.</i> , 2011
Canada	2009	<i>Salmonella</i>	Onion sprouts	20	Kozak <i>et al.</i> , 2013
Sweden	2009	<i>Shigella</i>	Snow peas	35	Lofdahl <i>et al.</i> , 2009
Norway and Denmark	2009	<i>Shigella</i>	Snow peas	33	EFSA Panel on Biological Hazards, 2013
USA	2010	<i>E. coli</i> O145	Lettuce	26	CDC, 2010a
USA	2010	<i>Salmonella</i>	Alfalfa sprouts	44	CDC, 2010b
USA	2010	<i>L. monocytogenes</i>	Fresh-cut produce (celery)	10 (5)	FDA, 2010
USA	2011	<i>Salmonella</i>	Alfalfa and mixed sprouts	140	CDC, 2011b
Denmark	2011	<i>Salmonella</i>	Tomatoes	43	EFSA Panel on Biological Hazards, 2013
USA	2011	<i>Salmonella</i>	Cantaloupe	20	CDC, 2011c
USA	2011	<i>Salmonella</i>	Papaya	106	CDC, 2011d

Denmark	2011	<i>E. coli</i> O27:H30	Snow peas	87	EFSA Panel on Biological Hazards, 2013
Europe	2011	<i>E. coli</i> O104:H4	Fenugreek sprouts	4075 (50)	EFSA, 2011
USA	2011	<i>L. monocytogenes</i>	Cantaloupe	146 (31)	CDC, 2011f
USA	2011	<i>E. coli</i> O157:H7	Strawberries	15 (1)	FDA, 2011
Germany	2011	<i>Salmonella</i>	Melons	17	EFSA Panel on Biological Hazards, 2013
USA	2011	<i>Salmonella</i>	Alfalfa sprouts	25	CDC, 2011e
USA	2011	<i>E. coli</i> O157:H7	Lettuce	60	CDC, 2011a
USA	2012	<i>E. coli</i> O26	Raw clover sprouts	29	CDC, 2012d
USA	2012	<i>E. coli</i> O157:H7	Spinach and salad	33	CDC, 2012c
USA	2012	<i>Salmonella</i>	Mango	127	CDC, 2012a
USA	2012	<i>Salmonella</i>	Cantaloupe	261 (3)	CDC, 2012b
USA	2013	<i>Salmonella</i>	Cucumbers	84	CDC, 2013a
USA	2013	<i>E. coli</i> O157:H7	Ready-to-eat salads	33	CDC, 2013b
USA	2014	<i>Salmonella</i>	Sprouted Chia powder	25	CDC, 2014a
USA	2014	<i>E. coli</i> O121	Raw clover Sprouts	19	CDC, 2014b

Updated from Olaimat and Holley (2012).

Shigella on peas and carrots (Table 4.1). Commodities most frequently associated with outbreaks include salad vegetables, lettuce, spinach, cantaloupe, cucumber, mamey, pawpaw corn, beans, peppers, soy, cabbage, raw watercress, radish, and onions (Ramos *et al.*, 2013). Considerable attention has been focused on food safety risks associated with leafy green vegetables due to recurrent outbreaks reported globally. *Salmonella* spp. and *E. coli* O157:H7 are the leading pathogens associated with food-borne illnesses caused by leafy green vegetables (WHO/FAO, 2008) and it is worth noting that ruminants, and particularly cattle, are the main reservoir for *E. coli* O157 (Hancock *et al.*, 2001), while birds including poultry are the main reservoir for *Salmonella* spp. (Vandeplas *et al.*, 2010).

Contamination of fresh produce may occur in the production environment, at harvest or during post-harvest operations such as washing, cutting, slicing, and packaging (Olaimat and Holley, 2012; Gil *et al.*, 2015). It has been reported that 47% and 32% of fresh produce outbreaks that occurred in Europe and the US between 1990 to 2009 were due to contamination during production, and that 10% and 6% of outbreaks, respectively, resulted from improper handling in food service establishments. Further, 7.5% and 3% of outbreaks in Europe and the US, respectively, were due to contamination during the processing of fresh produce. Unfortunately, the sources of contamination responsible for the remaining produce outbreaks were unknown (Soon *et al.*, 2012).

Fruits and vegetables are among the most perishable foods in the marketplace. Due to their high water activity, neutral to slightly acidic pH, and nutritional complexity, plant tissues can sustain the growth of many species of bacteria, yeasts, and molds. In addition, plant tissues can provide suitable habitats or transient niches for a range of human pathogens including bacteria (*Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes*, non-O157 STEC *E. coli*, *Shigella* spp., *Campylobacter* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Yersinia enterocolitica*), protozoa (*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Giardia* spp., *Entamoeba histolytica*), and

viruses including adenoviruses, enteroviruses, noroviruses, rotaviruses (Sivapalasingam *et al.*, 2004; Olaimat and Holley, 2012). The number and type of specific microorganisms present at harvest vary significantly depending on commodity, origin, growing practices, climate, factors associated with harvest, transportation, further processing, and subsequent handling (Brandl, 2006; Warriner *et al.*, 2009; Warriner and Namvar, 2010; Olaimat and Holley 2012).

In the absence of reliable and cost-effective means to decontaminate fresh produce, the prevention of contamination remains the focus of risk mitigation strategies. The low infectious doses of pathogens known to be transmitted via fresh produce and their ability to survive in agricultural, crop, or food environments underscore the importance of strategies to prevent contamination. However, sources of contaminants in the production environment are highly variable due to the range of geographies, climatic conditions, agricultural inputs, and technologies used to grow and harvest fruits and vegetables (WHO/FAO, 2008; Gil *et al.*, 2015; Strawn *et al.*, 2013a).

4.2 The production environment

Horticultural production is increasingly located near urban areas or animal production due to rapid population growth and the diminishing availability of arable land. Mitigating risks of contamination is challenging where fresh produce is grown in proximity to potential sources of microbiological hazards (Brandl, 2006). Pathogens originating in the digestive tract, respiratory system, skin, feet, hair, or feathers of animals may be transmitted to crops by direct or indirect routes (Liu *et al.*, 2013). Livestock, domestic animals, and wildlife can actively shed pathogens such as *E. coli* O157:H7, *Salmonella*, *Campylobacter*, or *Listeria* (Harris *et al.*, 2003; FDA, 2013). Consequently, animal intrusion in the production environment can result in the direct contamination of crops. While practical measures

to prevent access by livestock are available the movement of wild animals including birds is far more difficult to control (Harris *et al.*, 2003; Gil *et al.*, 2015). In labour intensive production systems the risk of direct transfer through human contact may be significant, necessitating close monitoring of worker health and sanitation (Gil *et al.*, 2015). Dissemination of stored animal or human wastes to farmland during flooding events has also contributed to historical outbreaks of food-borne illness (Davies *et al.*, 2008; Warriner *et al.*, 2009; Tirado *et al.*, 2010; Liu *et al.*, 2013). The latter is an example of indirect transmission of a pathogen by carrier-mediated transport from a reservoir. Other indirect routes of transmission have been examined in an effort to assess crop production risks associated with agricultural practices and inputs (WHO/FAO, 2008).

Weather conditions can also influence the probability that pathogens from animal reservoirs will be transmitted to the plant environment and to crops (Liu *et al.*, 2013). For example, surface water runoff containing *E. coli* O157:H7 contaminated a treated municipal water supply in Canada in 2000 and although crops did not appear to be involved, this led to 1286 human cases of illnesses. The surface water had become contaminated by cattle manure originating from a cluster of 13 nearby livestock farms (Public Health Agency Canada, 2000), and shows the vulnerability associated with weather events.

Changes in temperature can also affect fresh produce contamination in those circumstances where higher temperatures of water and air facilitate growth of food-borne pathogens (D'Souza *et al.*, 2004; Tirado *et al.*, 2010; Liu *et al.*, 2013). Increased precipitation on land at higher temperature was shown to increase the moisture content of the atmosphere by 7% per 1°C (Trenberth *et al.*, 2005), and consequently to increase the soil moisture content. These conditions may enhance survival of pathogens in both soil and manure (Warriner *et al.*, 2009). Work cited above has shown that the net level of produce contamination by bacteria is positively influenced by moisture accumulation (rainfall,

floods, irrigation, and soil splashes) and moderated by lethal effects of solar UV irradiation and nutrient limitation at the plant surface.

4.3 Soil

Soil is a natural environment for spore-forming pathogens such as *B. cereus*, *C. botulinum*, and *C. perfringens* as well as non-spore-forming pathogens like *Aeromonas* and *L. monocytogenes* (Warriner *et al.*, 2009). Survival of pathogens in soil depends on several factors including soil type, temperature, moisture, presence of plants, exposure to sunlight, and competition with endogenous organisms. Holley *et al.* (2006) found that high soil moisture and addition of hog manure slurry increased survival of a 6 serovar *Salmonella* cocktail (Agona, Hadar, Heidelberg, Montevideo, Oranienburg, and Typhimurium) in clay soil to >180 days during exposures to a sequence of summer–winter temperatures (days 1–45 at 25°C, days 46–90 at 10°C, days 91–135 at 4°C, followed by days 136–180 at –18°C). In contrast, Garcia *et al.* (2010) found that the addition of dairy cow manure reduced the survival of *S. Typhimurium* at 5 and 15°C, while there was no significant difference at 25°C. Nyberg *et al.* (2010) indicated that survival of *S. Typhimurium* and *E. coli* O157:H7 was significantly influenced by manure type when added to sandy soil. The decimal reduction times of *S. Typhimurium* and *E. coli* O157:H7 were 1.7 and 2.8 days, respectively, in clay soil amended with human urine, 5 and 3.6 days, respectively, with cattle slurry, or 9.7 and 13.5 days, respectively, with poultry manure. Overall, these values were slightly higher in sandy loam soil (Nyberg *et al.* 2010). Lang and Smith (2007) reported that *E. coli* O157:H7 survived in loam and clay soils for 175 days, but in sandy soils for only 56 days.

In general, survival of pathogens is greater in moist rather than dry soil. *S. Typhimurium* was reduced by only <2.0 log cfu/g in moist soil by 45 days at 20°C (Guo *et al.*, 2002). Ongeng *et al.* (2011) reported that *S. Typhimurium* and *E. coli*

O157:H7 survived for 98 and 77 days, respectively, in manure-amended soil at a moisture level of >80% compared to 70 and 56 days, respectively, in manure-amended soil protected from rainfall. Jiang *et al.* (2002) also reported that *E. coli* O157:H7 survived for 77, >226, and 231 days in manure-amended autoclaved soil (1 part manure: 50 parts autoclaved soil) at 5, 15, and 21 °C compared to 56, 109, and 174 days in similarly mixed manure-amended unautoclaved soil at the same temperatures, respectively. Bacterial predation by protozoa may have influenced this result.

Temperature is a critical factor affecting the survival of pathogens in soils where lower temperatures increase the survival of pathogens. The reduction in *S. Typhimurium* numbers in soil or cow manure-amended soil was higher at 23 to 25 °C compared to 5 to 15 °C (Garcia *et al.*, 2010; Semenov *et al.*, 2009). *E. coli* O157:H7 and *L. innocua* survived in soil amended with inoculated compost or sprinkle- and surface-irrigated with inoculated water for >63 days in fall and spring. However, a lower reduction was observed in the fall as a result of differences in temperature and humidity, which averaged 10 °C and 82%, respectively, in the fall and 17 °C and 62%, respectively, in the spring (Oliveira *et al.*, 2011, 2012).

The risk of pathogen transfer from soil to fresh produce in the field is influenced by the concentration of the pathogen in the soil and the distance from the soil to the edible parts of the plant. The use of manure-amended soil when germinating seeds can be an important route for transfer of pathogens to fresh produce. Semenov *et al.* (2010b) found that *S. Typhimurium* and *E. coli* O157:H7 colonized the rhizosphere and phyllosphere of cress and oat seedlings after seeds germinated in the soil were mixed with manure contaminated with either pathogen. Interestingly, numbers of *S. Typhimurium* or *E. coli* O157:H7 in the rhizosphere and phyllosphere of 5 day-old cress or 7 day-old oats were higher than those at the time of sowing cress or oat seeds, although the plants were not irrigated during this period (Semenov

et al., 2010b). *S. enterica* was able to colonize tomato plants from seedlings germinated in inoculated soil. Although *S. enterica* numbers on tomato leaves did not decrease during the first 5 weeks, numbers were significantly lower in the soil (Barak and Liang, 2008). Oliveira *et al.* (2011, 2012) observed in the spring and fall that *E. coli* O157:H7 and *L. innocua* were able to transfer from soil amended with inoculated compost to the inner and outer leaves of lettuce.

4.4 Fertilizers derived from animal wastes

Fertilizers derived from animal wastes, including raw manures, slurries, and their composts, are widely used in the production of food crops. The presence of zoonotic pathogens including *E. coli* O157:H7, *Salmonella* spp., and *Campylobacter* spp. and *L. monocytogenes* in manure in different countries has been reviewed by Olaimat and Holley (2012). It is worth noting that municipal wastewater and sewage sludge may contain a variety of pathogens including *Salmonella* sp., *Shigella* sp., *Yersinia* sp., *V. cholerae*, *C. jejuni*, and *E. coli* O157:H7 (Gerba and Smith 2005). Although manure slurries contain less dry matter, lower pH, less nitrogen, more dissolved organic matter, and have a less diversified microbial community than manure, they can also be important point sources of produce contamination (Semenov *et al.*, 2011).

Pathogen survival in slurry or manure varies significantly with temperature, aeration, and holding time, dry matter content, pH, and bacterial concentration (Ingram and Millner, 2007). *E. coli* O157:H7 survived in high moisture content bovine manure >70 days at 5 °C compared to 49 days at 30 °C (Semenov *et al.*, 2007). In another study, *E. coli* O157:H7 survived in bovine feces for 42 to 49 days at 37 °C, for 49 to 56 days at 22 °C, and for 63 to 70 days at 5 °C (Wang *et al.*, 1996). The decimal reduction time of a *Salmonella* cocktail containing *S. Typhimurium*, *S. Agona*, *S. Hadar* and *S. Oranienburg* in sow, feeder, and nursery

barn slurry stored at 37, 25, and 4°C ranged from 0.9 to 1.4 days, 8 to 19 days, and 22 to 60 days, respectively (Arrus *et al.*, 2006). Kudva *et al.* (1998) also found that *E. coli* O157:H7 survived for 47, 120, and 630 days in manure heaps of bovine, aerated ovine, and non-aerated ovine manure, respectively. Ongeng *et al.* (2011) found that *S. Typhimurium* and *E. coli* O157:H7 survived for 84 and 63 d, respectively, in manure at fluctuating temperatures (16 to 42°C) and a moisture level of >80% relative humidity. *S. Typhimurium* and *S. Derby* survived for 34 and 23 days, respectively, in pig slurry in the summer, and 58 and 46 days, respectively, in the winter (Mannion *et al.*, 2007). You *et al.* (2006) found that *S. Newport* survived for 184, 332, and 405 days in manure, manure-amended non-sterilized soil, and manure-amended sterilized soil, respectively. Anaerobic conditions may enhance the ability of pathogens to survive in manure. *E. coli* O157:H7 survived for 12 and 19 days in aerobic manure and slurry, respectively, compared to 184 and 46 days in anaerobic manure and slurry, respectively, at 16°C (Semenov *et al.*, 2011).

Hutchison *et al.* (2005) found that pathogens survived in fresh waste (slurries, dirty water) in the order *Campylobacter* < *Listeria* < *Salmonella* < *E. coli* O157. Nicholson *et al.* (2005) investigated the survival of *Salmonella*, *E. coli* O157:H7, *Listeria*, and *Campylobacter* in solid or liquid manures (dairy and pig manure solids, broiler litter, dairy slurry at 7% and 2% dry matter, and dirty water) and found that survival varied from 2 to 32 days in the order of *Campylobacter* < *Listeria* < *Salmonella* < *E. coli* O157:H7 in solid manure, and *Salmonella* < *Listeria* < *E. coli* O157:H7 < *Campylobacter* in liquid manure and dirty water. However, composting can effectively reduce pathogens commonly found in manure including those that have acquired abilities to resist environmental stresses such as acid tolerance by mutation (Warriner *et al.*, 2009). Millner (2003) found that in composting manure at 55°C for 3 days in an aerated heap or in a windrow heap that had been turned regularly to reach 55°C over 14 days, numbers of

E. coli and *Salmonella* were reduced by 6 log cfu/ml. In another study, *E. coli* O157:H7 was reduced by 4 log cfu/g in autoclaved commercial cow manure compost with a moisture content of 38% at 50, 55, 60, 65, and 70°C for 480, 180, 15, 2, and <1 min, respectively. Furthermore, less time was required to reduce the pathogen in unautoclaved compost than in autoclaved compost at 65 and 70°C (Jiang *et al.*, 2003), where differences may have been due to bacterial predation by protozoa and competition for nutrients.

It is evident that adequate composting of manures prior to field application can substantially reduce periods during which these pathogens would otherwise survive, remain viable, and pose a threat to the safety of produce. Nevertheless, the use of raw manures as fertilizers remains common in many jurisdictions. From a practical perspective, the means to determine the time required for pathogens to reach safe levels in manures, composts, or amended soils are essential for the formulation of guidelines to minimize the risk of transfer during crop production. To this end, mathematical modeling may be used to anticipate the fate of pathogens against the multiplicity of interacting factors that influence their behavior in these inherently complex biological systems. For example, Semenov *et al.* (2010a) developed a simulation model termed "COLIWAVE", which predicts the behaviour of *Escherichia coli* O157:H7 in manure and manure-amended soil in response to changes in temperature, oxygen concentration, substrate content, and competition by autochthonous microbial communities. The model can simulate *E. coli* O157:H7 survival under different manure/soil management strategies and may be used to examine the consequences of potential control strategies.

4.5 Irrigation

Large-scale production of fresh produce requires some form of irrigation during the growing season. Irrigation water has been

reported as a major potential risk factor for the contamination of fresh produce during production (Brandl, 2006; Pachepsky and Shelton, 2011). Greene *et al.* (2008) reported the occurrence of a multistate outbreak of *S. Newport* infection associated with tomatoes irrigated with contaminated pond water in the US. In addition, *S. Saintpaul*, which was isolated from irrigation water and from Serrano peppers, was linked to a large salmonellosis outbreak in the US and Canada in 2008 (CDC, 2008b). Furthermore, Söderström *et al.* (2008) reported that an *E. coli* O157:H7 outbreak in Sweden was caused by the consumption of lettuce irrigated with water from a small stream. Identical strains were isolated from the patients and cattle at a farm upstream from the irrigation point. Another *E. coli* O157:H7 outbreak, also associated with shredded lettuce in the US, was caused by accidental mixing of irrigation well water with water from a dairy manure pond (USFDA and California Food Emergency Response Team, 2008). Although not associated with a documented illness outbreak, Castro-Rosas *et al.* (2012) isolated *E. coli* from 85% (110/130) of samples and diarrheagenic *E. coli* from 8 samples of ready-to-eat salads purchased from restaurants in Pachuca-City, Mexico, where untreated sewage water was used for irrigation.

Irrigation water is susceptible to direct contamination from sewage or manure spills. Sources of irrigation water have been ranked in terms of increasing risk of microbial contamination in the following order: potable or rain water, groundwater from deep wells, groundwater from shallow wells, surface water, and finally raw or inadequately treated wastewater (Leifert *et al.*, 2008). Recycling of municipal wastewater for irrigation of produce has been practiced in a number of countries such as Australia, Germany, Spain, the Netherlands, and the US (Warriner *et al.*, 2009) and this may increase the risk of produce contamination by pathogens. Survey studies showed the presence of pathogens including *E. coli* O157:H7, *Salmonella*, *Campylobacter*, *L. monocytogenes*, and *Vibrio* in irrigation water in different

countries (Table 4.2). The level of contamination by these pathogens varied significantly and depended on the source of water. *Salmonella* and *E. coli* O157:H7 are the predominant pathogens that may contaminate water and their prevalence ranged from 3 to 79% and 1 to 28%, respectively, based on the surveys reported in Table 4.2.

Wild birds, pests, and other mammals are most likely to be involved in produce contamination in the field by direct contact or indirectly by way of the irrigation water (Kruse *et al.*, 2004; da Cruz *et al.*, 2006). In general, food-borne pathogens including *E. coli* O157:H7 and *Salmonella* have the ability to survive for long periods in water, although numbers of pathogen and indicator organisms in water usually decrease with time and declines are faster at higher temperatures (Table 4.3). *E. coli* O157 survived in sterile water for 91 days at 8°C (Wang and Doyle, 1998), while *Salmonella* survived in sterile water for 152 days at 18°C (Mitscherlich and Marth, 1984). *E. coli* O157:H7 survived in water from different rivers for 32 to 51 days at 6°C and 21 to 32 days at 24°C. However, the survival period increased when *E. coli* O157:H7 was inoculated in muddy water and reached 49 to 65 days at 6°C and 26 to 60 days at 24°C (Czajkowska *et al.*, 2005). Vital *et al.* (2008) found that *E. coli* O157 grew in sterile fresh water at low carbon concentrations at 30°C.

Food-borne pathogens transmitted from irrigation water to produce remain viable for variable periods of time depending on the environmental challenge (Delaquis *et al.*, 2007). Pathogens may reach the edible parts of produce through the root system (Bernstein *et al.*, 2007a, 2007b) or via the stomata, scar tissue, or wounds as a consequence of irrigation water contacting leaf surfaces or from raindrop splashes from the soil surface (Kroupitski *et al.*, 2009; Mitra *et al.*, 2009). Guo *et al.* (2001) reported that *Salmonella* migrated from soil directly into the stem scar tissue of green tomatoes. *E. coli* O157:H7 and *L. innocua* were transferred from inoculated sprinkle-applied

Table 4.2 Prevalence of food-borne pathogens in different water sources.

Year	Country	Water source	Pathogens	Prevalence (%)	Reference
2005–2006	USA	Surface water	<i>Salmonella</i>	57/72 (79)	Haley et al., 2009
2008–2010	USA	Streams, ponds	<i>Salmonella</i>	6/96 (6)	Benjamin et al., 2013
2008–2010	USA	Streams, ponds	Generic <i>E. coli</i>	199/255 (78)	Benjamin et al., 2013
2008–2010	USA	Streams, ponds	<i>E. coli</i> O157:H7	4/256 (2)	Benjamin et al., 2013
2011–2013	USA	Lakes, rivers, streams, ponds	<i>E. coli</i> O157:H7	110/1386 (8)	Cooley et al., 2014
2011–2013	USA	Lakes, rivers, streams, ponds	<i>Salmonella</i>	908/1405 (65)	Cooley et al., 2014
2011–2013	USA	Lakes, rivers, streams, ponds	<i>L. monocytogenes</i>	576/1405 (43)	Cooley et al., 2014
2011–2013	USA	Lakes, rivers, streams, ponds	Non-O157 <i>E. coli</i>	110/1405 (11)	Cooley et al., 2014
2011–2012	USA	Ponds	<i>C. jejuni</i>	54/280 (19)	Gu et al., 2013
2012	USA	Surface water	<i>L. monocytogenes</i>	22/74 (30)	Strawn et al., 2013b
2012	USA	Surface water	<i>Salmonella</i>	8/74 (11)	Strawn et al., 2013b
1999–2000	Canada	Surface water	<i>E. coli</i> O157:H7	13/1483 (1)	Johnson et al., 2003
1999–2000	Canada	Surface water	<i>Salmonella</i>	88/1429 (6)	Johnson et al., 2003
2000–2001	Canada	River, irrigation canal water	<i>E. coli</i> O157:H7	27/1608 (2)	Gannon et al., 2004
2001	Canada	River, irrigation canal water	<i>Salmonella</i>	166/1629 (10)	Gannon et al., 2004
2003–2005	Canada	Rivers, creeks	<i>Salmonella</i>	91/116 (78)	Thomas et al., 2013
2006	Netherlands	Surface water	<i>Salmonella</i>	7/49 (14)	Heuvelink et al., 2008
2006	Netherlands	Surface water	<i>Campylobacter</i>	14/49 (29)	Heuvelink et al., 2008
2006	Netherlands	Surface water	<i>E. coli</i> O157:H7	1/49 (2)	Heuvelink et al., 2008
2011–2012	Belgium	Irrigation water	Generic <i>E. coli</i>	71/120 (59)	Holvoet et al., 2013
2008	Australia	Ponds, creeks	<i>C. jejuni</i>	8/32 (25)	Ahmed et al., 2009
2008	Australia	Ponds, creeks	<i>Salmonella</i>	1/32 (3)	Ahmed et al., 2009
2008	Australia	Ponds, creeks	<i>E. coli</i> O157:H7	9/32 (28)	Ahmed et al., 2009
2002	Nigeria	River	<i>E. coli</i> O157:H7	2/96 (2)	Chigor et al., 2010
2003	Nigeria	Irrigation water	<i>Salmonella</i>	16/196 (8)	Okafo et al., 2003
2003	Nigeria	Irrigation water	<i>Vibrio</i>	41/196 (21)	Okafo et al., 2003
2003	Nigeria	Irrigation water	Generic <i>E. coli</i>	45/196 (23)	Okafo et al., 2003
2007	Egypt	River, canal, wells, drains sewage, underground water	Generic <i>E. coli</i>	14/50 (28)	El-Jakee et al., 2009

Table 4.3 Survival of *E. coli* O157 and *Salmonella* in water from different sources.

Pathogen	Water source	Temperature	Survival	Reference
<i>Salmonella</i>	Sterile municipal water	23	≥45 days	Santo-Domingo et al., 2000
	River water	23	≥45 days	Santo-Domingo et al., 2000
	Sterile well water	18	152 days	Mitscherlich and Marth, 1984
	Saline water	4	121	Thomas et al., 2013
	Lake fresh water and brackish water	20 to 30	≥34	Chandran et al., 2013
<i>E. coli</i> O157:H7	Fresh water	23	54	Moore et al., 2003
	Sterile municipal water	8	91 days	Wang and Doyle, 1998
	Sterile municipal water	25	49 days	Wang and Doyle, 1998
	Well water and filtered well water (0.3 µm filter)	15	≤64	Artz and Killham, 2002
	Filtered well water (0.2 µm filter) and autoclaved well water	15	≥64	Artz and Killham, 2002
	Lake water, faecal contaminated puddle water, river water, and animal drinking trough water	10	≥64	Avery et al., 2008
	Farm water (stored outdoor in the field)	-2 to 15	14	McGee et al., 2002
	Farm water (stored indoor in the lab)	15	31	McGee et al., 2002
	Fecal contaminated farm water (stored outdoor in the field)	-2 to 15	24	McGee et al., 2002
	Sterile distilled municipal water (stored indoor in the lab)	15	>31	McGee et al., 2002
	Sterile distilled municipal water (stored outdoor in the field)	-2 to 15	17	McGee et al., 2002
	Sterile deionized water	4	210	Dinu and Bach, 2011

and surface water to the inner and outer leaves of lettuce in fall and spring (Oliveira *et al.*, 2011, 2012). *S. enterica* colonized tomato plants from seedlings irrigated with inoculated water, and the highest numbers of the pathogen were found in tomato plants when exposed to contaminated irrigation water than when exposed to contaminated soil (Barak and Liang, 2008).

The methods used for application of irrigation water can affect the presence of pathogens on fresh produce. Commonly used methods include gravity (flood) irrigation, spray, drip, and subsurface irrigation. However, flood and spray irrigation represent the greatest risk for fresh produce contamination where the water directly contacts the edible parts of crops (FDA, 1998). It has been found that 90% of lettuce plants irrigated with water containing 7.0 log cfu/ml *E. coli* O157 using spray irrigation were contaminated with *E. coli* O157, while only 19% of plants were contaminated using flood irrigation (Solomon *et al.*, 2002). Greater numbers of pathogens in irrigation water increase the potential for produce contamination. Erickson *et al.* (2008) found a positive relationship between the *E. coli* O157:H7 level in irrigation water and its prevalence on spinach. No *E. coli* O157:H7 was detected on spinach plants spray-irrigated with water containing 2.0 log cfu/ml *E. coli* O157:H7; however, the pathogen was detected on spinach that was irrigated with water containing 4.0 log cfu/ml *E. coli* O157:H7 (Erickson *et al.*, 2008). In another study, *Salmonella* was not detected on spinach plants irrigated for 6 weeks with water containing 3.0 log cfu/ml *Salmonella*, but it was found at 4.0 log cfu/g when plants were irrigated with water containing 6.0 log cfu/ml (Patel and Darlington, 2010). When rocket salad plants were irrigated with water containing 5.6, 6.6, or 7.6 log cfu/ml *E. coli* O157:H7, 58.5, 67.4, and 73.4%, respectively, of *E. coli* O157:H7 cells were transferred from irrigation water on to rocket leaves after 72h (Alam *et al.*, 2014). It is likely that lower levels of pathogens in irrigation water result in a lower prevalence of produce contamination. On the other hand, Mootian *et al.* (2009) found that

30% of lettuce irrigated with water containing 1.0 or 2.0 log cfu/ml *E. coli* O157:H7 for 15 days became contaminated. It was suggested that for produce contamination the pathogen level in irrigation water may not be as important as the ability of the specific microorganism to internalize and colonize the plant (Pachepsky and Shelton, 2011).

Although food-borne pathogens may contaminate plant surfaces by irrigation water, the adherence to and internalization of pathogens within produce is both strain and plant specific (Pachepsky and Shelton, 2011). Guo *et al.* (2002) found that there were significant differences in the ability of *Salmonella* serotypes to survive on and in tomato plants (surface, stem scar tissue, pulp). Through quantitative risk assessment models, Hamilton *et al.* (2006) found that contamination risk associated with the use of reclaimed water varied among crops. Lettuce, broccoli, and cabbage were found to pose a higher risk than cucumbers. In another study, *E. coli* and *C. perfringens* were detected on the surfaces of cantaloupe and lettuce irrigated with contaminated water, but not on similarly treated bell peppers (Song *et al.*, 2006). It seems that edible parts of plants grown at the ground surface, such as lettuce and parsley, were more often internally contaminated with pathogens than plants that grew higher above the soil surface like tomatoes (Melloul *et al.*, 2001).

Predictive water quality models are widely used to anticipate the dissemination of pollutants within defined watersheds using spatially variable governing factors, such as land use, soil conditions, and distance to hydrologic features. The reader is referred to the review by Parajuli and Ouyang (2013) for a description of modeling approaches used for this purpose. The most widely employed to date is the watershed-scale, physically-based, and continuous-time Soil and Water Assessment Tool (SWAT), which has been used to simulate the fate and transport of fecal indicators (primarily fecal coliforms or *E. coli*) from non-point sources in agricultural environments (Parajuli *et al.*, 2009; Kim *et al.*, 2010). SWAT models distinguish the

effects of multiple factors including weather, surface runoff, evapotranspiration, crop growth, nutrient loading and water routing, and long-term consequences of varying agricultural management practices such as grazing operations or manure application. A bacterial transport subroutine within SWAT considers the effect of temperature or bacterial growth or die-off and the model can be applied to the estimation of pathogen loading at specific points, such as irrigation water intake. A SWAT model was employed to characterize factors that influence the prevalence of specific pathogens (*E. coli* O157:H7, *Salmonella*, *Campylobacter*, *Cryptosporidium*, and *Giardia*) in a recent study performed in a Canadian agricultural watershed (Frey *et al.*, 2013).

4.6 Harvesting and handling

Harvesting is a critical period in the fresh produce chain for product contamination because it links field production to postharvest operations. Fresh produce may be harvested mechanically or by hand (WHO/FAO, 2008). The delicate nature of some leafy vegetables and herbs leads easily to damage and bruising, which cause nutrient leaching and facilitate growth of pathogens (Brandl and Mandrell, 2002; WHO/FAO, 2008). In addition, mechanical injury of plant tissues can expose internal surfaces to microbial contamination and growth (Bartz *et al.*, 2013).

Operations including sorting, trimming, washing, drying, grading, packing, precooling and storage may take place in the open field or in a packing house normally located on or adjacent to horticultural plots, and this may lead to edible plant material being exposed to dust and wildlife carrying food-borne pathogens (WHO/FAO, 2008). Johnston *et al.* (2006) isolated *L. monocytogenes* from 7% (3/43) of cabbages entering packing houses. Prazak *et al.* (2002) also found 4.8% (6/125) of cabbages were positive for *L. monocytogenes* on arrival at the packing house. The presence of pathogens on

produce at harvest is related to the risk of exposure during preharvest plant growth from contact with soil, water, and fertilizer.

The harvested produce may be hand sorted and may undergo some minor processing such as removal of the outer wrap leaves or be subjected to coring, washing, or spraying. Fresh produce may be placed in bulk containers or packed ready for dispatch in boxes or covered with plastic film (WHO/FAO, 2008; EFSA, 2011). During these steps, employees, soil, equipment, cold storage facilities, packaging materials, and water may cause contamination (Gorny, 1999; James, 2006; WHO/FAO, 2008). Direct contact by workers in the field using contaminated utensils and tools or physical damage to products by handling equipment can contaminate fresh produce. Field containers may also contribute pathogens from mud, industrial lubricants, metal fasteners, or splinters. Workers infected with hepatitis A or those who have nausea, vomiting, or diarrhea while in the fields are likely to transfer pathogens to the harvested produce (Gorny, 1999; James, 2006). The health status and hygienic practices of employees involved in field preparation, planting, weeding, and pruning, as well as harvesting, will influence product contamination (James, 2006; Gil *et al.*, 2015). Poor hygiene practices of farm workers and handlers were suspected to be the route of contamination in investigations of several food-borne outbreaks associated with fresh produce (De Roeve, 1998; Harris *et al.*, 2003; Erickson, 2010). In particular, with outbreaks caused by viruses, the potential role of workers in contamination of fresh produce has been highlighted (Gorny, 1999; Soon *et al.*, 2012) and frequently employees in retail foodservice establishments who wash their hands improperly or at inappropriate times during preparation and serving of food have been implicated. In a hand washing study, it was found that when employees washed their hands they only used 1 or 2 steps of the acceptable 3-step hand washing procedure described in the 2005 Food Code (Paez *et al.*, 2007). Since coliforms can occur naturally on plant materials, they have limited value as a

sanitation indicator. More appropriately for these products, fecal coliforms are used as an indicator of animal and human fecal contamination. Johnston *et al.* (2005) isolated coliforms from leafy greens, herbs, cantaloupe, and other vegetables at different stages of production and processing with numbers from 2.1 to 4.3 log cfu/g. *L. monocytogenes* and *E. coli* O157:H7 were not detected in any of the 398 produce items tested; however, *Salmonella* was detected on 3.3% of cantaloupe samples. In this report the observation that with some vegetables (cilantro and parsley) coliform numbers increased twofold during the packing process was useful in improving the process.

The microbial safety of fresh produce is highly dependent on preharvest factors and the conduct of the harvesting process itself. Controls must be put in place to minimize contamination during harvesting by soil, water, workers, and harvesting equipment. Proper hygienic procedures are a crucial factor that influences the safety of fresh produce and the health status of employees should be monitored.

4.7 Postharvest processing

Fresh produce may be exposed to microbial contamination during any of the processing operations used (grading, separating, coring, cutting, slicing, shredding, chopping, washing, sanitizing, chilling, and packaging). Produce can be contaminated by contact with food workers, surfaces in the facility including containers, conveyors, equipment, and utensils or other contaminated produce during processes of grading, sorting, and cutting, or by the water used in washing. However, other steps such as chilling and packaging may arrest bacterial growth and afford some protection against additional contamination, while sanitizing can reduce bacterial numbers (FDA, 2013; WHO/FAO, 2008).

Danyluk and Schaffner (2011) generated Monte Carlo simulations to model the risk

associated with spinach processing steps using data from the 2006 US outbreak involving *E. coli* O157:H7 that resulted in more than 200 illnesses. When it was assumed that *E. coli* present on the spinach had exclusively come from the field, the model predicted temperature abuse could have yielded a 1 log cfu/day increase in its numbers, and if present initially at 0.1% of samples, would have been adequate to have caused the outbreak. Therefore, better temperature control would have led to a different outcome.

Cross-contamination between fresh produce and equipment surfaces during cutting and preparation has been demonstrated. For example, contaminated shredding equipment, water baths, processing water, and food preparation environments were identified as sources of contamination in several salmonellosis outbreaks (Stafford *et al.*, 2002). When *S. Bovismorbificans* caused an outbreak in 2001 in Australia linked to shredded lettuce, the organism was detected on cutting blades used in processing and this confirmed that the shredding equipment was the source of *Salmonella* contamination (Stafford *et al.*, 2002). Another study examining cross-contamination during preparation of chicken salads found *Campylobacter* strains in 29% of food preparation events where the organisms were isolated from the salads, cleaning materials, and food-contact surfaces. Molecular typing results showed that the same *Campylobacter* strains found in the prepared chicken salads were also isolated from raw chicken pieces, and since the chicken was adequately cooled, results indicated that microbial transfer during food preparation had taken place (Redmond *et al.*, 2004). Pérez-Rodríguez *et al.* (2011) developed a cross-contamination simulation model to estimate the number of contaminated packaged units resulting from the introduction of lettuce contaminated with *Escherichia coli* O157:H7 in a fresh-cut lettuce processing line. The model enables predictions for different initial pathogen concentrations and considers the impact of interventions including pathogen testing,

chlorination, irradiation, and cleaning and disinfection procedures on the extent of cross-contamination. Puerta-Gomez *et al.* (2013) developed a quantitative risk assessment model, which indicated that for low (1 cfu/g) levels of cross-contamination, sanitizing steps including chlorine washing of baby spinach would be an effective method for hazard control. However, at *Salmonella* concentrations of more than 3 cfu/g, these steps would be inadequate and alternate processing techniques such as irradiation should be considered.

Food processing environments may provide an opportunity for microorganisms from raw materials to access moisture and nutrients sufficient to allow biofilm formation. Biofilms can be aggregates of single or multiple types of bacteria, fungi, algae, or protozoa enclosed in a self-produced extracellular polymeric matrix that are attached to a biotic or abiotic surface (Jacques *et al.*, 2010; Vogeeler *et al.*, 2014). Food-borne pathogens such as *Salmonella*, *E. coli* O157:H7, *Listeria*, *Pseudomonas*, and *Bacillus* spp. may colonize surfaces of food processing equipment. When present as one component of a mixture of organisms, inactivating or removing them by water washing or effectively treating them with a disinfectant or sanitizer is made more difficult because the cells are protected from these exposures (Carpentier and Cerf, 1993). Similarly, studies have shown that bacteria may form biofilms on the surfaces of a wide variety of fresh produce (Fett and Cooke, 2003; Morris and Monier, 2003). Lindow and Brandl (2003) reported that 30 to 80% of bacteria on plant surfaces exist within biofilms. There are also reports that *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* were able to form biofilms on surfaces of fresh cantaloupes, lettuce, spinach, and cilantro stored at 4 to 22 °C after 24 h (Brandl and Mandrell, 2002; Annous *et al.*, 2005; Niemira and Cooke, 2010; Olmez and Temur, 2010). It is likely that formation of biofilms on produce surfaces is a survival strategy by pathogens to deal with harsh environments including wide temperature changes, desiccation, UV, and oxidative stress.

Some pathogens enter into a viable but non-culturable (VBNC) state in which bacteria can remain in stressful environments such as at low temperatures for long periods of time and maintain their virulence potential (Dinu and Bach, 2013; Tan *et al.*, 2014). Dreux *et al.* (2007) reported that *L. monocytogenes* became VBNC on parsley leaves under dry conditions. Dinu and Bach (2011, 2013) found that the number of culturable *E. coli* O157:H7 on lettuce and spinach surfaces decreased with time and bacterial cells evolved towards VBNC within 7 to 16 days at 8 °C.

Food-borne pathogens may adhere to plant surfaces and internalize in damaged and cut tissues during production, harvesting, and processing (Olaimat and Holley, 2012; Vogeeler *et al.*, 2014). Although several studies reported that internalization is a common consequence of the contamination of produce with pathogens (Kroupitski *et al.*, 2009; Niemira and Cooke, 2010; Olmez and Temur, 2010; Saldaña *et al.*, 2011), other studies indicated that internalization does not occur frequently and depends on several factors including the type of fresh produce, the pathogen strain, contamination level, and plant maturity (Jablasone *et al.*, 2005; Mitra *et al.*, 2009; Pu *et al.*, 2009; Golberg *et al.*, 2011; Wright *et al.*, 2013). Further, other studies reported that pathogens are not able to internalize within fresh produce tissues (Sharma *et al.*, 2009; Zhang *et al.*, 2009a, 2009b; Erickson *et al.*, 2010a, 2010b).

Biofilm formation and internalization by food-borne pathogens are considered to be food safety issues because under these conditions pathogens are less likely to be removed during washing and sanitizing. Consequently, chemical sanitizers commonly used in the fresh produce industry as the main hurdle to combat contamination may not consistently eliminate this problem (WHO/FAO, 2008; Olaimat and Holley, 2012). A comprehensive overview of sanitizers and washing systems used for the disinfection of fresh or fresh-cut produce in Gómez-López (2012) clearly shows that the antimicrobial effects attainable with current

technologies are finite. For example, considerable efforts have been directed at the development of improved treatments or processes for the elimination of pathogens such as *Salmonella* and *E. coli* O157:H7 from leafy vegetables. Irrespective of sanitizer or mode of application, maximum population reductions achieved without compromising product quality generally range between 1 and 2 log cfu/g.

Washing can be done by spraying with potable water and then immersion in cold (1 to 5°C) sanitized water containing 50 to 150 ppm sodium hypochlorite acidified with 150 to 200 ppm citric acid to adjust pH between 6.5 and 7.5 to optimize the antimicrobial action of chlorine (Artes *et al.*, 2009). The use of chemical or natural antimicrobial solutions and physical treatment of water have shown promising antimicrobial activity against pathogens on fresh produce. In addition to hypochlorite (Simmons *et al.*, 2006; Baert *et al.*, 2009), chlorine dioxide (Kim *et al.*, 2008; Keskinen and Annous, 2011; Chen *et al.*, 2014), peroxyacetic acid (Martínez-Sánchez *et al.*, 2006; Baert *et al.*, 2009; Al-Nabulsi *et al.*, 2014), peracetic acid (Hellström *et al.*, 2006; Samadi *et al.*, 2009; Dai *et al.*, 2012), electrolyzed water (Fishburn *et al.*, 2012; Forghani and Oh, 2013; Gómez-López *et al.*, 2013), organic acids (Phungamngoen *et al.*, 2013; Ganesh *et al.*, 2012; Nastou *et al.*, 2012), plant essential oils (Karagözlü *et al.*, 2011; Moore-Neibel *et al.*, 2013; Todd *et al.*, 2013), plant extracts (Gomes *et al.*, 2011; Orue *et al.*, 2013; Su and D'Souza, 2013), and ozonized water (Singla *et al.*, 2011; Fishburn *et al.*, 2012; Sengun, 2013) have been evaluated.

Physical interventions including mild heat (Delaquis and Austin, 2007), irradiation (Mahmoud 2010a, 2010b; Mahmoud *et al.*, 2010; Mukhopadhyay *et al.*, 2013; Moosekian *et al.*, 2014), ultraviolet light (Birmipa *et al.*, 2013; Mukhopadhyay *et al.*, 2013), ultrasound (Elizaquível *et al.*, 2012; Alexandre *et al.*, 2013; Birmipa *et al.*, 2013), and cold plasma (Misra *et al.*, 2014; Ziuzina *et al.*, 2014) have also been examined for their ability to control food-borne pathogens on fresh produce. In general, physical

interventions were more inhibitory than chemical agents against pathogens on produce. These methods can yield a rapid and effective reduction of pathogens, do not usually affect the flavor of fresh produce, and can be conducted either as a part of the packaging process or be used after packaging, which can prevent cross-contamination. However, their disadvantages (cost, suspicion by consumers, and physico-chemical changes in produce that can result) have limited their adoption by the produce industry (Olaimat and Holley, 2012; Ramos *et al.*, 2013).

4.8 Packaging, storage, and transportation

Produce may be packaged in retail containers in the field or placed in bulk bins, baskets, or bags for transport to packaging sites. Damage caused to produce during transfer from the field to processing locations or from these sites to market may enhance internalization of pathogens (WHO/FAO, 2008). Refrigeration during produce distribution and prior to consumption is believed to decrease the risk of food-borne illness; however, pathogens with low infectious doses may remain viable and cause illness (WHO/FAO, 2008; FDA, 2013). Contamination of fresh produce during transportation and marketing may occur by contact with contaminated surfaces in vehicles and storage equipment, by the airflow during cooling and storage, or by contaminated condensate dripping from cooling equipment (James, 2006; WHO/FAO, 2008).

Packaging is frequently used to avoid water loss and maintain a high humidity during distribution and storage of fresh produce, particularly with fresh-cut fruits and vegetables. This practice positively influences the survival and growth of pathogens (Brandl and Mandrell, 2002). Usually the fresh produce industry uses modified atmosphere packaging (MAP) with low O₂ and high CO₂ concentrations to preserve the quality of fresh-cut produce

(Francis *et al.*, 2012). Growth and survival of pathogens on fresh produce after processing are influenced by the pathogen strain, produce species, temperature, relative humidity, atmosphere, and packaging (Harris *et al.*, 2003). Damage or cutting fresh produce reduces shelf-life when compared to the intact item due in part to a higher respiration rate (Francis *et al.*, 2012), which may change the proportions of O₂ and CO₂ in the packaging headspace and influence the ability of food-borne pathogens to grow. Temperature is the most important factor affecting the antimicrobial nature of package atmospheres and directly contributes to bacterial growth and survival (FDA, 2013); therefore, temperature control and maintenance of an adequate cold chain are critical to food safety. Oliveira *et al.* (2010) found that *E. coli* O157:H7 and *Salmonella* on shredded lettuce decreased 1.0 log cfu/g while *L. monocytogenes* increased 1.0 log cfu/g after 10 days at 5°C under MAP. However, numbers of the three pathogens increased between 2.4 and 4.2 log cfu/g at 3 days when stored at 25°C. Similar results were observed by Ergönül (2011), who found that numbers of *E. coli* O157:H7 and *Salmonella* on MAP shredded lettuce were reduced 1.0 log cfu/g at 7 days and 4°C, while at 20°C, *E. coli* O157:H7 increased 1.5 log cfu/g and *Salmonella* numbers remained constant. Posada-Izquierdo *et al.* (2014) reported that *E. coli* O157:H7 was able to grow at ≥8°C on MAP fresh-cut lettuce. It is evident that growth of mesophilic pathogens on fresh produce declined under refrigerated temperatures but psychrotrophic pathogens like *L. monocytogenes* were able to grow under these conditions.

The research outcomes summarized above indicate that pathogen survival in packaged produce is a dynamic process characterized by periods of temperature-induced population expansion or decline. It has been reported that temperature variation can occur during transportation and retail display (Estrada-Flores *et al.*, 2006; Estrada-Flores and Platt, 2007). Dallaire *et al.* (2006) found that the core temperature of boxed broccoli from two farms in

four retail stores located in the same geographic region varied between 2.2 and 8.2°C. McKellar *et al.* (2014) continuously recorded time and temperature data in packaged fresh-cut leafy vegetables during storage in a processing facility, transport, and storage at the point of delivery in two retail distribution chains. Temperatures were found to fluctuate at different stages along the chain and occasions where temperatures exceeded 5°C occurred occasionally during transport and retail storage. Interestingly, the overall amount of time spent at temperature > 5°C was higher during the summer season than in winter. Studies carried out by Zeng *et al.* (2014) in the US revealed that the temperature of fresh-cut lettuce in refrigerated trailers, retail storage, and display ranged between −0.3 to 7.7°C, 0.6 to 15.4°C, and −1.1 to 9.7°C, respectively. Temperatures of fresh produce in display cabinets and domestic refrigerators in Europe and the US may reach up to 16°C (EFSA, 2007).

Experimentation under conditions that simulate time and temperature scenarios at each stage along food chains can provide realistic assessments of pathogen survival, but this approach is labour intensive when a broad range of times and temperatures must be considered. Predictive models that consider the influence of several simultaneous parameters on the behavior of pathogens in packaged produce provide an alternative for the quantitative assessment of survival in dynamic food systems. The value of this approach was demonstrated by McKellar and Delaquis (2011) in the development of a combined growth/death model that predicts *E. coli* O157:H7 behavior in packaged fresh-cut leafy vegetables stored under isothermal and non-isothermal conditions. The model was used by McKellar *et al.* (2012) to simulate the fate of *E. coli* O157:H7 under time–temperature profiles obtained from measurements performed in commercial distribution systems. Results indicated an overall decline in population of 0.983 log cfu/g and that the extent of cell death was proportional to the total time spent in the cold chain examined for this work. However, slight growth was predicted

in a few instances when the temperature was maintained above 5°C. The combined growth/death model was also used by Posada-Izquierdo *et al.* (2013, 2014) to anticipate the effects of alternative sanitation and packaging techniques and by Zeng *et al.* (2014) to examine the consequences of temperature fluctuation in distribution systems for packaged fresh-cut lettuce. Under the time–temperature profiles recorded in the latter study, *E. coli* O157:H7 populations could increase by up to 3.0 log cfu/g.

4.9 Conclusions

Food-borne illness outbreaks associated with fresh fruit and vegetables have increased worldwide, focusing attention on factors that affect the microbial safety of fresh produce, including agricultural and hygienic practices during growing, harvesting, and handling. In addition, the quality of wash water, processing technologies used, packaging, processing and storage temperatures, transportation, and distribution can each affect the extent of produce contamination. The effect of pre- and postharvest operations on the contamination and safety of fresh produce has been discussed in this chapter. Internalization, biofilm formation, and the VBNC state are the major bacterial strategies that extend the survival of food-borne pathogens on fresh produce. In these instances, interventions involving some form of chemical agent such as chlorine may not have sufficient residual activity to adequately control pathogens on produce. Other alternative strategies including physical methods have the potential to reduce pathogens on produce and it appears that some are more active than chemical agents.

The best method to reduce food-borne pathogens on fresh produce is prevention of contamination through the combined use of good agricultural and management practices, good quality irrigation water, properly composted animal manure, proper worker hygiene and sanitation practices, and proper handling, preparation, and storage; however, this is not always

possible. Since fresh produce is minimally processed and consumed without application of techniques to eliminate pathogens, unit process operations including sorting, cleaning, trimming, peeling, cutting, shredding, and packaging must be designed to reduce contamination and prevent bacterial growth.

References

- Abadias, M., Usall, J., Anguera, M., Solsona, C., and Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, **123**, 121–129.
- Ahmed, W., Sawant, S., Huygens, F., Goonetilleke, A., and Gardner, T. 2009. Prevalence and occurrence of zoonotic bacterial pathogens in surface waters determined by quantitative PCR. *Water Research*, **43**, 4918–4928.
- Alam, M., Ahlström, C., Burleigh, S., *et al.* 2014. Prevalence of *Escherichia coli* O157:H7 on spinach and rocket as affected by inoculum and time to harvest. *Scientia Horticulturae*, **165**, 235–241.
- Alexandre, E.M.C., Brandao, T.R.S., and Silva, C.L.M. 2013. Impact of non-thermal technologies and sanitizer solutions on microbial load reduction and quality factor retention of frozen red bell peppers. *Innovative Food Science and Emerging Technologies*, **17**, 199–205.
- Al-Nabulsi, A.A., Osaili, T.M., Obaidat, H.M., Shaker, R.R., Awaisheh, S.S., and Holley, R.A. 2014. Inactivation of stressed *Escherichia coli* O157:H7 cells on the surfaces of rocket salad leaves by chlorine and peroxyacetic acid. *Journal of Food Protection*, **77**, 32–39.
- Annous, B.A., Solomon, E.B., Cooke, P.H., and Burke, A. 2005. Biofilm formation by *Salmonella* spp. on cantaloupe melons. *Journal of Food Safety*, **25**, 276–287.
- Arrus, K.M., Holley, R.A., Ominski, K.H., *et al.* 2006. Influence of temperature on *Salmonella* survival in hog manure slurry and seasonal temperature profiles in farm manure storage reservoirs. *Livestock Science*, **102**, 226–236.

- Artes, F., Gómez, P., Aguayo, E., Escalona, V., and Artés-Hernández, F. 2009. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology*, **51**, 287–296.
- Artz, R.R.E. and Killham, K. 2002. Survival of *Escherichia coli* O157:H7 in private drinking water wells: influences of protozoan grazing and elevated copper concentrations. *FEMS Microbiology Letters*, **216**, 117–122.
- Avery, L.M., Williams, A.P., Killham, K., and Jones, D.L. 2008. Survival of *Escherichia coli* O157:H7 in waters from lakes, rivers, puddles and animal drinking troughs. *Science of the Total Environment*, **389**, 378–385.
- Baert, L., Vandekinderen, I., Devlieghere, F., Van Coillie, E., Debevere, J., and Uyttendaele, M. 2009. Efficacy of sodium hypochlorite and peroxyacetic acid to reduce murine norovirus 1, B40-8, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 on shredded iceberg lettuce and in residual wash water. *Journal of Food Protection*, **72**, 1047–1054.
- Barak, J.D. and Liang, A.S. 2008. Role of soil, crop debris, and a plant pathogen in *Salmonella enterica* contamination of tomato plants. *PLoS One*, **3**, e1657.
- Bartz, J.A., Sargent, S.A., and Mahovic, M. 2013. Guide to identifying and controlling postharvest tomato diseases in Florida. IFAS/Extension HS 866, University of Florida.
- Benjamin, L., Atwill, E.R., Jay-Russell, J., et al. 2013. Occurrence of generic *Escherichia coli*, *E. coli* O157 and *Salmonella* spp. in water and sediment from leafy green produce farms and streams on the central California coast. *International Journal of Food Microbiology*, **165**, 65–76.
- Bernstein, N., Sela, S., and Neder-Lavon, S. 2007a. Effect of irrigation regimes on persistence of *Salmonella enterica* serovar Newport in small experimental pots designed for plant cultivation. *Irrigation Science*, **26**, 1–8.
- Bernstein, N., Sela, S., and Neder-Lavon, S. 2007b. Assessment of contamination potential of lettuce by *Salmonella enterica* serovar Newport added to the plant growing medium. *Journal of Food Protection*, **70**, 1717–1722.
- Birmpa, A., Sfika, V., and Vantakaris, A. 2013. Ultraviolet light and ultrasound as non-thermal treatments for inactivation of micro-organisms in fresh ready-to-eat foods. *International Journal of Food Microbiology*, **167**, 96–102.
- Brandl, M.T. 2006. Fitness of human enteric pathogens on plants and implications for food safety. *Annual Review of Phytopathology*, **44**, 367–392.
- Brandl, M.T. and Mandrell, R.E. 2002. Fitness of *Salmonella enterica* serovar Thompson in the cilantro phyllosphere. *Applied and Environmental Microbiology*, **68**, 3614–3621.
- Carpentier, B. and Cerf, O. 1993. Biofilms and their consequences with particular reference to hygiene in the food industry. *Journal of Applied Bacteriology*, **75**, 499–511.
- Castro-Rosas, J., Cerna-Cortés, J.F., Méndez-Reyes, E., Lopez-Hernandez, D., Gómez-Aldapa, C.A., and Estrada-Garcia, T. 2012. Presence of faecal coliforms, *Escherichia coli* and diarrheagenic *E. coli* pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water. *International Journal of Food Microbiology*, **156**, 176–180.
- Centers for Disease Control and Prevention (CDC). 2006a. Salmonellosis – outbreak investigation, October 2006 [Online]. Available at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salmonellosis_2006/110306_outbreak_notice.htm (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2006b. Update on multi-state outbreak of *E. coli* O157:H7 infections from fresh spinach, October 6, 2006 [Online]. Available at: <http://www.cdc.gov/ecoli/2006/september/updates/100606.htm> (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2007. Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants – United States, 2005–2006. *Morbidity and Mortality Weekly Report*, **56**, 909–911.
- Centers for Disease Control and Prevention (CDC). 2008a. Investigation of outbreak of infections caused by *Salmonella* Litchfield [Online]. Available at: <http://www.cdc.gov/salmonella/litchfield/> (15 Jul 2014).

- Centers for Disease Control and Prevention (CDC). 2008b. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items – United States, 2008. *Morbidity and Mortality Weekly Report*, 57, 929–934.
- Centers for Disease Control and Prevention (CDC). 2009a. Investigation of an outbreak of *Salmonella* Saintpaul infections linked to raw alfalfa sprouts [Online]. Available at: <http://www.cdc.gov/salmonella/saintpaul/alfalfa/> (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC) 2009b. Investigation update: outbreak of *Salmonella* Typhimurium infections, 2008–2009 [Online]. Available at: www.cdc.gov/salmonella/typhimurium/update.html (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2010a. Investigation update: multistate outbreak of human *E. coli* O145 infections linked to shredded romaine lettuce from a single processing facility [Online]. Available at: http://www.cdc.gov/ecoli/2010/ecoli_o145/index.html (9 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2010b. Investigation update: multistate outbreak of human *Salmonella* Newport infections linked to raw alfalfa sprouts [Online]. Available at: <http://www.cdc.gov/salmonella/newport/index.html> (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2011a. Investigation announcement: multistate outbreak of *E. coli* O157:H7 infections linked to romaine lettuce [Online]. Available at: <http://www.cdc.gov/ecoli/2011/ecoliO157/romainelettuce/120711/index.html> (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2011b. Investigation update: multistate outbreak of human *Salmonella* I 4,[5],12:i: – infections linked to alfalfa sprouts [Online]. Available at: <http://www.cdc.gov/salmonella/i4512i-/021011/index.html> (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2011c. Investigation update: multistate outbreak of *Salmonella* Panama infections linked to cantaloupe [Online]. Available at: <http://www.cdc.gov/salmonella/panama0311/062311/index.html> (15 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2011d. Multistate outbreak of human *Salmonella* Agona infections linked to whole, fresh imported papayas [Online]. Available: <http://www.cdc.gov/salmonella/agona-papayas/index.html> (22 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2011e. Multistate outbreak of human *Salmonella* Enteritidis infections linked to alfalfa sprouts and spicy sprouts [Online]. Available at: <http://www.cdc.gov/salmonella/sprouts-enteritidis0611/070611/index.html> (22 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2011f. Multistate outbreak of listeriosis linked to whole cantaloupes from Jensen farms, Colorado [Online]. Available at: <http://www.cdc.gov/listeria/outbreaks/cantaloupes-jensen-farms/120811/index.html> (22 Jul 2014).
- Centers for Disease Control and Prevention (CDC). 2012a. Multistate outbreak of *Salmonella* Braenderup infections associated with mangoes (final update) [Online]. Available at: <http://www.cdc.gov/salmonella/braenderup-08-12/index.html> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2012b. Multistate outbreak of *Salmonella* Typhimurium and *Salmonella* Newport infections linked to cantaloupe (final update) [Online]. Available at: <http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2012c. Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to organic spinach and spring mix blend (final update) [Online]. Available at: <http://www.cdc.gov/ecoli/2012/O157H7-11-12/index.html> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2012d. Multistate outbreak of Shiga toxin-producing *Escherichia coli* O26 infections linked to raw clover sprouts at Jimmy John's restaurants (final update) [Online]. Available at: <http://www.cdc.gov/ecoli/2012/O26-02-12/index.html> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2013a. Multistate outbreak of *Salmonella*

- Saintpaul infections linked to imported cucumbers (final update) [Online]. Available at: <http://www.cdc.gov/salmonella/saintpaul-04-13/> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2013b. Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to ready-to-eat salads (final update) [Online]. Available at: <http://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2014a. Multistate outbreak of *Salmonella* infections linked to organic sprouted chia powder [Online]. Available at: <http://www.cdc.gov/salmonella/newport-05-14/index.html> (10 Aug 2014).
- Centers for Disease Control and Prevention (CDC). 2014b. Multistate outbreak of Shiga toxin-producing *Escherichia coli* O121 infections linked to raw clover sprouts (final update) [Online]. Available at: <http://www.cdc.gov/ecoli/2014/O121-05-14/index.html> (10 Aug 2014).
- Chandran, A., Suson, P.S., Thomas, A.P., Hatha, M., and Mazumder, A. 2013. Survival of multi-drug resistant enteropathogenic *Escherichia coli* and *Salmonella paratyphi* in Vembanadu Lake as a function of saltwater barrier along southwest coast of India. *Journal of Water and Health*, **11**, 324–332.
- Chen, Q., Wang, Y., Chen, F., Zhang, Y., and Liao, X. 2014. Chlorine dioxide treatment for the removal of pesticide residues on fresh lettuce and in aqueous solution. *Food Control*, **40**, 106–112.
- Chigor, V.N., Umoh, V.J., and Smith, S.I. 2010. Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria. *African Journal of Biotechnology*, **9**, 178–182.
- Compton, J.A., Baney, J.A., Donaldson, S.C., et al. 2008. *Salmonella* infections in the common raccoon (*Procyon lotor*) in western Pennsylvania. *Journal of Clinical Microbiology*, **46**, 3084–3086.
- Cooley, M.B., Quiñones, B., Oryang, D., Mandrell, R.E., and Gorski, L. 2014. Prevalence of Shiga toxin producing *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* at public access water shed sites in a California Central Coast agricultural region. *Frontiers in Cellular and Infection Microbiology*, **4**, 30. doi: 10.3389/fcimb.2014.00030.
- Czajkowska, D., Witkowska-Gwiazdowska, A., Sikorska, I., Boszczyk-Maleszak, H., and Horoch, M. 2005. Survival of *Escherichia coli* serotype O157:H7 in water and in bottom-shore sediments. *Polish Journal of Environmental Studies*, **14**, 423–430.
- da Cruz, A.G., Sergio, A.C., and Maia, M.C.A. 2006. Quality assurance requirements in produce processing. *Trends in Food Science and Technology*, **17**, 406–411.
- Dai, X., Luo, H., Jiang, L., Xue, Y., and Yu, Z. 2012. Efficacy of different sanitizing agents and their combination on microbe population and quality of fresh-cut Chinese chives. *Journal of Food Science*, **77**, M348–M352.
- Dallaire, R., LeBlanc, D.I., Tranchant, C.C., Vasseur, L., Delaquis, P., and Beaulieu, C. 2006. Monitoring the microbial populations and temperatures of fresh broccoli from harvest to retail display. *Journal of Food Protection*, **69**, 1118–1125.
- Danyluk, M.D. and Schaffner, D.W. 2011. Quantitative assessment of the microbial risk of leafy greens from farm to consumption: preliminary framework, data, and risk estimates. *Journal of Food Protection*, **74**, 700–708.
- Davies, R.H., Newell, D.G., Sayers, A.R., et al. 2008. Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. *Epidemiology and Infection*, **136**, 739–751.
- De Jong, A. 2007. Epidemiology of enteric pathogens in the vegetable production chain: current state. fruit and vegetables: unsafe to eat? Workshop towards Microbiological Safety of Vegetables, October 29, 2007, Marijkeweg 20, Wageningen.
- Delaquis, P. and Austin, J.W. 2007. The effect of heat treatments on the fate of foodborne pathogens in horticultural produce. *Stewart Postharvest Review*, **3**, 9.1–9.5.

- Delaquis, P., Bach, S., and Dinu, L.-D. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. *Journal of Food Protection*, **70**, 1966–1974.
- Denny, J., Threlfall, J., Takkinen, J., *et al.* 2007. Multinational *Salmonella* Paratyphi B variant Java (*Salmonella* Java) outbreak, August–December 2007. *Eurosurveillance*, **12**, pii=3332 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3332> (25 Aug 2014).
- De Roever, C. 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control*, **9**, 321–347.
- Dinu, L. and Bach, S. 2011. Induction of viable but non-culturable *Escherichia coli* O157:H7 on the phyllosphere of lettuce: a food safety risk factor. *Applied and Environmental Microbiology*, **77**, 8295–8302.
- Dinu, L. and Bach, S. 2013. Detection of viable but nonculturable *Escherichia coli* O157:H7 from vegetable samples using quantitative PCR with propidium monoazide and immunological assays. *Food Control*, **31**, 268–73.
- Dreux, N., Albagnac, C., Federighi, M., Carlin, F., Morris, C.E., and Nguyen-the, C. 2007. Viable but non-culturable *Listeria monocytogenes* on parsley leaves and absence of recovery to a culturable state. *Journal of Applied Microbiology*, **103**, 1272–1281.
- D'Souza, R.M., Becker, N.G., Hall, G., and Moodie, K.B.A. 2004. Does ambient temperature affect foodborne disease? *Epidemiology*, **15**, 86–92.
- Elizaquivel, P., Sanchez, G., Selma, M.V., and Aznar, R. 2012. Application of propidium monoazide-qPCR to evaluate the ultrasonic inactivation of *Escherichia coli* O157:H7 in fresh-cut vegetable wash water. *Food Microbiology*, **30**, 316–320.
- El-Jakee, J., Moussa, E.I., Mohamed, K.F., and Mohamed, G. 2009. Using molecular techniques for characterization of *Escherichia coli* isolated from water sources in Egypt. *Global Veterinaria*, **3**, 354–362.
- Elviss, N.C., Little, C.L., Hucklesby, L., *et al.* 2009. Microbiological study of fresh herbs from retail premises uncovers an international outbreak of salmonellosis. *International Journal of Food Microbiology*, **134**, 83–88.
- Emberland, K.E., Ethelberg, S., Kuusi, M., *et al.* 2007. Outbreak of *Salmonella* Weltevreden infections in Norway, Denmark and Finland associated with alfalfa sprouts, July–October 2007. *Eurosurveillance*, **12**, pii=3321 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3321> (25 Aug 2014).
- Ergönül, B. 2011. Survival characteristics of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in minimally processed lettuce during storage at different temperatures. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, **6**, 339–342.
- Erickson, M.C. 2010. Microbial risks associated with cabbage, carrots, celery, onions, and deli salads made with these produce items. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 602–619.
- Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., *et al.* 2008. Surface and internalized *Escherichia coli* O157:H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. *Journal of Food Protection*, **73**, 1023–1029.
- Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., *et al.* 2010a. Infrequent internalization of *Escherichia coli* O157:H7 into field-grown leafy greens. *Journal of Food Protection*, **73**, 500–506.
- Erickson, M.C., Webb, C.C., Diaz-Perez, J.C., *et al.* 2010b. Surface and internalized *Escherichia coli* O157:H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. *Journal of Food Protection*, **73**, 1023–1029.
- Estrada-Flores, S. and Platt, G. 2007. Electricity usage in the Australian cold chain. *Food Australia*, **59**, 382–394.
- Estrada-Flores, S., Eddy, A., and Smale, N. 2006. Evaluation of the thermal performance of five refrigerated vans. In: *Proceedings of the Conference on Innovative Equipment and Systems for Comfort and Food Preservation*, pp. 369–377. International Institute of Refrigeration, Auckland, NZ.
- European Food Safety Authority (EFSA). 2007. Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat

- foods and the related risk for human illness. Adopted on 6 December 2007. *EFSA Journal*, **599**, 1–42.
- European Food Safety Authority (EFSA) 2011. Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. *EFSA Journal*, **9**, 2274, 50 pp.
- European Food Safety Authority (EFSA) 2013. Panel on Biological Hazards (BIOHAZ) Panel Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA Journal*, **11**, 3025, 138 pp.
- Fett, W.F. and Cooke, P.H. 2003. Scanning electron microscopy of native biofilms on mung bean sprouts. *Canadian Journal of Microbiology*, **49**, 45–50.
- Fishburn, J.D., Tang, Y., and Frank, J.F. 2012. Efficacy of various consumer-friendly produce washing technologies in reducing pathogens on fresh produce. *Food Protection Trends*, **32**, 456–466.
- Food and Drug Administration (FDA) 1998. Guidance for industry, Guide to minimize microbial food safety hazards for fresh fruit and vegetables [Online]. Available at: <http://www.fda.gov/downloads/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/UCM169112.pdf> (22 Aug 2014).
- Food and Drug Administration (FDA) 2007. FDA and states closer to identifying source of *E. coli* contamination associated with illnesses at Taco John's restaurants [Online]. Available at: <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108827.htm> (18 Jul 2014).
- Food and Drug Administration (FDA) 2010. Recall – State press release, DSHS orders Sangar produce to close, recall products [Online]. Available at: <http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2010/ucm230709.htm> (18 Jul 2014).
- Food and Drug Administration (FDA) 2011. Recall – State/Local press release, fresh strawberries from Washington county farm implicated in *E. coli* O157 outbreak in NW Oregon [Online]. Available at: <http://www.fda.gov/Safety/Recalls/ucm267667.htm> (20 Jul 2014).
- Food and Drug Administration (FDA) 2013. Chapter IV. Outbreaks associated with fresh and fresh-cut produce. Incidence, growth, and survival of pathogens in fresh and fresh-cut produce [Online]. Available at: <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm091265.htm> (18 Jul 2014).
- Food and Drug Administration (FDA) and California Food Emergency Response Team. 2008. Investigation of the Taco John's *Escherichia coli* O157:H7 outbreak associated with iceberg lettuce. California Department of Public Health Food and Drug Branch, Sacramento, CA, USA [Online]. Available at: <http://www.cdph.ca.gov/pubsforms/Documents/fdb%20eru%20IceLet%20TacoJohn022008.pdf> (27 Aug 2014).
- Forghani, F. and Oh, D.-H. 2013. Hurdle enhancement of slightly acidic electrolyzed water antimicrobial efficacy on Chinese cabbage, lettuce, sesame leaf and spinach using ultrasonication and water wash. *Food Microbiology*, **36**, 40–45.
- Francis, G.A., Gallone, A., Nychas, G.J., *et al.* 2012. Factors affecting quality and safety of fresh-cut produce. *Critical Reviews in Food Science and Nutrition*, **52**, 595–610.
- Frey, S.K., Topp, E., Edge, T.A., *et al.* 2013. Using SWAT, Bacteroidales microbial source tracking markers, and fecal indicator bacteria to predict waterborne pathogen occurrence in an agricultural watershed. *Water Research*, **47**, 6326–6337.
- Friesema, I., Sigmundsdottir, G., van der Zwaluw, K., *et al.* 2008. An international outbreak of Shiga toxin-producing *Escherichia coli* O157 infection due to lettuce, September–October 2007. *Eurosurveillance*, **13**, pii=19065 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19065> (24 Aug 2014).
- Ganesh, V., Hettiarachchy, N.S., Griffis, C.L., Martin, E.M., and Ricke, S.C. 2012. Electrostatic spraying of food-grade organic and inorganic acids and plant extracts to decontaminate *Escherichia coli* O157:H7 on spinach and iceberg lettuce. *Journal of Food Science*, **77**, M391–M396.

- Gannon, V.P., Graham, T.A., Read, S., *et al.* 2004. Bacterial pathogens in rural water supplies in Southern Alberta, Canada. *Journal of Toxicology and Environmental Health*, **67**, 1643–1653.
- Garcia, R., Baelum, J., Fredslund, L., *et al.* 2010. Influence of temperature and predation on survival of *Salmonella enterica* serovar Typhimurium and expression of *invA* in soil and manure-amended soil. *Applied and Environmental Microbiology*, **76**, 5025–5031.
- Gerba, C.P. and Smith, J.E. Jr. 2005 Sources of pathogenic microorganisms and their fate during land application of wastes. *Journal of Environmental Quality*, **34**, 42–48.
- Gil, M.I., Selma, M.V., Suslow, T., Jacxsens, L., Uyttendaele, M., and Allende, A. 2015. Pre- and post-harvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Critical Reviews in Food Science and Nutrition*, **55**, 453–468. doi: 10.1080/10408398.2012.657808 [Online]. Available at: <http://dx.doi.org/10.1080/10408398.2012.657808> (23 Aug 2014).
- Golberg, D., Kroupitski, Y., Belausov, E., Pinto, R., and Sela, S. 2011. *Salmonella* Typhimurium internalization is variable in leafy vegetables and fresh herbs. *International Journal of Food Microbiology*, **145**, 250–257.
- Gomes, C., Moreira, R.G., and Castell-Perez, E. 2011. Microencapsulated antimicrobial compounds as a means to enhance electron beam irradiation treatment for inactivation of pathogens on fresh spinach leaves. *Journal of Food Science*, **76**, E479–E488.
- Gómez-López, V.M. (ed.). 2012. *Decontamination of Fresh and Minimally Processed Produce*. Blackwell Publishers, Ames, Iowa, USA.
- Gómez-López, V.M., Gobet, J., Selma, M.V., Gil, M.I., and Allende, A. 2013. Operating conditions for the electrolytic disinfection of process wash water from the fresh-cut industry contaminated with *E. coli* O157:H7. *Food Control*, **29**, 42–48.
- Gorny, J.R. 1999. Food safety for fresh horticultural produce. In: *Small-Scale Postharvest Technology: Economic Opportunities, Quality and Food Safety* (eds Kitinoja, L. and Gorny, J.R.), pp. 10.1–10.12. Postharvest Horticulture Series No. 21. Department of Pomology, University of California, Davis, CA, USA.
- Grant, J., Wendelboe, A.M., Wendel, A., *et al.* 2008. Spinach-associated *Escherichia coli* O157:H7 outbreak, Utah and New Mexico, 2006. *Emerging Infectious Disease*, **14**, 1633–1636.
- Greene, S.K., Daly, E.R., Talbot, E.A., *et al.* 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. *Epidemiology and Infection*, **136**, 157–165.
- Gu, G.Y., Luo, Z.Y., Cevallos-Cevallos, J.M., *et al.* 2013. Factors affecting the occurrence of *Escherichia coli* O157 contamination in irrigation ponds on produce farms in the Suwannee River Watershed. *Canadian Journal of Microbiology*, **59**, 175–182.
- Guo, X., Chen, J.R., Brackett, R.E., and Beuchat, L.R. 2001. Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Applied and Environmental Microbiology*, **67**, 4760–4764.
- Guo, X., Chen, J., Brackett, R.E., and Beuchat, L.R. 2002. Survival of *Salmonella* on tomatoes stored at high relative humidity, in soil, and on tomatoes in contact with soil. *Journal of Food Protection*, **65**, 274–279.
- Haley, B.J., Cole, D.J., and Lipp, E.K. 2009. Distribution, diversity, and seasonality of waterborne salmonellae in a rural watershed. *Applied and Environmental Microbiology*, **75**, 1248–1255.
- Hamilton, A.J., Stagnitti, F., Premier, R., Boland, A.-M., and Hale, G. 2006. Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Applied and Environmental Microbiology*, **72**, 3284–3290.
- Hancock, D., Besser, T., Lejeune, J., Davis, M., and Rice, D. 2001. The control of VTEC in the animal reservoir. *International Journal of Food Microbiology*, **66**, 71–78.
- Harris, L.J., Farber, J.N., Beuchat, L.R., *et al.* 2003. Outbreaks associated with fresh produce: incidence, growth and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety*, **2**, 78–141.

- Hellström, S., Kervinen, R., Lyly, M., Ahvenainen-Rantala, R., and Korkeala, H. 2006. Efficacy of disinfectants to reduce *Listeria monocytogenes* on precut iceberg lettuce. *Journal of Food Protection*, **69**, 1565–1570.
- Heuvelink, A.E., Zwarkuis, J.T.M., Van Heerwaarden, C., Arends, B., Stortelder, V., and de Boer, E. 2008. Pathogenic bacteria and parasites in wildlife and surface water. *Tijdschrift Voor Diergeneeskunde*, **133**, 330–335.
- Holley, R.A., Arrus, K.M., Ominski, K.H., Tenuta, M., and Blank, G. 2006. *Salmonella* survival in manure-treated soils during simulated seasonal temperature exposure. *Journal of Environmental Quality*, **35**, 1170–1180.
- Holvoet, K., Samplers, I., Callens, B., Dewulf, J., and Uyttendaele, M. 2013. Moderate prevalence of antimicrobial resistance in *Escherichia coli* isolates from lettuce, irrigation water, and soil. *Applied and Environmental Microbiology*, **79**, 6677–6683.
- Hutchison, M.L., Walters, L.D., Moore, A., and Avery, S.M. 2005. Declines of zoonotic agents in liquid livestock wastes stored in batches on-farm. *Journal of Applied Microbiology*, **99**, 58–65.
- Ingram, D.T. and Millner, P.D. 2007. Factors affecting compost tea as a potential source of *Escherichia coli* and *Salmonella* on fresh produce. *Journal of Food Protection*, **70**, 828–834.
- Jablasone, J., Warriner, K., and Griffiths, M. 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. *International Journal of Food Microbiology*, **99**, 7–18.
- Jacques, M., Aragon, V., and Tremblay, Y.D. 2010. Biofilm formation in bacterial pathogens of veterinary importance. *Animal Health Research Reviews*, **11**, 97–121.
- James, J. 2006. Overview of microbial hazards in fresh fruit and vegetables operations. In: *Microbial Hazards Identification in Fresh Fruit and Vegetables* (ed. James, J.), pp. 1–36. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- Jiang, X., Morgan, J., and Doyle, M. 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Applied and Environmental Microbiology*, **68**, 2605–2609.
- Jiang, X.P., Morgan, J., and Doyle, M.P. 2003. Thermal inactivation of *Escherichia coli* O157:H7 in cow manure compost. *Journal of Food Protection*, **66**, 1771–1777.
- Johnson, J.Y.M., Thomas, J.E., Graham, T.A., et al. 2003. Prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources. *Canadian Journal of Microbiology*, **49**, 326–335.
- Johnston, L.M., Jaykus, L., Moll, D., et al. 2005. A field study of the microbiological quality of fresh produce. *Journal of Food Protection*, **68**, 1840–1847.
- Johnston, L.M., Jaykus, L.A., Moll, D., Anciso, J., Mora, B., and Moe, C.L. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *International Journal of Food Microbiology*, **112**, 83–95.
- Karagözlü, N., Ergönül, B., and Özcan, D. 2011. Determination of antimicrobial effect of mint and basil essential oils on survival of *E. coli* O157:H7 and *S. Typhimurium* in fresh-cut lettuce and purslane. *Food Control*, **22**, 1851–1855.
- Keskinen, L.A. and Annous, B.A. 2011. Efficacy of adding detergents to sanitizer solutions for inactivation of *Escherichia coli* O157:H7 on Romaine lettuce. *International Journal of Food Microbiology*, **147**, 157–161.
- Kim, Y.-J., Lee, S.-H., Park, J., et al. 2008. Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on stored iceberg lettuce by aqueous chlorine dioxide treatment. *Journal of Food Science*, **73**, M418–M422.
- Kim, J.-W., Pachepsky, Y.A., Shelton, D.R., and Coppock, C. 2010. Effect of streambed bacteria release on *E. coli* concentrations: monitoring and modeling with the modified SWAT. *Ecological Modelling*, **221**, 1592–1604.
- Kozak, G.K., MacDonald, D., Landry, L., and Farber, J.M. 2013. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *Journal of Food Protection*, **76**, 173–183.
- Kroupitski, Y., Golberg, D., Belausov, E., et al. 2009. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and

- penetration through open stomata. *Applied and Environmental Microbiology*, **75**, 6076–6086.
- Kruse, H., Kirkemo, A.M., and Handeland, K. 2004. Wildlife as source of zoonotic infections. *Emerging Infectious Diseases*, **10**, 2067–2072.
- Kudva, I.T., Blanch, K., and Hovde, C.J. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology*, **64**, 3166–3174.
- Landry, L., Phan, Q., Kelly, S., et al. 2007. *Salmonella* Oranienburg infections associated with fruit salad served in health-care facilities – northeastern United States and Canada, 2006. *Morbidity Mortality Weekly Report*, **56**, 1025–1028.
- Lang, N.L. and Smith, S.R. 2007. Influence of soil type, moisture content and biosolids application on the fate of *Escherichia coli* in agricultural soil under controlled laboratory conditions. *Journal of Applied Microbiology*, **103**, 2122–2131.
- Leifert, C., Ball, K., Volakakis, N., and Cooper, C. 2008. Control of enteric pathogens in ready-to-eat vegetable crops in organic and “low input” production systems: a HACCP-based approach. *Journal of Applied Microbiology*, **105**, 931–950.
- Lewis, H.C., Kirk, M., Ethelberg, S., et al. 2007. Outbreaks of shigellosis in Denmark and Australia associated with imported baby corn, August 2007 – final summary. *Eurosurveillance*, **12**, pii–3279 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3279> (24 Aug 2014).
- Lindow, S.E. and Brandl, M.T. 2003. Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, **69**, 1875–1883.
- Liu, C., Hofstra, N., and Franz, E. 2013. Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by *Escherichia coli* O157 and *Salmonella* spp. *International Journal of Food Microbiology*, **163**, 119–128.
- Lofdahl, M., Ivarsson, S., Andersson, S., Langmark, J., and Plym-Forshell, L. 2009. An outbreak of *Shigella dysenteriae* in Sweden, May–June 2009, with sugar snaps as the suspected source. *Eurosurveillance*, **14**, pii–19268 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19268> (23 Aug 2014).
- Lynch, M.F., Tauxe, R.V., and Hedberg, C.W. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infection*, **137**, 307–315.
- Mahmoud, B.S.M. 2010a. Effects of X-ray radiation on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Shigella flexneri* inoculated on shredded iceberg lettuce. *Food Microbiology*, **27**, 109–114.
- Mahmoud, B.S.M. 2010b. The effects of X-ray radiation on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri* inoculated on whole Roma tomatoes. *Food Microbiology*, **27**, 1057–1063.
- Mahmoud, B.S.M., Bachman, G., and Linton, R.H. 2010. Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Shigella flexneri* on spinach leaves by X-ray. *Food Microbiology*, **27**, 24–28.
- Mannion, C., Lynch, P.B., Egan, J., and Leonard, F.C. 2007. Seasonal effects on the survival characteristics of *Salmonella* Typhimurium and *Salmonella* Derby in pig slurry during storage. *Journal of Applied Microbiology*, **103**, 1386–1392.
- Martínez-Sánchez, A., Allende, A., Bennett, R.N., Ferreres F., and Gil, M.I. 2006. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharvest Biology and Technology*, **42**, 86–97.
- McGee, P., Bolton, D.J., Sheridan, J.J., Earley, B., Kelly, G., and Leonard, N. 2002. Survival of *Escherichia coli* O157:H7 in farm water: its role as a vector in the transmission of the organism within herds. *Journal of Applied Microbiology*, **93**, 706–713.
- McKellar, R.C. and Delaquis, P. 2011. Development of a dynamic growth–death model for *Escherichia coli* O157:H7 in minimally processed leafy green vegetables. *International Journal of Food Microbiology*, **51**, 7–14.
- McKellar, R.C., LeBlanc, D.I., Lu, J., and Delaquis, P. 2012. Simulation of *Escherichia coli* O157:H7 behaviour in fresh-cut lettuce under dynamic temperature conditions during distribution from processing to retail *Foodborne Pathogens and Disease*, **9**, 239–244.

- McKellar, R.C., LeBlanc, D.I., Pérez Rodríguez, F., and Delaquis, P. 2014. Comparative simulation of *Escherichia coli* O157:H7 behaviour in packaged fresh-cut lettuce distributed in a typical Canadian cold chain in the summer and winter. *Food Control*, **35**, 192–199.
- Melloul, A.A., Hassani, L., and Rajouk, L. 2001. *Salmonella* contamination of vegetables irrigated with untreated wastewater. *World Journal of Microbiology Biotechnology*, **17**, 207–209.
- Millner, P. 2003. Composting: improving on a time-tested technique. *Agricultural Research*, **51**, 20–21.
- Misra, N.N., Keener, K.M., Mosnier, J.P., Bourke, P., and Cullen, P.J. 2014. In-package atmospheric pressure cold plasma treatment of cherry tomatoes. *Journal of Bioscience and Bioengineering*, **118**, 177–182. doi: 10.1016/j.jbiosc.2014.02.005.
- Mitra, R., Cuesta-Alonso, E., Wayadande, A., Talley, J., Gilliland, S., and Fletcher, J. 2009. Effect of route of introduction and host cultivar on the colonization, internalization, and movement of the human pathogen *Escherichia coli* O157:H7 in spinach. *Journal of Food Protection*, **72**, 1521–1530.
- Mitscherlich, E. and Marth, E.H. 1984. Microbial survival in the environment. In: *Bacteria and Rickettsiae Important in Human and Animal Health* (eds Mitscherlich, E. and Marth, E.H.), pp. 802–854. Springer, New York, USA.
- Mody, R.K., Greene, S.A., Gaul, L., et al. 2011. National outbreak of *Salmonella* serotype Saintpaul infections: importance of Texas restaurant investigations in implicating jalapeño peppers. *PLoS One*, **6**, e16579.
- Moore, B.C., Martinez, E., Gay, J.M., and Rice, D.H. 2003. Survival of *Salmonella enterica* in freshwater and sediments and transmission by the aquatic midge *Chironomus tentans* (Chironomidae: Diptera). *Applied and Environmental Microbiology*, **69**, 4556–4560.
- Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M., Jaroni, D., and Ravishankar, S. 2013. Antimicrobial activity of oregano oil against antibiotic-resistant *Salmonella enterica* on organic leafy greens at varying exposure times and storage temperatures. *Food Microbiology*, **34**, 123–129.
- Moosekian, S.R., Jeong, S., and Ryser, E.T. 2014. Inactivation of sanitizer-injured *Escherichia coli* O157:H7 on baby spinach using X-ray irradiation. *Food Control*, **36**, 243–247.
- Mootian, G., Wu, W.H., and Matthews, K.R. 2009. Transfer of *Escherichia coli* O157:H7 from soil, water, and manure contaminated with low numbers of the pathogen to lettuce plants. *Journal of Food Protection*, **72**, 2308–2312.
- Morris, C.E. and Monier, J.-M. 2003. The ecological significance of biofilm formation by plant-associated bacteria. *Annual Review of Phytopathology*, **41**, 429–253.
- Mukhopadhyay, S., Ukuku, D., Fan, X., and Juneja, V.K. 2013. Efficacy of integrated treatment of UV light and low dose gamma irradiation on inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on grape tomatoes. *Journal of Food Science*, **78**, 1049–1056.
- Munnoch, S.A., Ward, K., Sheridan, S., et al. 2008. A multi-state outbreak of *Salmonella* Saintpaul in Australia associated with cantaloupe consumption. *Epidemiology and Infection*, **137**, 367–374.
- Nastou, A., Rhoades, J., Smirniotis, P., Makri, I., Kontominas, M., and Likotrafiti, E. 2012. Efficacy of household washing treatments for the control of *Listeria monocytogenes* on salad vegetables. *International Journal of Food Microbiology*, **159**, 247–253.
- Nicholson, F.A., Groves, S.J., and Chambers, B.J. 2005. Pathogen survival during livestock manure storage and following land application. *Bioresource Technology*, **96**, 135–143.
- Niemira, B.A. and Cooke, P.H. 2010. *Escherichia coli* O157:H7 biofilm formation on Romaine lettuce and spinach leaf surfaces reduces efficacy of irradiation and sodium hypochlorite washes. *Journal of Food Science*, **75**, M270–M277.
- Nyberg, K.A., Vinnerås, B., Ottoson, J.R., Aronsson, P., and Albiñ, A. 2010. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in manure-amended soils studied in outdoor lysimeters. *Applied Soil Ecology*, **46**, 398–404.
- Okafo, C.N., Uoh, V.J., and Galadima, M. 2003. Occurrence of pathogens on vegetables harvested

- from soils irrigated with contaminated streams. *Science of the Total Environment*, **311**, 49–56.
- Olaimeat, A.N. and Holley, R.A. 2012 Factors influencing the microbial safety of fresh produce: a review. *Food Microbiology*, **32**, 1–19.
- Oliveira, M., Usall, J., Solsona, C., Alegre, I., Viñas, I., and Abadias, M. 2010. Effect of packaging type and storage temperature on the growth of foodborne pathogens on shredded “Romaine” lettuce. *Food Microbiology*, **27**, 375–380.
- Oliveira, M., Usall, J., Vinas, I., Solsona, C., and Abadias, M. 2011. Transfer of *Listeria innocua* from contaminated compost and irrigation water to lettuce leaves. *Food Microbiology*, **28**, 590–596.
- Oliveira, M., Viñas, I., Usall, J., Anguera M., and Abadias M. 2012. Presence and survival of *Escherichia coli* O157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *International Journal of Food Microbiology*, **156**, 133–140.
- Olmez, H. and Temur, S.D. 2010. Effects of different sanitizing treatments on biofilms and attachment of *Escherichia coli* and *Listeria monocytogenes* on green leaf lettuce. *LWT Food Science and Technology*, **43**, 964–970.
- Ongeng, D., Muyanja, C., Geeraerd, A.H., Springael, D., and Ryckeboer, J. 2011. Survival of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium in manure and manure-amended soil under tropical climatic conditions in Sub-Saharan Africa. *Journal of Applied Microbiology*, **110**, 1007–1022.
- Orue, N., Garcia, S., Feng, P., and Heredia, N. 2013. Decontamination of *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7 from leafy green vegetables using edible plant extracts. *Journal of Food Science*, **78**, M290–M296.
- Pachepsky, Y. and Shelton, D. 2011. *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. *Critical Reviews in Environmental Science and Technology*, **41**, 1067–1110.
- Paez, P., Strohhahn, C.H., and Sneed, J. 2007. Developing benchmarks for handwashing in retail foodservice operations: a pilot study in delicatessens. *Food Protection Trends*, **27**, 903–908.
- Painter, J.A., Hoekstra, R.M., Ayers, T., *et al.* 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, **19**, 407–415.
- Parajuli, P.B. and Ouyang, Y. 2013. Watershed-scale hydrological modeling methods and applications. In: *Current Perspectives in Contaminant Hydrology and Water Resources Sustainability* (ed. Bradley, P.M.), pp. 57–80. InTech, [Online]. Available at: <http://www.intechopen.com/books/current-perspectives-in-contaminant-hydrology-and-water-resources-sustainability/watershed-scale-hydrological-modeling-methods-and-applications> (11 Sep 2014).
- Parajuli, P.B., Mankin, K.R., and Barnes, P.L. 2009. Source specific fecal bacteria modeling using soil and water assessment tool model. *Bioresource Technology*, **100**, 953–963.
- Patel, J.R. and Darlington, K.H. 2010. Survival of *Salmonella* on spinach leaves treated with contaminated irrigation water. In: *International Association for Food Protection Annual meeting*, pp. 3–29. Book of Abstracts, Anaheim, CA, USA.
- Pérez-Rodríguez, F., Campos, D., Ryser, E.T., Buchholz, A.L., Posada-Izquierdo, G.D., and Marks, B.P. 2011. A mathematical risk model for *Escherichia coli* O157:H7 cross-contamination of lettuce during processing. *Food Microbiology*, **28**, 694–701.
- Pezzoli, L., Elson, R., Little, C., *et al.* 2007. International outbreak of *Salmonella* Senftenberg in 2007. *Eurosurveillance*, **12**, pii=3218. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3218>.
- Phungamngoen, C., Chiewchan, N., and Devahastin, S. 2013. Effects of various pretreatments and drying methods on *Salmonella* resistance and physical properties of cabbage. *Journal of Food Engineering*, **115**, 237–244.
- Posada-Izquierdo, G. D., Pérez-Rodríguez, F., López-Gálvez, E., *et al.* 2013. Modelling growth of *Escherichia coli* O157:H7 in fresh-cut lettuce submitted to commercial process conditions: chlorine washing and modified atmosphere packaging. *Food Microbiology*, **33**, 131–138.

- Posada-Izquierdo, G.D., Pérez-Rodríguez, F., López-Gálvez, F., Allende, A., Gil, M.I., and Zurera, G. 2014. Modeling growth of *Escherichia coli* O157:H7 in fresh-cut lettuce treated with neutral electrolyzed water and under modified atmosphere packaging. *International Journal of Food Microbiology*, **177**, 1–8.
- Prazak, A.M., Murano, E.A., Mercado, I., and Acuff, G.R. 2002. Prevalence of *Listeria monocytogenes* during production and post-harvest processing of cabbage. *Journal of Food Protection*, **65**, 1728–1734.
- Pu, S., Beaulieu, J.C., Prinyawiwatukul, W., and Ge, B. 2009. Effects of plant maturity and growth media bacterial inoculum level on the surface contamination and internalization of *Escherichia coli* O157:H7 in growing spinach leaves. *Journal of Food Protection*, **72**, 2313–2320.
- Public Health Agency Canada. 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May–June, 2000, Canada Communicable Disease Report, **26**, p. 20 [Online]. Available at: <http://www.collectionscanada.gc.ca/webarchives/20071214052929/http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/00vol26/dr2620eb.html> (13 Jul 2014).
- Puerta-Gomez, A.F., Kim, J., Moreira, R.G., Klute, G.A., and Castell-Perez, M.E. 2013. Quantitative assessment of the effectiveness of intervention steps to reduce the risk of contamination of ready-to-eat baby spinach with *Salmonella*. *Food Control*, **31**, 410–418.
- Ramos, B., Miller, F.A., Brandão, T.R.S., Teixeira, P., and Silva, C.L.M. 2013. Fresh fruits and vegetables – an overview on applied methodologies to improve its quality and safety. *Innovative Food Science and Emerging Technologies*, **20**, 1–15.
- Redmond, E.C., Griffith, C.J., Slader, J., and Humphrey, T. 2004. Microbiological and observational analysis of cross-contamination risks during domestic food preparation. *British Food Journal*, **106**, 581–597.
- Rimhanen-Finne, R., Niskanen, T., Lienemann, T., *et al.* 2011. A nationwide outbreak of *Salmonella* Bovismorbificans associated with sprouted alfalfa seeds in Finland, 2009. *Zoonoses and Public Health*, **58**, 589–596.
- Rohekar, S., Tsui, F.W., Tsui, H.W., *et al.* 2008. Symptomatic acute reactive arthritis after an outbreak of *Salmonella*. *The Journal of Rheumatology*, **35**, 1599–1602.
- Saldaña, Z., Sánchez, E., Xicohtencatl-Cortes, J., Puente, J.L., and Girón, J.A. 2011. Surface structures involved in plant stomata and leaf colonization by Shiga-toxigenic *Escherichia coli* O157:H7. *Frontiers in Microbiology*, **2**, 119. doi: 10.3389/fmicb.2011.00119.
- Samadi, N., Abadian, N., Bakhtiari, D., Fazell, M.R., and Jamalifar, H. 2009. Efficacy of detergents and fresh produce disinfectants against microorganisms associated with mixed raw vegetables. *Journal of Food Protection*, **72**, 1486–1490.
- Santo-Domingo, J.W., Harmon, S., and Bennett, J. 2000. Survival of *Salmonella* species in river water. *Current Microbiology*, **40**, 409–417.
- Scharff, R.L. 2010. Health-related costs from foodborne illness in the United States. The Produce Safety Project at Georgetown University [Online]. Available at: <http://www.pewtrusts.org/en/research-and-analysis/reports/0001/01/01/healthrelated-costs-from-foodborne-illness-in-the-united-states> (11 Sep 2014).
- Semenov, A.V., van Bruggen, A.H.C., van Overbeek, L., Termorshuizen, A.J., and Semenov, A.M. 2007. Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in cow manure. *FEMS Microbiology Ecology*, **60**, 419–428.
- Semenov, A.V., van Overbeek, L., and van Bruggen, A.H.C. 2009. Percolation and survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in soil amended with contaminated dairy cattle manure or slurry. *Applied and Environmental Microbiology*, **75**, 3206–3215.
- Semenov, A.V., Franz, E., and van Bruggen, A.H.C. 2010a. COLIWAVE: a simulation model for survival of *E. coli* O157:H7 in dairy manure and manure-amended soil. *Ecological Modelling*, **221**, 599–609.
- Semenov, A.V., Kuprianov, A.A., and van Bruggen, A.H.C. 2010b. Transfer of enteric pathogens to successive habitats as part of microbial cycles. *Microbial Ecology*, **60**, 239–249.

- Semenov, A.V., van Overbeek, L., Termoshuizen A.J., and Van Bruggen, A.H.C. 2011. Influence of aerobic and anaerobic conditions on survival of *Escherichia coli* O157:H7 and *Salmonella enteric* serovar Typhimurium in Luria–Bertani broth, farm-yard manure and slurry. *Journal of Environmental Management*, **92**, 780–787.
- Sengun, I.Y. 2013. Effects of ozone wash for inactivation of *S. Typhimurium* and background microbiota on lettuce and parsley. *Journal of Food Safety*, **33**, 273–281.
- Sharma, M., Ingram, D.T., Patel, J.R., et al. 2009. A novel approach to investigate the uptake and internalization of *Escherichia coli* O157:H7 in spinach cultivated in soil and hydroponic medium. *Journal of Food Protection*, **72**, 1513–1520.
- Simmons, J.L., Ryu, J.-H., and Beuchat, L.R. 2006. Comparison of treatment of fresh-cut lettuce and diced tomatoes with sodium hypochlorite and calcium hypochlorite for effects on microbiological and sensory qualities. *Food Protection Trends*, **26**, 662–667.
- Singla, R., Ganguli, A., and Ghosh, M. 2011. An effective combined treatment using malic acid and ozone inhibits *Shigella* spp. on sprouts. *Food Control*, **22**, 1032–1039.
- Sivapalasingam, S., Friedman, C.R., Cohen, L., and Tauxe, R.V. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection*, **67**, 2342–2353.
- Söderström, A., Österberg, P., Lindqvist, A., et al. 2008. A large *Escherichia coli* O1577 outbreak in Sweden associated with locally produced lettuce. *Foodborne Pathogen Disease*, **5**, 339–348.
- Solomon, E.B., Yaron, S., and Mathews, K.R. 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology*, **68**, 397–400.
- Song, I., Stine, S.W., Choi, C.Y., and Gerba, C.P. 2006. Comparison of crop contamination by microorganisms during subsurface drip and furrow irrigation. *Journal of Environmental Engineering*, **132**, 1243–1248.
- Soon, J.M., Manning, L., Davies, W.P., and Baines, R. 2012. Fresh produce-associated outbreaks: a call for HACCP on farms? *British Food Journal*, **114**, 553–597.
- Stafford, R.J., McCall, B.J., Neill, A.S., et al. 2002. A statewide outbreak of *Salmonella bovis* morbi-ficans phage type 32 infection in Queensland. *Communicable Disease Intelligence*, **26**, 568–573.
- Strawn, L.K., Fortes, E.D., Bihn, E.A., et al. 2013a. Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. *Applied and Environmental Microbiology*, **79**, 588–600.
- Strawn, L.K., Grohn, Y.T., Warchocki, S., Worobo, R.W., Bihn, E.A., and Wiedmann, M. 2013b. Risk factors associated with *Salmonella* and *Listeria monocytogenes* contamination of produce fields. *Applied and Environmental Microbiology*, **79**, 7618–7627.
- Su, X. and D'Souza, D.H. 2013. Grape seed extract for foodborne virus reduction on produce. *Food Microbiology*, **34**, 1–6.
- Takkinen, J., Nakari, U.-M., Johansson, T., Niskanen, T., Siitonen, A., and Kuusi, M. 2005. A nationwide outbreak of multiresistant *Salmonella* Typhimurium var Copenhagen DT104B infection in Finland due to contaminated lettuce from Spain, May 2005. *Eurosurveillance*, **10**, pii=2734 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2734> (22 Aug 2014).
- Tan, S.Y.E., Chew, S.C., Tan, S.Y.Y., Givskov, M., and Yang, L. 2014. Emerging frontiers in detection and control of bacterial biofilms. *Current Opinion in Biotechnology*, **26**, 1–6.
- Thomas, J.L., Slawson, R.M., and Taylor, W.D. 2013. *Salmonella* serotype diversity and seasonality in urban and rural streams. *Journal of Applied Microbiology*, **114**, 907–922.
- Tirado, M.C., Clarke, R., Jaykus, L.A., McQuatters-Gollop, A., and Frank, J.M. 2010. Climate change and food safety: a review. *Food Research International*, **43**, 1745–1765.
- Todd, J., Friedman, M., Patel, J., Jaroni, D., and Ravishankar, S. 2013. The antimicrobial effects of cinnamon leaf oil against multi-drug resistant *Salmonella* Newport on organic leafy greens.

- International Journal of Food Microbiology*, **166**, 193–199.
- Trenberth, K.E., Fasullo, J., and Smith, L. 2005. Trends and variability in column-integrated water vapor. *Climate Dynamics*, **24**, 741–758.
- Vandeplas, S., Dubois Dauphin, R., Beckers, Y., Thonart, P., and Théwis, A. 2010. *Salmonella* in chicken: current and developing strategies to reduce contamination at farm level. *Journal of Food Protection*, **174**, 774–785.
- Vital, M., Hammes, F., and Egli, T. 2008. *Escherichia coli* O157 can grow in natural freshwater at low carbon concentrations. *Environmental Microbiology*, **10**, 2387–2396.
- Vogeleer, P., Tremblay, Y.D.N., Mafu, A.A., Jacques, M., and Harel, J. 2014. Life on the outside: role of biofilms in environmental persistence of Shiga-toxin producing *Escherichia coli*. *Frontiers in Microbiology*, **5**, 317. doi: 10.3389/fmicb.2014.00317.
- Wang, G. and Doyle, M.P. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection*, **61**, 662–667.
- Wang, G., Zhao, T., and Doyle, M.P. 1996. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. *Applied and Environmental Microbiology*, **62**, 2567–2570.
- Warriner, K. and Namvar, A. 2010. The tricks learnt by human enteric pathogens to persist within the plant environment. *Current Opinion in Biotechnology*, **21**, 131–136.
- Warriner, K., Huber, A., Namvar, A., Fan, W., and Dunfield, K. 2009. Recent advances in the microbial safety of fresh fruits and vegetables. *Advances in Food and Nutrition Research*, **57**, 155–208.
- Werner, S., Boman, K., Einemo, I., et al. 2007. Outbreak of *Salmonella* Stanley in Sweden associated with alfalfa sprouts, July–August 2007. *Eurosurveillance*, **12**, pii=3291 [Online]. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3291> (23 Aug 2014).
- World Health Organization (WHO) 2000. CINDI dietary guide, Health Documentation Services, WHO Regional Office for Europe, Scherfigsvej 8, 2100 Copenhagen Ø, Denmark [Online]. Available at: http://www.euro.who.int/_data/assets/pdf_file/0010/119926/E70041.pdf (01 Aug 2014).
- World Health Organisation/Food and Agriculture Organization (WHO/FAO) 2008. Microbiological hazards in fresh leafy vegetables and herbs, Microbiological Risk Assessment Series, Meeting Report. 20, Avenue Appia, CH-1211 Geneva 27, Switzerland [Online]. Available at: http://www.fao.org/ag/agn/agns/jemra/Jemra_Report%20on%20fresh%20leafy%20vegetables%20and%20herbs.pdf (30 Jun 2014).
- Wright, K., Chapman, S., McGeachy, K., et al. 2013. The endophytic lifestyle of *Escherichia coli* O157:H7: quantification and internal localization in roots. *Phytopathology*, **103**, 333–340.
- You, Y., Rankin, S.C., Aceto, H.W., Benson, C.E., Toth, J.D., and Dou, Z. 2006. Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Applied and Environmental Microbiology*, **72**, 5777–5783.
- Zhang, G., Ma, L., Beuchat, L.R., Erickson, M.C., Phelan, V.H., and Doyle, M.P. 2009a. Heat and drought stress during growth of lettuce (*Lactuca sativa* L.) does not promote internalization of *Escherichia coli* O157:H7. *Journal of Food Protection*, **72**, 2471–2475.
- Zhang, G., Ma, L., Beuchat, L.R., Erickson, M.C., Phelan, V.H., and Doyle, M.P. 2009b. Lack of internalization of *Escherichia coli* O157:H7 in lettuce (*Lactuca sativa* L.) after leaf surface and soil inoculation. *Journal of Food Protection*, **72**, 2028–2037.
- Zeng, W., Vorst, K., Brown, W., et al. 2014. Growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in packaged fresh-cut Romaine mix at fluctuating temperatures during commercial transport, retail storage, and display. *Journal of Food Protection*, **77**, 197–206.
- Ziuzina, D., Patil, S., Cullen, P.J., Keener, K.M., and Bourke, P. 2014. Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* inoculated on fresh produce. *Food Microbiology*, **42**, 109–116.

CHAPTER 5

Impact of heating operations on the microbial ecology of foods

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5.1 Background and basic information of heating operations

There is a wide variety of food heating processes aiming at accomplishing several missions. By heating a food matrix we may ensure its safety due to pathogens elimination, to extend the shelf life time, enhance the texture, the taste, and other sensory properties. On the contrary, there are several drawbacks of heat processes as their application may also lead to nutritional and quality deterioration of the food product. For this purpose, unit operation on heat processes is a critical area and has to be adapted to the singularity of each product. The fields described in the paragraphs of this chapter often overlap, but for simplicity purposes we will follow a specific norm of presenting. Herein, in the first part of the chapter basic information regarding the heating processes and unit operations will be given, while in the second part the impact of these operations on food-borne microorganisms will be thoroughly covered.

5.1.1 Cooking (grilling, broiling, griddle, roasting, frying, stewing, sous-vide, braising)

The cooking process can be defined as a food preparation process by heating the ingredients (Dictionary of Food Science and Technology,

2005). The human is the only animal who applies the cooking process for its dietetic habits. Scientists support the fact that there is archeological evidence pointing out that cooking has been practiced for about a million years (Miller and Wetterstrom, 2000). The reasons that forced humans to invent cooking are not clear, but may be connected with the needs of sterilization, preventing deterioration, or enhancing the softness and digestibility of their food acquired from hunting or fishing. From the early stages of the human until recent centuries the only source of heating was the fire. During all these years several cooking techniques arose all over the world, based on the technological achievements of each era and creating local cuisines. Cooking methods can be divided into five basic categories. Food may be immersed in liquids such as water, stocks or wine (boiling, poaching, stewing), immersed in fats or oils (frying), exposed to vapor (steaming and braising), exposed to dry heat (grilling, broiling, griddling, roasting, baking), and subjected to contact with hot fats (sautéing) (Dictionary of Food Science and Technology, 2005). Since there are a lot of variations of these cooking techniques, a cacophony appears regarding their definitions in literature. In this part of the chapter the main cooking methods will be described as well as some recent representative studies concerning their impact on the inactivation of food-borne microorganisms.

Grilling and broiling refer to similar cooking processes. In grilling, the heat source is below (as with a barbecue grill) while heat transfer during broiling is achieved from the upper surface of the food. Both grilling and broiling involve intense heat by direct and by radiation mechanisms, respectively. Griddling is described as a cooking method where a food matrix is cooked on a broad flat metallic or alloy surface heated directly or indirectly by means of an open flame or electrical resistance, respectively (Jiménez-Monreal *et al.*, 2009; Gomes *et al.*, 2014).

Frying can be defined as a cooking method where fat or oil is used as the heat transfer medium in direct contact with the food. Frying methods can be divided into three different techniques: (a) pan frying, where a thin flat piece of food is cooked by contact with a hot fatty layer, commonly without agitation, (b) stir frying, where food pieces are cooked in a hot fatty phase with constant agitation, and (c) deep frying, where food sliced into pieces are immersed into a boiling fatty phase with a heat transfer over the entire surface of the pieces (Zeki, 2013). Roasting is defined as a cooking method where food is heated simultaneously by convection, conduction, and radiation in an oven. More specifically, the convection mechanism is related to the heat transfer through the circulation of the hot gases. Heat transfer by conduction occurs through contact of the food and the hot surfaces of the oven, whereas radiation heating occurs by radiant heat emanating from the walls or the bodies installed in the oven (Zeki, 2013).

Stewing can be defined as the method where foods are cooked slowly at a relatively low temperature and for a long period of time in a small amount of liquid in a closed cooking pot (Dictionary of Food Science and Technology, 2005). Stewing tenderizes tough pieces of meat and allows the flavors of the ingredient components to blend. Similarly, braising stems from the French word “*braiser*”, which refers to cooking slowly under mild heating. In particular, braising refers to a combination of two other cooking methods, i.e., light frying initially,

followed by stewing in a small amount of liquid at low heat for a lengthy period of time in a closed container (Dictionary of Food Science and Technology, 2005). Another cooking method, which nowadays is often commercially utilized due to its advantages, such as ease and appropriateness for the management of pre-cooked foodstuff and limited exposure to microbial contaminants, is sous-vide (Roldán *et al.*, 2013). Sous-vide originates from the French term meaning under-vacuum, and as a method can be defined as cooking in vacuumized heat-stable plastic pouches at precisely controlled temperatures followed by rapid cooling (Baldwin, 2012; Roldán *et al.*, 2013).

The thermal stability of heat-shocked and non-heat-shocked spores of the virulence-attenuated Sterne strain of *Bacillus anthracis* at selected temperatures in irradiated raw ground beef heated in a water bath or cooked using two commercial grills was recently evaluated (Juneja *et al.*, 2010). More specifically, in the first approach, thermal inactivation was conducted by placing the inoculated samples in a basket that was completely submerged in a temperature-controlled, circulating water bath stabilized at 65, 70, 75, and 80°C ± 0.5°C. The total heating times ranged from 180 min at 65°C to 5 min at 80°C. The second approach involved the use of a commercial open flame gas grill or a clamshell electric grill. The inoculated ground beef patties were heated to the target temperatures of 71.1, 82.2, or 93.3°C. According to the authors, their water bath approach was not feasible when compared to the grilling methods that followed. Moreover, they validated that cooking ground beef patties on a commercial grill at a temperature of 71.1°C, which is considered as “well done” (*bien cuit*), is sufficient to appreciably lessen the potential risk of illness associated with this spore-forming bacterium. Another study investigated whether commonly used cooking temperatures are lethal for *Escherichia coli* O157:H7 translocated into beef steaks cut from mechanically blade-tenderized subprimals (Luchansky *et al.*, 2009). Cooking of the samples was carried out on an open flame

commercial gas grill using natural gas and with four burners set at the maximum burning level to achieve the target internal temperatures of 48.9, 54.4, and 60°C. The beef steaks were flipped over at the mid-point temperature during cooking, in an attempt for both sides of each steak to be equally cooked. In this study, which went further than the grill treatment at different temperatures, they investigated the impact of cooking on beef steaks with different thicknesses. In this study, although the target temperatures were in the cooking level of “blue” (*bleu*), “rare” (*saignant*), and “medium rare” (*à point*) a noticeable reduction of *Escherichia coli* O157:H7 was observed. In 2011, Shen *et al.* (2011) determined the extent of *Escherichia coli* O157:H7 inactivation in non-intact beef cooked up to a “medium–rare” level (65°C final internal temperature) by pan-broiling or roasting with various cooking appliances set at different temperatures. Pan-broiling was carried out with the use of an electric skillet or an indoor barbecue grill, whilst for roasting a stainless steel toaster oven or a standard kitchen oven was used. The appliances were preheated and remained stable at 149 or 204°C for the electric skillet, 149 or 218°C for the grill, 149 or 232°C for the toaster oven, and 149, 204, or 260°C for the kitchen oven. The beef samples were placed on the pan-broiling surface or in the center of the oven for roasting. The pan-broiled samples were flipped over when the temperature in the geometric center reached the temperature of 42°C. Beef samples required cooking for 33 to 45 min and 27 to 60 min by pan-broiling and roasting, respectively, in order to reach the target internal temperature of 65°C. Under the conditions of their study, the results indicated that the type of appliance and the setting temperature affected significantly the thermal inactivation of *Escherichia coli* O157:H7 in beef samples.

Konagaya *et al.* (2009) investigated the thermal inactivation of *Clostridium botulinum* spores during cooking of rice. The cooking of the inoculated rice was performed in a sealed retail container following one or two steps of cooking. More specifically, the containers were

heated at 100°C for 15 min immediately followed by 105°C for 15 min or at 100°C for 30 min. During storage food-grade oxygen-absorbing agents were present in order to prevent spores of aerobic microbes from germinating. The results acquired indicated that after the two-step cooking process no viable spores were detected, while the one-step approach was followed by less efficient inactivation of *Clostridium botulinum* spores.

De Paula *et al.* (2005) published their study regarding the impact of two different methods of boiling and of a typical frying of eggs on the inactivation of *Salmonella enteritidis* ATCC11076. According to the first method of boiling, the contaminated eggs were placed in a saucepan and covered with 1.5 l of water (water level 3 cm over the eggs) at 21°C. The pan was then placed on the burner and the samples were collected and analyzed after 1, 3, and 10 min of boiling. In the second method, the eggs were immersed directly in the boiling water with 1, 3, and 10 min of dwell times. The main differences between these two methods can be focused on the heating rates and the final reached temperature of the samples. Following the first method, the heating rates of the egg yolks are much higher than in the second procedure, whereas the final temperature reached after holding them for 10 min in boiling water was $76.2 \pm 2.0^\circ\text{C}$ and $94.77 \pm 1.2^\circ\text{C}$ for the first method and the second method, respectively. In the same study the authors then investigated the impact of frying. According to their protocol they placed the contaminated content of the eggs in a covered frying pan containing preheated vegetable oil (140–160°C) and were fried for 1.5, 2.0, and 10 min.

Recently, two studies regarding the microbiological quality of sous-vide processed food matrices were published (Roldán *et al.*, 2013; Rinaldi *et al.*, 2013). Roldán *et al.* (2013) studied the effect of sous-vide cooking of lamb loins at different temperature–time combinations on microbiological and other features. Initially the authors packaged the samples in vacuum plastic bags and subsequently cooked

them in thermostated water baths for different times (6, 12, and 24 h) and temperatures (60, 70, and 80 °C) combinations. By the end of the cooking process the samples were submerged in ice-cold water (2 °C) for 1 h and then stored under refrigeration overnight at 2 °C. Rinaldi *et al.* (2013) followed a different approach for their investigation regarding the effect of sous-vide cooking on microbiological counts of carrots and Brussels sprouts. Vegetables were vacuum packed in plastic bags and cooked for 20 min in an air/steam oven under steam at 100 °C. After rapid chilling of the bags, samples were stored at 4 °C in the dark for 1, 5, and 10 days and then reheated for 20 min in a water bath at 60 °C. In both studies the sous-vide cooking processes showed the ability to decrease the microbial populations efficiently.

5.1.2 Blanching

Blanching is a thermal pretreatment where the food is exposed to high temperatures for a brief period of time. The blanching method is a popular process not only in an industrial but also in a domestic level. The main function of blanching is to inactivate or to stem bacterial and enzyme action, which could otherwise cause rapid degradation in terms of quality. Commonly, blanching is used prior to appertization, drying, canning, freezing, and lyophilization of fruits and vegetables. Moreover, two additional effects of blanching may include the release of air and gases in the product, which may lead to a product volume reduction.

The main techniques of blanching involve either direct or indirect heating systems. Blanching can be performed in boiling water and/or in steam, while recently dry blanching has been developed such as microwave and infrared blanching (Vishwanathan *et al.*, 2013). This may depend on the product. Direct conventional heating is normally made by immersion in hot water, at temperatures ranging from 80 to 100 °C, or by exposure to steam (steaming). The operation of blanching is usually carried out in horizontal chambers. The time of pretreatment

in the blancher commonly varies from 1 to 5 minutes. Like most conventional thermal processes applied to food, blanching is highly related to the time–temperature combination. These two factors have to be considered carefully with respect to the kind of food matrix that is to be blanched. The main critical factors, regarding the food matrix, that affect the success of blanching are the size and shape, the thermal conductivity, which can vary by type and maturity of the food matrices, the method of heating, and the convective heat transfer coefficient. The target enzymes of blanching that are responsible for quality degradation during storage of fruits and vegetables include mainly lipoxygenases, polyphenoloxidases, polygalacturoxidases, polygalacturonases, chlorophyllases, as well as two more heat-resistant enzymes such as catalases and peroxidases.

Numerous studies have been carried out regarding the impact of several blanching techniques on the aforementioned enzyme deactivation and the quality attributes when applied on a wide range of fruits and vegetables. On the contrary, unfortunately, literature lacks studies regarding the impact of blanching on food-borne microbial populations. Herein are a few recent studies with respect to the lethal effects of conventional blanching on food-borne pathogenic microorganisms.

The influence of modified blanching treatments on inactivation of *Salmonella* Typhimurium strains during drying and storage of carrot samples were investigated by DiPersio *et al.* (2007). They applied blanching treatments by water (88 °C, 4 min) and steam (88 °C, 10 min) to inoculated carrot samples and in two different contents of food-grade citric acid solutions (88 °C, 4 min). Subsequent to blanching, the samples were dried for 6 h at 60 °C and stored for up to 30 days at 25 ± 3 °C and 30 ± 6 % relative humidity. The authors reckoned that compared to the traditional home-drying methods, the blanching treatments proposed from their study were more effective in reducing *Salmonella* populations on inoculated carrot slices. In 2012, Harris *et al.* (2012) carried out a study regarding

the behavior of *Salmonella enteritidis* on the surface of almond kernels during hot water blanching. More specifically, they evaluated methods for recovery of *Salmonella* from inoculated almonds before and after exposure to hot water as well as determining the heat resistance of *Salmonella* and *Enterococcus faecalis* on almond kernels exposed to hot water. The inoculated almonds were submersed and remained, with the use of a wire mesh basket, in the hot water. The almonds were gently agitated during treatment and water bath temperatures that were tested ranged from 60 to 88°C. The almonds were blanched from 30 s to 12 min and the water volume was maintained constant at 25 l. Interesting results were obtained, which can give information regarding the lethal impact of blanching pretreatment, although they are not directly comparable with the commercial post-harvest processes in which almond skin is removed and subsequently almonds are being dried in temperatures ranging from 104 to 106°C. On the contrary, Phungamngoen *et al.* (2013) investigated the combined effects of pretreatment and drying methods on the resistance of *Salmonella anatum* attached to cabbage surfaces. More specifically, boiling water and steaming were tested as blanching methods, whilst hot air and vacuum were employed as drying methods. The blanching treatment times were 4 min and 2 min for hot water and steam, respectively, and the drying of the samples in both techniques was carried out at 60°C. In this study, the authors claimed that the combination of blanching and drying led to a more microbiologically safe product as the pretreatment helped to reduce the heat resistance of *Salmonella* during drying.

5.1.3 Pasteurization

Although undoubtedly boiling of milk or other liquid food products had been used by housekeepers over a very long period of time, in the 1860s Louis Pasteur carried out the first scientific study regarding the application of heat in controlling the fermentation and preserving liquid products. During his studies he found

that by heating wine and beer to a sufficient temperature and time it was possible to inactivate the spoilage microorganisms without greatly influencing the basic character of the product (Steele, 2000). The developed process, pasteurization, takes its name from Pasteur. More specifically, pasteurization traditionally was defined as the process of heating a (typically a liquid) food product for a specific time at a specific temperature (usually in the range of 65 to 95°C) followed by rapid cooling. The aim of pasteurization is first to kill the pathogens and substantially to lower the microbial load responsible for spoilage through a proper time–temperature combination.

Recently, according to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the Food Safety and Inspection Service of the United States Department of Agriculture, a new broader definition for pasteurization was introduced (<http://www.fsis.usda.gov/wps/portal/fsis/home>). Pasteurization was defined as any process, treatment, or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage. By this definition, it can be assumed that pasteurization could also include non-thermal processing methods such as high-pressure or high-intensity pulsed electric field (PEF) treatments. In this part of the chapter recent studies regarding conventional pasteurization heat processes oriented towards the microbiological lethal impact in food matrices will be discussed.

Vegara *et al.* (2013) investigated the effects of two pasteurization processes on the microbial population and on the shelf life of pomegranate juices. More specifically, during their study, both cloudy and clarified juices of pomegranate were subjected to heat treatments at 65°C for 30 s or at 90°C for 5 s as low-temperature pasteurization (LTP) and high-temperature pasteurization (HTP) processes, respectively. The treatments were carried out by means of

a semitubular pasteurizer (25 l/h), whilst after the treatment, for the needs of the shelf life study, the samples were stored in cooled incubators at 25 and 5°C for 45 and 120 days, respectively. Results obtained from their study showed that the combination of LTP treatment and the subsequent refrigeration may help to ensure the microbiological safety of pasteurized pomegranate juices by avoiding a dramatic impact on sensory parameters and nutritional properties. In 2014, Skara *et al.* (2014a) designed and validated a model system for the steam surface pasteurization of cooked surimi gels inoculated with realistic populations of the bacteria *Listeria innocua* (Skara *et al.*, 2014a). The model fish products were contaminated by two different inoculation methods (contact and drop inoculation) at low and high levels. For the needs of their study, the surfaces of the samples were thermally treated perpendicularly by a heating or cooling medium in four chambers. The process time varied from 3 to 23 s, while the temperature distribution of the process, as the authors reported in a separate study of the surface temperature reached 99°C after 6 s of treatment (Skara *et al.*, 2014b). The temperature was observed to be significantly lower under the surface in these processing times.

Benlloch-Tinoco *et al.* (2014) applied conventional thermal pasteurization by means of a circulating thermostatic water bath in kiwifruit purée contaminated with *Listeria monocytogenes*. The samples consisted of 20 g of kiwifruit purée in stainless steel tubes and the treatments that were studied were at temperatures of 50, 55, and 60°C for 90–1200 s periods of time. Their work aimed at the comparison of conventional thermal pasteurization with the microwave heating pasteurization and their results will be later discussed in the corresponding paragraph on dielectric heating applications.

5.1.4 Commercial sterilization

As aforementioned, the conservation of food and feed products is mainly achieved by killing the microorganisms that are present. The most

commonly used techniques in the food, drink, and milk (FDM) industries relate to heat treatments. Depending on the product, its physical and chemical properties as well as the respective shelf-life requirements, heat treatment processes in various time–temperature combinations can be applied. Further to blanching and pasteurization, one more thermal process is commercial sterilization, which can be defined as another controlled heating process that is used to eliminate viable forms and spores of microorganisms with public health significance. Commercial sterilization should not be confused with the term “sterilization”, which generally involves the elimination of the entire microbial load and its spores (Holdsworth, 2009). This treatment can be attained by means of moist or dry heat, filtration, irradiation, or by chemical methods. In comparison to pasteurization, commercial sterilization is a more intense heat treatment as temperatures in the range of 110 to 120°C are applied for a longer time period in order to lead to a stable product with a longer shelf life. One critical factor regarding the application of commercial sterilization is the impact on nutritional and sensory qualities of the treated food. Therefore, several efforts have been carried out in order to achieve minimal effects. Most of the recent research studies regarding the impact of commercial sterilization on foods are focused on the quality attributes. Herein, some recent results concerning the efficiency of each treatment to eliminate the microbial populations will be presented.

Witthuhn *et al.* (2011) carried out a study to determine the heat resistance of aerobic spore formers isolated from dairy products and cocoa powder and tried to give an overview of the occurring highly heat-resistant spores. The thermal inactivation of selected strains at different temperatures was determined. The thermal treatment of the samples was carried out by means of a batch-heating system and performed for 30 min at 110, 120, and 125°C. For the determination of inactivation kinetics of *Bacillus amyloliquefaciens* ultrafiltration permeate made

by spray-dried sweet whey powder was used. The authors also used ultrafiltration permeate as the heating medium in order to simulate the inactivation in milk. The temperature ranged from 105 to 140°C up to 35 min of treatment. More recently, Stoeckel *et al.* (2014) carried out a study in order to examine the inactivation of *Bacillus amyloliquefaciens* and *Bacillus smithii* spores in a batch heating system, which was compared with the inactivation of *Bacillus amyloliquefaciens* spores inactivated in a continuous heating system. The batch heating system consisted of a pressure vessel with inlets and outlets for the steam and iced water. During the experiments, the tubes that contained the spore suspension were heated and cooled by direct contact with saturated steam condensation and with iced water, respectively. The treatment temperatures ranged from 110 to 140°C for 4 s to 30 min. The continuous sterilization system consisted of a double-pipe tubular heat exchanger, a sterilized feed tank, an eccentric screw pump, a prewarming section with two plate heat exchangers, a holding section and an iced water cooling unit. The product was heated in the double heat exchanger with the use of pressurized hot water. In this system time-temperature combinations of 110–140°C and 0–256 s were applied while the maximum difference of temperature at the exit of the holding section was 5°C. About 10 s were needed to increase the temperature from 40°C to the target point. In the continuous system the highest increase in temperature was measured in the first third of the heating section.

More studies regarding thermal processes in the temperature ranges of pasteurization and commercial sterilization with the application of different technologies are discussed in the following paragraphs.

5.1.5 Ohmic heating

Ohmic heating, which may also be encountered under the terms of “resistance heating”, “electroconductive heating”, or “Joule heating” is a process in which electrical current passes through a food product having electrical resistance

(Fellows, 2009; Yildiz-Turp *et al.*, 2013). The fundamental principle of ohmic heating is the dissipation of electrical energy in the form of heat using an electrical conductor; the generated energy is proportional to the square power of the local electric field strength and the electrical conductivity (Goullieux and Pain, 2014). The local electric field is mainly related to the operating parameters of the applied voltage and the intensity, whilst the electrical conductivity is connected with the food matrix and is measured by the quantity of electricity transferred across a unit area, per unit potential gradient and per unit of time (Goullieux and Pain, 2014). Food matrices may be classified into different categories with respect to their conductivity. The lower the conductivity of the material, the higher electrical field strength it requires. Hitherto, ohmic heating has been applied in several food processes such as cooking, blanching, pasteurization, sterilization, and extraction. In this section of the chapter, recent studies regarding the applications of ohmic heating as an alternative method for microbial and enzyme inactivation on food will be documented.

Recently, a study regarding the effect of ohmic heating on the inactivation of the enzymes alkaline phosphatase (EC 3.1.1.11), peroxidase (EC 1.11.1.7), and pectine methylesterase (EC 3.1.1.11) was published (Jakób *et al.*, 2010). The working materials used for this study were apple juice, cloudberry jam, bovine and caprine milk, and three vegetable mixtures consisting of carrot, broccoli, potatoes, butter, milk, salt, and pepper. For the needs of their investigation the samples were poured into a horizontal cylindrical glass reactor and capped by stainless steel electrodes. The voltage was adjusted by a power relay and generated an alternating current with a frequency of 50 Hz. Since the objective of their study was to compare the inactivation effects of their ohmic heating method with the conventional approach, their system was controlled to have similar heating rates with the conventional heating in each case. During their experiments the temperature at

the geometrical center of the reactor ranged from 54 to 66°C for industrial jam, consumer jam, and apple juice, from 52 to 64°C for bovine and caprine milk, and from 58 to 78°C for broccoli, potatoes, and carrot samples. According to the authors, the results acquired from their multitemperature approach indicated that ohmic heating can be a useful alternative method for sterilization or pasteurization of food products and may also enhance the enzyme inactivation rates.

Sagong *et al.* (2011) studied the efficacy of ohmic heating for inactivating *Salmonella Typhimurium*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in orange juice and tomato juice with various exposure times and electric field strengths. Their study was carried out by means of an ohmic heating chamber consisting of a 2-liter Pyrex glass container and two titanium plate electrodes. The supplied power in the chamber was alternating current at 60 Hz and the voltage ranged from 100 to 200 V. The time intervals of treatments needed in order for the temperature at the central section of the chamber to be raised from 28 to 80°C were circa 100, 200, 570, and 120 s, 260 s, and 660 s under 10 V/cm, 15 V/cm, and 20 V/cm electric field strengths for tomato juice and orange juice, respectively. The heating rates of tomato juice were considerably higher than those of orange juice and the authors asserted that this phenomenon may be related to higher conductivity of tomato juice at all temperatures due to the added salt during processing. The same research group went one step further as they applied ohmic heating on a lab-scale continuous processing of tomato and orange juices (Lee *et al.*, 2012). In this study the juices were treated with electric field strengths in the range of 25–40 V/cm. The samples were pumped through the horizontal chamber with a constant flow rate of 120 ml/min. The temperature history showed that the treatment with 30 V/cm has similar heating rates to those of the conventional heating process. The results obtained from both of their studies indicated that ohmic heating could be potentially used to pasteurize

juices even at an industrial level. More recently, Lee *et al.* (2013) investigated the effect of frequency of alternating current during ohmic heating on electrode corrosion, heating rate, inactivation of food-borne pathogens, and quality of tomato-based salsa. Seven frequencies (in the range of 60 Hz to 20 kHz) and three wave forms (sine, square, and sawtooth) were applied to each sample under 12.5 V/cm constant electric field strength. According to the authors, when the frequency transcended 1 kHz, electrode corrosion was not observed. The heating rate of the samples was dependent on frequency up to 500 Hz, but there was no significant difference when the frequency was increased over 1 kHz. Electrical conductivity of the sample increased in tandem with a rise in the frequency. At a frequency of 60 Hz, the square wave produced a lower heating rate than the other types of waves. The heating rate between waveforms was not significantly different when the frequency was greater than 500 Hz.

Three recent studies regarding the application of ohmic heating for the inactivation of vegetative bacterial spores were published (Somavat *et al.*, 2012, 2013; Park *et al.*, 2013). Somavat *et al.* (2012, 2013) applied the ohmic heating aiming to inactivate spores from *Geobacillus stearothermophilus* and *Bacillus coagulans* in tomato juice, while Park *et al.* (2013) applied ohmic heating combined with pressure for the inactivation of suspended *Bacillus amyloliquefaciens* and *Geobacillus stearothermophilus* in green pea purée, carrot purée, and tomato juice. In both studies Somavat *et al.* used the same experimental setup. The experimentations were carried out by means of a prototype heater-cooler system equipped with two titanium electrodes for the application of the alternate current to capillary cell holders. The samples were treated ohmically at frequencies of 10 kHz and 60 Hz with a square waveform pulse mode. Treatment temperatures were 121, 125, 130°C and 95, 100, 105°C for *Geobacillus stearothermophilus* and for *Bacillus coagulans* spores, respectively. Promising results were acquired concerning the lethal

effect of ohmic treatments compared to the conventional thermal methods. Park *et al.* (2013) used a laboratory scale specially designed high-pressure ohmic thermal sterilization cell and the alternate (60 Hz) current was applied with the use of platinum-plated titanium electrodes. According to their approach the samples were initially pressurized up to 600 MPa and then the temperature was raised with constant electric field strength up to the target temperature of 105°C. Afterwards, the samples remained at the target temperature for various times (0, 1, 5, 10, 20, and 30 min) by adjusting the electric field strength in the range from 0 to 50 V/cm. Their results indicated that a combined process with the synergistic application of pressure, heat, and electrical treatment may efficiently inactivate pressure thermal resistant bacterial spores under the proper conditions.

5.1.6 Dielectric heating

Since Michael Faraday first evidenced the existence of electromagnetic fields, J.C. Maxwell mathematically described the behavior of the electromagnetic waves, and Heinrich Hertz experimentally verified Maxwell's theory, almost two centuries have already passed. These great discoveries were a real revolution in science and humans never stopped inventing new

applications based on the electromagnetic fields that significantly influence the evolution of the human life. For the needs of this book we will try to give an overview and to localize the applications of electromagnetic fields in food thermal processes.

Dielectric radiation is a form of non-ionizing radiation that is able to produce thermal effects in food matrices. The basic principle is that in the dielectric range of frequencies the electromagnetic waves penetrate into the food and are then transformed into heat within the matrix. Although there is often a discordance regarding the spectral boundaries within the scientific community, dielectric heating methods are usually classified into two individual categories. Radiofrequency (RF) heating uses lower frequencies in the range of 1 to 300 MHz, while the second category is heating generated by microwave radiation in higher frequency waves ranging from 300 to 3000 MHz (Figure 5.1). As the latter ranges of frequencies are also used for telecommunication purposes, selected frequencies (table 1) are allowed to be used for industrial, scientific, and medical use (ISM radio bands) according to the Federal Communication Commission (FCC) and the European Telecommunication Standards Institute (ETSI) (Pereira and Vicente, 2010; Leadley, 2008).

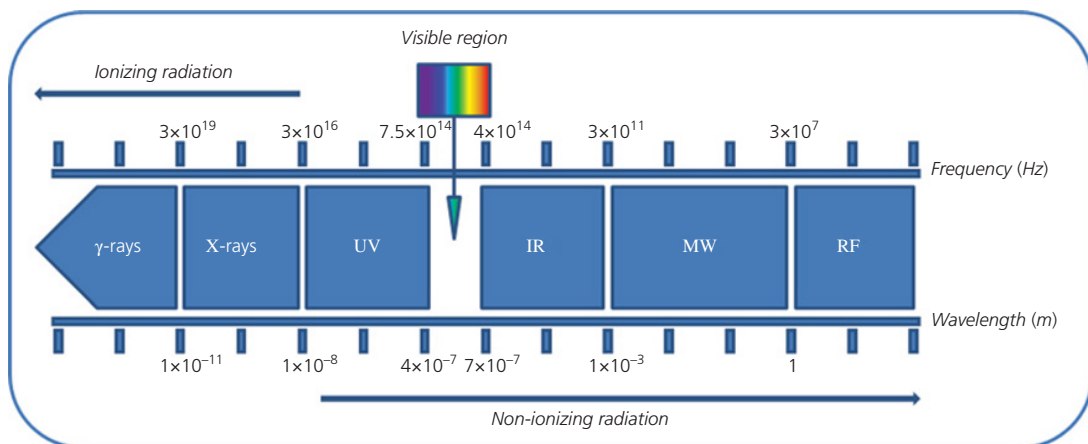


Figure 5.1 Electromagnetic spectrum with its various regions ordered by frequency and wavelength.

Table 5.1 Frequencies available for industrial, scientific, and medical uses (ISM radio bands).

	Central frequency (MHz)	Frequency range (MHz)
Radiofrequencies	13.560	13.553–13.567
	27.120	26.957–27.283
	40.680	40.660–40.700
Microwaves	915	902–928
	2450	2400–2500

5.1.6.1 Radiofrequency heating

As aforementioned, RF heating of food is carried out with the application of electromagnetic fields in the permitted frequencies shown in Table 5.1. RF heating commonly involves the application of a high-voltage alternating electric field to a medium. Dielectric materials contain electrons, atoms, ions, and molecules. Therefore, different types of interactions, both intrinsically as well as between the applied electromagnetic field and the constituent charged moieties, occur within the material. These interactions are described as electronic, atomic, ionic, and molecular displacements. More specifically the move of the electrons with respect to the positive nucleus of each atom can be defined as electronic displacement. Atomic displacement involves interactions between the atoms of each molecule or just between the atoms in the case of a material with a crystalline structure.

The two main mechanisms related to the RF heating are the ionic and the molecular displacements. When an electric field is applied to a dielectric material, the positive ions are forced to move to one direction while the negative charged ions move to the opposite direction. Therefore, in the case of an alternate electric field the ions tend to move forwards and backwards led by the enduring antistrophe of the polarity according to the frequency of the applied field. This internal turmoil driven by the indecisive external factor leads to the generation of heat within the product by friction. The aforementioned phenomenon is described as heat generated by ionic displacement. Molecular displacement involves molecules with a native

asymmetrical form and unbalanced charge distribution. These molecules are characterized by electric moment in addition to their autogenous electric field when an external electric field is applied. The molecules are polarized and attempt to realign with respect to the electric field vector. The oscillation of the polar molecules when an alternate electric field is applied is able to produce heat within the material due to friction forces.

Several studies have been carried out regarding the investigation of the dielectric properties, such as dielectric constant (ϵ'), dielectric loss factor (ϵ''), and penetration depth of various food matrices. The dielectric properties, which are often temperature-dependent, should be taken into account in order for a proper dielectric heating process to be designed.

Wang *et al.* (2003) applied radiofrequencies for heating whey protein gel, liquid whey protein mixture, macaroni noodles, cheese sauce, and macaroni and cheese. The frequencies of the waves they used were 27 and 40 MHz. The temperature ranged from 20 to 121.1°C, which are typical conditions used in commercial pasteurization and sterilization processes, whereas 121.1°C is commonly used as a reference temperature in thermal food processes. Although the authors did not provide any information regarding the microbiological impact of the heat treatments, they estimated extensively the dielectric properties of the aforementioned food matrices in a wide range of temperatures. In this study it was proposed that an RF sterilization process would be suitable for low acid foods, while fewer changes in terms

of quality were noticed compared to the retort-sterilized products.

A similar approach was taken in the study of Al-Holy *et al.* (2005). They determined the dielectric properties of untreated sturgeon and salmon caviar. The range of temperatures tested was 20–80°C, which are temperatures commonly used in pasteurization protocols. The impact of salt concentrations on the dielectric properties was studied. They reported that a large increase in ϵ' and ϵ'' was observed while increasing the temperature and the salt content, whereas the frequency of the applied waves had a greater influence on the penetration depth. The authors claimed that the results obtained from the thermobacteriological study provided the basis for designing a dielectric pasteurization protocol for the food matrices they tested. Nonetheless, no specific data for microbiological results are provided regarding the impact of the proposed process.

The main issues for all the thermal food processes by means of irradiation are the penetration depth and the uniformity of heating. Therefore, it would be helpful to provide microbiological data in processes that will verify the efficiency of each proposed food process.

More recently, Wainwright *et al.* (2010) evaluated the efficacy of RF power as an alternative to chemical disinfectants to inactivate *T. gondii* oocysts in water intended for public consumption. For their experiments they used a parallel plate cavity RF system, which consisted of two parallel metallic aluminum plates (46 cm × 60 cm × 2.5 cm) connected to a copper (coil resonance inductor and a capacitor) matching network. An RF system signal generator was connected with a wideband RF amplifier to deliver RF energy to the enclosed cavity. The samples of water with increased turbidity were treated as the turbidity had been shown to maximize the efficiency of the RF heating. In this study the different contaminated samples were heated in a range of temperatures from 50 to 80°C and the RF treating time ranged from 7 to 17 min with respect to the target temperature. Interesting results were acquired from

this study regarding the efficacy of RF treatment to the inactivation of *T. gondii*.

In 2013, Schlisselberg *et al.* carried out a research concerning the inactivation of food-borne pathogens in ground beef by cooking with highly controlled radiofrequency energy. Further than applying RF energy for cooking, the authors used a conventional heating cooking method for reference samples but they also combined RF with conventional heating for their study. The microbial populations used for the needs of this study were *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica*, whereas the contaminated food matrix was ground beef (20% w/w fat). The samples were shaped into balls (~4 cm radius). Unfortunately, their report is not supported by a detailed description of their experimental setup. The lowest and the highest transmitted frequencies were 800 and 1000 MHz, respectively, with a maximal energy of 1000 W. The authors compared three different cooking procedures, RF cooking, convection cooking (external heat only), and RF with convection cooking (combined treatment). All the samples were treated until the temperature on the core of them reached the target temperature of 73°C (±1°C). The heating times of the samples were 1 to 7.5 min, 1 to 40 min, and 1 to 5.5 min for RF, convection, and combined treatments, respectively. The RF cooking technology according to the authors combines the advantage of a reduced processing time and lower energy demands compared to conventional issues. The critical issue for all the new thermal food treatments that have to be covered is the safety of the product.

5.1.6.2 Microwave heating

During the last few decades, microwave radiation has been applied to various heating applications related to foods. Microwave heating has been used on drying, thawing, tempering, blanching as well as pasteurization and sterilization.

Microwave heating occurs due to the ability of some materials to convert the absorbed

microwave energy into heat. Mediated dipolar and ionic mechanisms are mainly responsible for the microwave heating of food matrices. The presence of water in the majority of the foods is related to the dielectric heating as the water dipole attempts to continuously reorient in the electromagnetic radiation's oscillating electric field.

Depending on the frequency, the dipole may move in time to the field, lag behind it, or remain apparently unaffected. In the case of free liquid water this movement occurs at GHz frequencies (microwaves) whereas it occurs at MHz (short radiowaves) and at kHz frequencies (long radiowaves) in "bound" water and ice, respectively (Chaplin, 2014). The high frequency of the applied electromagnetic radiation attempts to realign the water molecules numerous times per second. This vigorous realignment causes volumetric heating, mainly due to the internal friction of molecules (Chandrasekaran *et al.*, 2013). Biomolecules may also be influenced at microwave frequencies, but it is not considered that such resonant coupling is significant due to their relatively low energy (Chaplin, 2014).

For the needs of this chapter we will discuss the unit operations of microwave heating food processes in recent studies focused on microbiological impacts. Rodríguez-Marval *et al.* (2009) have published a research study regarding the impact of different power and time combinations of microwave oven on the inactivation of *Listeria monocytogenes* on inoculated and stored frankfurters. The samples consisted of 60% pork and 40% beef. A domestic microwave oven with 1100 W maximum power output at 2450 MHz was used for the experiments of this study. High or medium power levels of microwave treatments were followed. Time periods of microwave application were 30, 45, 60, 75 s and 60, 75 s at high (1100 W) and medium (550 W) power levels, respectively. The aforementioned heating treatments were carried out on samples that had been held in aerobic storage at 7°C for 0, 3, and 7 days. As the frankfurters were immersed in distilled water

during treatment, it can be assumed that the majority of the radiation energy was absorbed by water. This fact may be responsible for the limitations in temperature under some tested conditions.

In 2010, Cinquanta *et al.* (2010) proposed an improved pilot-scale microwave oven for the pasteurization of orange juice. The term pasteurization of orange juice mainly refers to the inactivation of pectin methyl esterase (PME; EC3.1.1.11), which is responsible for quality degradation and is more thermal resistant than the spoilage microorganisms contained in the juice. The pilot scale microwave oven was composed of a magnetron with 3 kW of absorbed electrical power and operated at a standard frequency of 2.45 GHz. The magnetron was centered at the side of an aluminum cubic chamber (1 m × 1 m × 1 m). The 250 ml orange juice samples were placed in the middle of the chamber in cylindrical beakers (7 cm diameter). The time-temperature combinations of the stirred orange juice treatment into the MW oven prototype were at 60°C for 2 and 5 min, at 70°C and at 75°C for 1, 2.5, and 5 min, and at 85°C for 0.25, 0.5, 1, and 2 min. The authors reported that under specific time-temperature combinations the proposed microwave process constitutes an effective technology for the stabilization of fresh orange juice through the reduction of PME activity.

The application of microwave radiation for the control of aflatoxins produced by the fungi *Aspergillus parasiticus* in hazelnuts was used by Basaran and Akran (2010). A household microwave oven was used in their study (1250 W; 2450 GHz). As samples of this study both kernels and in-shell hazelnuts were used. Samples were placed in the center of the rotated tray and forced air was used inside the cavity (0.2 m/s). They tested several different durations of microwave treatment (0, 30, 60, 90, 120 s). In all cases, each 30 s of radiation was followed by a time interval in which the samples were cooled to the initial temperature. The maximum temperature reached during the process was 55°C. The authors reported their

satisfaction concerning the efficiency of the proposed process regarding the reduction of the fungi's population, which in turn would lead to the minimization of the aflatoxin load.

A water-assisted microwave heating process has been employed for the inactivation of *Salmonella* Typhimurium in fresh vegetables (De la Vega-Miranda *et al.*, 2012). The working vegetables of this study were jalapeño pepper (*C. annuum* L.) and coriander foliage (*C. sativum* L.). The vegetables were immersed in distilled water at 1:1 and 1:1.5 (w/w) ratios for jalapeño pepper and coriander foliage, respectively. The output power of the microwave oven was 1200 W and the frequency was 2450 MHz. The authors treated the samples until the temperature of 63°C, which has been reported by the FDA as the target temperature for *Salmonella* inactivation. Maximum microwave power was used during the process aiming at minimizing the duration of the treatment. Their results seem to be promising regarding the inactivation of *Salmonella*, while their proposed process for preventing bacterial infections would be easy to follow in domestic use.

During the last few years microwave heating finds more and more applications in blanching of fruits and vegetables. Recently, an interesting study was published by Ruiz-Ojeda and Peñas (2013). They compared the conventional industrial hot water with microwave blanching of green beans (*Phaseolus vulgaris* L.). The microwave blanching of the green bean pods was carried out in a domestic microwave oven (900 W; 2450 GHz). Two beakers of water (50 ml) had been placed in the cavity of the oven in order to provide a water-saturated atmosphere and absorb part of the transmitted energy. Different combinations of nominal power level (650, 750, and 900 W) and processing time (50, 100, 150, 200, 250, and 300 s) were performed. In the conditions of their study, the authors reported that the optimal treatment time for microwave blanching of green beans was 125, 150, and 170 s at 900, 750, and 650 W nominal power, respectively. According to this study, the proposed process is advantageous because the

processing times are shorter and the energy demands lower compared to conventional blanching, whereas the overall quality of the blanched red beans was comparable to that of the commercial product.

In 2014, the inactivation of *Listeria monocytogenes* in a kiwifruit purée by conventional and microwave heating was studied by Benlloch-Tinoco *et al.* (2014). Like most studies in this field, a domestic microwave oven provided with a temperature recording system was used. Tests were performed at three different nominal power levels of 600, 900, and 1000 W with processing times varying between 50 and 340 s. The maximum temperature of treatment was circa 60°C. Their results showed that microwave treatment can potentially be more effective and a faster process for the inactivation of *Listeria monocytogenes* in a kiwifruit purée. It has to be mentioned that the temperature that was taken into account in this study was the temperature recorded at the coldest parts of the samples, although it would be interesting if the study also provided data about the temperature profile of the entire sample. Another recent study dealt with the inactivation of *Cronobacter sakazakii* in reconstituted powder infant formula milk by means of microwave treatment (Pina-Pérez *et al.*, 2014). The same experimental setup was used in the study of Benlloch-Tinoco *et al.* (2014). The processing conditions of the treatments were in the range of 400–900 W and 0–120 s for the nominal power level and time, respectively. The temperature at the cold point of the samples at the final time point (120 s) varied from 58.1 ± 3.1 to 88.1 ± 1.5 for the 400 to 900 W power levels. Their results indicated that MW use could be assumed as an effective method to inactivate *Cronobacter sakazakii* in powder infant formula milk when optimum conditions of treatment are being followed. According to their study, an application of 900 W for a time period of 60–120 s at 2.450 GHz would be the optimal conditions.

Valero *et al.* (2014) investigated the inactivation of *Salmonella enterica* serovar Enteritidis

in inoculated pasteurized potato omelet at different microwave heating treatments. The samples consisted of 67% moisture, 16.9% carbohydrates, 11% fat, and 5.1% proteins. Microwave heating was performed at four nominal power levels–time combinations: 300 W for 150 s, 450 W for 90 s, 600 W for 60 s, and 800 W for 45 s, by means of a commercial microwave oven. Temperatures reached levels above 70°C at 120, 60, and 40; with 35 s for treatment at 300, 450, 600, and 800 W, respectively. The aforementioned combinations of microwave treatment were mentioned as efficient for inactivation of the bacterium.

5.1.7 Infrared heating

Infrared (IR) is an invisible electromagnetic radiation (0.78–1000 μm) that is located between the visible light (0.38–0.78 μm) and the region of microwaves (1–1000 mm) in the electromagnetic spectrum (Figure 5.1). The region of infrared radiation is separated into three different subregions, the shorter wavelengths (0.78–1.4 μm) are called near-infrared (NIR), the medium wavelengths (1.4–3.0 μm) are called mid-infrared (MIR) and the longest infrared wavelengths (3.0–1000 μm) are known as far-infrared (FIR). Following the IR spectrum classification, the emitters used for IR applications can be generally divided into the three categories of short, medium, or long wave emitters. Infrared heating is an indirect method in which energy is applied to the surface of a material by radiation and then converted to heat. Radiation at wavelengths of FIR is referred to as advantageous for food processing due to the fact that most food components absorb radiative energy in this region (Sandu, 1986). Food substances such as water, proteins, and starches absorb far-IR energy most efficiently via the mechanism of changes in the molecular vibrational state (Rastogi, 2012). IR technology has found several applications, predominantly in the world of electronics. During the last few years, IR technology has attracted the interest of the scientific community more and more regarding new applications of infrared radiation on thermal food processes. In this subsection, modern studies

concerning the applications of infrared heating on food processes focusing on the impact on food-borne microorganisms will be presented. Power intensity, peak wavelength, temperature, type of microorganism, kind, and the size and shape of the food matrix are the main critical parameters related to microbial inactivation using IR heating methods.

Hamanaka *et al.* (2006) investigated the effect of the wavelength of IR heaters on the inactivation of bacterial spores (*Bacillus subtilis*) at various water activities (α_w). For the needs of their study an experimental chamber equipped with an IR heater was used. Three types of IR heaters were used (950, 1100, and 1150 nm; 0.5 kW). A stainless steel Petri dish inoculated with spores was positioned 15 cm below the IR source. The authors reported that the temperature of the stainless steel Petri dish reached circa 100°C after 120 s of IR irradiation in all the tested wavelengths. Unfortunately, this study is not provided with additional temperature data regarding the temperature of the organic matter. It is mentioned that the temperature of the organic material was expected to be considerably higher as it has a greater ability to absorb infrared rays. Their results regarding the survival of the bacterial spores indicated that the inactivation of *Bacillus subtilis* by IR heating was affected by both the wavelength of the IR radiation and the initial value of α_w . According to their study, the inactivating efficacy of IR heating against the bacterial spores was greater in the case of the shorter IR wavelength heater (950 nm).

In 2009, Huang *et al.* published their research concerning the inactivation kinetics of yeast cells, for which study the authors used a suite of predictive models with data obtained from infrared heating and drying experiments. The samples consisted of a *Saccharomyces cerevisiae* suspension and an infrared heater incorporated with an electronic balance was used as the experimental setup for this study. The maximum temperature reached during the experiments was 90°C. The authors verified the models for predicting the survival of yeast. It was found

that the simple Arrhenius equation could be used for the prediction of yeast survival when heat was applied without dehydration occurring. Recently, Ha and Kang (2013, 2014a, 2014b) published three interesting studies regarding the application of IR heating against food-borne microorganisms. More specifically, they investigated the inactivation kinetics of *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on ready-to-eat sliced ham by near-infrared (NIR) heating as a function of the radiation intensity (Ha and Kang, 2014a). At the sample location the radiation intensity of four NIR emitters was adjusted to around 100, 150, and 200 $\mu\text{W}/\text{cm}^2\text{nm}$. The times needed for reaching the target temperature of 87°C were 50, 75, and 95 s of treatments under 200, 150, and 100 $\mu\text{W}/\text{cm}^2\text{nm}$, respectively. These temperatures correspond to the surface temperature of the samples, but no data regarding the penetration depth was provided, as unfortunately they were slices of 2 mm. In their other two studies Ha and Kang (2013, 2014b) investigated the synergistic bactericidal effects of the simultaneous application of NIR heating and UV radiation on *Cronobacter sakazakii* in a powdered infant formula as well as on *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7 in powdered red pepper (*capsicum annuum* L.). For both studies they used the same equipment suitable for their powdered substrates. Their experimental setup consisted of a stainless chamber with a rotational mixer equipped with a quartz halogen infrared heating lamp and a UV germicidal lamp. The maximum wavelength of the NIR emitting source was about 1300 nm. Both the radiation sources were placed within aluminum reflectors in order for the radiation to be redirected and to be more efficiently and uniformly distributed. In the case of the red pepper powder differences between the temperature of the chamber and the core of the substrate during treatment were observed, whilst during the treatment of the powdered milk the disparity of the temperatures between the core of the sample

and the chamber's cavity was eliminated after the second minute of irradiation. More specifically, the infant powder milk core and the chamber reached circa 73°C after 7 min of treatment, while the red pepper powder core and the chamber reached 62°C and 75°C, respectively, after 5 min of treatment. In both studies the equal heating rate was observed between the simultaneous NIR-UV and the singlet NIR treatments. According to the authors the proposed method, based on the NIR-UV combined irradiation, can be applied as a potential alternative to other interventions that are used for the decontamination of powders.

5.2 Quantitative aspects and how unit operations impact on food-borne microorganisms

Quantitative methodologies, i.e., the use of mathematical and statistical models, are the main tools to describe the impact of a process as well as the storage conditions post-processing on the food stability. The food stability could be related to quality aspects such as appearance, color, aroma, taste, freshness, organoleptically assessed attributes, vitamin content, enzyme activity, level of spoilage microorganisms, as well as safety aspects such as the level of pathogenic microorganisms. For that purpose monitoring and control of critical parameters are essential factors under consideration when applying any type of thermal process. In this chapter the focus is on analyzing the responses of the microbiological attributes in relation to the applied thermal processing parameters.

Any quantitative analysis requires the careful selection and further assessment of specific parameters and their applied ranges on the studied microbial safety attributes. These parameters can be categorized in extrinsic and intrinsic parameters. An overview of these parameters for the presented unit operations of this chapter have been introduced in the previous sections and are summarized in Tables 5.2 and 5.3.

Table 5.2 Main extrinsic parameters of thermal processes.

Cooking/blanching pasteurization/ commerical sterilization	Ohmic heating	Radiofrequency	Microwave	Infrared heating
Time Temperature	Time Temperature Voltage gradient	Time Temperature Frequency	Time Temperature Frequency	Temperature Time

Table 5.3 Main intrinsic parameters of thermal processes.

Cooking/blanching pasteurization/ commerical sterilization	Ohmic heating	Radiofrequency	Microwave	Infrared heating
Time Temperature	pH Water activity Fat content Electric conductivity Composition	Ionic density Moisture Density Specific heat Dielectric constant Food composition Dielectric loss factor Penetration depth	Ionic density Moisture Density Specific heat Dielectric constant Food composition	Composition Moisture

As reported by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) all pasteurization processes need to be validated through the use of process authorities, challenge studies, predictive modeling, and/or safe harbors (Anonymous, 2006). Reference to predictive mathematical modeling is also made by the Commission Regulation No. 2073/2005 on the microbiological criteria for foodstuffs. These principles (i.e., challenge studies, predictive modeling, and/or safe harbors) could be considered for any of the thermal treatments described in this chapter, even if they are not only focusing on thermal pasteurized food products. Hereunder, a number of different quantitative approaches are reviewed.

5.3 Application of F-value concept

Traditional calculations of heat processes (sterilization, pasteurization, and cooking) are based on the popular *F-value* concept. This concept

was firstly introduced by Ball (1923). The *F-value* is defined as the time needed to achieve a required or observed reduction in microbial numbers at a reference temperature, or as any equivalent time–temperature combination yielding the same reduction:

$$F = 10^{(T(t)-T_{ref})/z} t \quad (5.1)$$

This parameter then reflects the efficiency of a time–temperature combination and is a relevant indicator for comparing the intensity of two heat treatments (Mafart, 2005; Mafart *et al.*, 2010). As the same authors discussed, if the actual *F-value* is measured after a thermal cycle, it should then be regarded as an assessment tool whereas the required *F-value*, which is determined from a necessary safety level, acts more as an optimization tool. Similarly, the *P-value* and the *C-value* are the corresponding thermal death values under pasteurization and cooking conditions, respectively (Pittia *et al.*, 2008; Mafart *et al.*, 2010; Valdramidis *et al.*, 2011).

If the process conditions are isothermal then the corresponding F-, P-, and C-values are given by Equation (5.1) (presented as the F-value).

This equation is transformed to the following equation when thermal treatments are applied under dynamic temperatures:

$$F = \int_0^t 10^{(T(t)-T_{ref})/z} dt \quad (5.2)$$

Application of this equation, also called the accumulated lethality can be found for different heat treatments (e.g., ohmic heating: Kamonpatana *et al.*, 2013; microwave heating: Benlloch-Tinoco *et al.*, 2014).

These two equations are valid if the survival curve obeys first-order kinetics. This means that microbial inactivation processes are described as log-linear and therefore a linear relationship between the logarithm of the microbial population level (in absolute value or relative to the initial value) and the treatment time are considered. This log linearity is traditionally described by the following equation:

$$\log \frac{N}{N_0} = -\frac{t}{D} \quad (5.3)$$

Herein, N represents the microbial cell density (cfu/ml), N_0 the initial microbial cell density (cfu/ml), and D (min) the decimal reduction time. If log-linearity is indeed observed, the thermal death time F-value is used as a basis for comparing heat sterilization procedures. This approach has been widely used, especially in the canning industry, for the so-called “12D process” of the proteolytic strains (Group I) of *Clostridium botulinum* spores (ICMSF, 1996; Stumbo, 1965). Despite the worldwide use, a lot of deviations from log-linearity have been observed (Geeraerd *et al.*, 2005). As other authors acknowledge, the success of the canning industry in using the F-value as a measure of the heat processes efficacy could be attributed to overprocessing and not to the calculation method’s correctness (Corradini and Peleg, 2006). These deviations are evident, particularly at lower temperatures than the

sterilization ones and for vegetative cells (Huang, 2009; Miller *et al.*, 2009; Valdramidis *et al.*, 2006, 2011).

5.4 Dealing with non-linearity

A way to tackle the non-linearity of the microbial survival or the lack of complete kinetic data is by relating the survival fraction directly to the process parameters. In order to assess the efficacy of a specific processing technology, the estimation of the time to achieve a certain number of log reductions can be applied. This has been previously discussed by some researchers (Buchanan *et al.*, 1993; Valdramidis *et al.*, 2005; Xiong *et al.*, 1999; Velliou *et al.*, 2011). Hulsheger *et al.* (1981) and Peleg (1995) applied these approaches either by using Fermi’s kinetic model representing the survivors fraction as a function of both the electric field strength and the number of pulses employed or by applying Hulsheger’s kinetic model to describe the kinetics of survival curves. In the latter case a linear relationship between the natural logarithm of the survivor’s fraction and the electric field strength as well as a linear relationship between the natural logarithm of the survivor’s fraction and the natural logarithm of the PEF treatment time was assumed.

In the last years the focus is on describing the complete kinetic data by using different types of non-linear curves typically discussed in Predictive Microbiology. The advantage of this approach is that the developed (validated) models can be applied to predict the microbial population at any time of the process within the experimental range of the conditions tested. An overview of inactivation models portraying eight common types of curves is given by Geeraerd *et al.* (2005). The main modeling structures include the use of Weibul type models, the shoulder log-linear with tail models and their reduced versions, the biphasic models, etc. In Table 5.4 some representative examples of models used to describe microbial kinetics during the discussed thermal processes are illustrated.

Table 5.4 Representative examples of kinetic models to describe microbial inactivation kinetics during thermal processing of food (model) systems.

Process	Model	Food (model) systems	Microorganisms	Reference
Cooking/ Blanching	Log-linear	Milk	<i>S. thermophiles</i> <i>Viable aerobes</i>	Sun <i>et al.</i> , 2008
Pasteurization/ commercial sterilization	Weibul	Soup	<i>B. sporothermodurans</i>	Periago <i>et al.</i> , 2004
Ohmic heating	Log-linear	carrot juice Milk	<i>B. licheniformis</i> <i>S. thermophiles</i> <i>Viable aerobes</i>	Tola and Ramaswamy, 2014 Sun <i>et al.</i> , 2008
Radiofrequency	Log-linear	Apple cider Dry milk	<i>E. coli</i> <i>C. sakazakii</i> <i>Salmonella</i> spp.	Geveke and Brunkhorst, 2008 Michael <i>et al.</i> , 2014
Microwave	Log-linear	Kiwifruit puree Apple juice	<i>L. monocytogenes</i> <i>S. cerevisiae</i> / <i>L. plantarum</i>	Benlloch-Tinoco <i>et al.</i> , 2014 Tajchakavit <i>et al.</i> , 1999
	Shoulder + log-linear	Alginate gel	<i>E. coli</i>	Hamoud-Agha <i>et al.</i> , 2013
Infrared heating	Log-linear + tail Log-logistic Weibul	Rice Sliced-ham	<i>A. flavus</i> <i>E. coli</i> / <i>S. Typhimurium</i> / <i>L. monocytogenes</i>	Wang <i>et al.</i> , 2014 Ha and Kang, 2014a

Table 5.5 Examples of comparative studies between conventional heating and other thermal processes applying log-linear inactivation kinetics (MH: microwave heating, CT: conventional thermal process, OH: ohmic heating).

Food product	Microorganism	Process	D-values	References
Kiwifruit puree	<i>L. monocytogenes</i>	MH (900 W) MH (1000 W) CT (60 °C)	$D_{60^\circ\text{C}} = 17.35 \text{ s}$ $D_{60^\circ\text{C}} = 17.04 \text{ s}$ $D_{60^\circ\text{C}} = 37.45 \text{ s}$	Benlloch-Tinoco <i>et al.</i> , 2014
Apple cider	<i>L. plantarum</i>	MH (700 W) CT (60 °C)	$D_{60^\circ\text{C}} = 3.8 \text{ s}$ $D_{60^\circ\text{C}} = 22 \text{ s}$	Tajchakavit <i>et al.</i> , 1999
Carrot juice (pH=6.2 with citric acid)	<i>B. licheniformis</i>	OH (4 kHz) CH	$D_{97^\circ\text{C}} = 1.6 \text{ min}$ $D_{97^\circ\text{C}} = 2 \text{ min}$	Tola and Ramaswamy, 2014
Milk	<i>S. thermophiles</i>	OH (20 kHz) CH	$D_{80^\circ\text{C}} = 0.16 \text{ min}$ $D_{80^\circ\text{C}} = 0.20 \text{ min}$	Sun <i>et al.</i> , 2008
Tomato juice	<i>S. coagulans</i>	OH (10 kHz) CH	$D_{95^\circ\text{C}} = 8.81 \text{ min}$ $D_{95^\circ\text{C}} = 10.1 \text{ min}$	Somavat <i>et al.</i> , 2008

Evidently the comparison between the different thermal processes in relation to their efficacy to inactivate microbial populations requires the use of similar modeling approaches or the estimation of parameters that can permit

a direct comparison. Current literature is based on log-linear kinetic approaches for comparison of thermal processes (see Table 5.5). From the presented examples it is evident that conventional heating is less efficient than the other

processes. Nevertheless, for the optimization and application of these technologies, incorporating knowledge of the impact on other food quality stability indices (vitamins, enzymes, proteins, etc.) is necessary.

Considering that for all these thermal processes the temperature inside the food product is not uniform, the microbial evaluation is better dealt with as a function of space coordinates. Thus, the overall destruction of bacteria during conventional, ohmic, radiofrequency, infrared, and microwave heating needs to be evaluated by coupling microbial models with heat transfer models. Some relevant examples appear in the literature where Hamoud-Agha *et al.* (2013) integrated the ordinary differential equations of the Geeraerd *et al.* (2000) model into the three-dimensional finite element model over the entire course of the heating process by microwave. Another example was also presented in the literature in the case of a continuous flow ohmic heater system (Kamonpatana *et al.*, 2013). Other numerical computation fluid dynamics-based models for thermal technologies are available in the literature but their further coupling with microbial models will be required in the future.

5.5 Development of new concepts to assess heat processes

The development of non-linear kinetic models raises the need of redefining the thermal death time by a modeling approach that includes the possibility of non-log-linear microbial survival curves for any kind of thermal treatment. As Mafart and Coroller (2012) discussed in these cases, the F-value (and similarly the P- or C-value) loses its additive character and conventional calculations can no longer be applied. Consequently, any assessment or optimization calculation requires a suitable model for determining the log-decrease values of the specific studied microorganisms, which retain their additive character. On that basis recent studies in the broader field of heat processing suggested

alternative approaches for evaluating the efficacy of a process when assuming that microbial heat resistances follow a Weibulian frequency distribution model (Corradini and Peleg, 2006; Mafart *et al.*, 2002; Sant'Ana *et al.*, 2009). These approaches took into account the convex and concave types of non-log-linearity. Similarly, Mafart and Coroller (2012) also demonstrated the determination of the F-value from a Weibul model. On the same line, Valdramidis *et al.* (2011) introduced the concept of the MH-value (mild heat value) as an alternative approach to the commonly used sterilization, pasteurization, and cook value (F -, P -, C -value), which is calculated to estimate the efficiency of a mild heat process. The MH-value is the time needed to achieve a predefined microbial reduction at a reference temperature and a known thermal resistant constant, z , for log-linear or specific types of non-log-linear microbial inactivation kinetics. The MH-value was developed by using the Geeraerd *et al.* (2000) shoulder-log linear kinetic model and a Bigelow type model (Bigelow, 1921) that describe the influence of the inactivation rate k_{max} in relation to the temperature. The equations read as follows:

$$MH = \int_0^t \left(\ln 10 \exp \frac{\ln 10}{z} (T(t) - T_{ref}) \right) \left(\frac{1}{1 + C_c(t)} \right) dt \quad (5.4)$$

$$\frac{dC_c}{dt} = -k_{max} (T(t)) C_c(t) \quad (5.5)$$

Herein, N represents the microbial cell density (cfu/ml), C_c is related to the physiological state of cells (-), k_{max} denotes the specific inactivation rate (1/min), while the asymptotic decimal reduction time (*AsymD*-value) (Juneja *et al.*, 2001) and the thermal resistance constant (z -value), i.e., the number of degrees Celsius change required to achieve a tenfold change in the *AsymD*-value, are the thermal inactivation parameters. Observe that the MH-value is given by a set of two equations in which the second describes the evolution of the microbial physiological state of the cells.

As Mafart and Coroller (2012) highlighted, this modification of the standard F-concept also changes its meaning; while the conventional F-value is a tool for the standard comparison of processes regardless of any target strain, the MH-value rather reflects the efficiency of a process on the reduction of a specific target strain. In future similar approaches will need to be developed or validated for a range of different microorganisms in order to be further considered and serve as alternatives to the classical F-, P-, and C-values of any thermal process.

5.6 Microbial safety and stability of heating operations: challenges and perspectives

The most common process in the food industry is the thermal process. In this chapter a number of different thermal processes has been reviewed and critical issues in relation to their efficacy and to their impact on the microbial inactivation is outlined. There are still some challenges that need to be addressed in relation to the microbial safety and stability of heating operations. As Condron *et al.* (2015) recently discussed, a harmonized methodology for the study of heat resistance of food pathogens is lacking. This may also require the harmonization of the experimental design protocols and the validation procedures for heat treatment studies. All these could provide information to allow international agreement on appropriate risk management of emerging potential hazards for human and animal health. In the same paper the authors proceeded by proposing steps of harmonization to be considered for the selection and preparation of challenge organisms, the preparation of challenge culture(s), the development of experimental design, the thermal treatment apparatus, the analysis/interpretation of data and the thermal inactivation models. If this harmonization is implemented during the coming years it will also permit a more direct comparison of the efficacy of all the thermal processes discussed in this chapter.

Finally, and as discussed before, considering that for all these thermal processes the temperature inside the food product is not uniform, the microbial evaluation is better to be dealt with as a function of space coordinates. Thus, the overall destruction of bacteria during conventional, ohmic, radiofrequency, infrared, and microwave heating will need to be further evaluated by coupling microbial models with heat transfer models.

References

- Al-Holy, M., Wang, Y., Tang, J., and Rasco, B. 2005. Dielectric properties of salmon (*Oncorhynchus keta*) and sturgeon (*Acipenser transmontanus*) caviar at radio frequency (RF) and microwave (MW) pasteurization frequencies. *Journal of Food Engineering*, **70**, 564–570.
- Anonymous. 2006. Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization — National Advisory Committee on Microbiological Criteria for Foods. *Journal of Food Protection*, **69**, 1190–1216.
- Baldwin, D.E. 2012. Sous vide cooking: a review. *International Journal of Gastronomy and Food Science*, **1**, 15–30.
- Ball, C. 1923. *Thermal Process Time for Canned Food*. Bull, Washington, DC, USA.
- Basaran, P. and Akhan, Ü. 2010. Microwave irradiation of hazelnuts for the control of aflatoxin producing *Aspergillus parasiticus*. *Innovative Food Science and Emerging Technologies*, **11**, 113–117.
- Benlloch-Tinoco, M., Pina-Pérez, M.C., Martínez-Navarrete, N., and Rodrigo, D. 2014. *Listeria monocytogenes* inactivation kinetics under microwave and conventional thermal processing in a kiwifruit puree. *Innovative Food Science and Emerging Technologies*, **22**, 131–136.
- Bigelow, W. 1921. The logarithmic nature of thermal death curves. *Journal of Infectious Diseases*, **29**, 528–538.
- Buchanan, R.L., Golden, M.H., and Whiting, R.C. 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics

- of *Listeria monocytogenes*. *Journal of Food Protection*, **56**, 474–478.
- Chandrasekaran, S., Ramanathan, S., and Basak, T. 2013. Microwave food processing – a review. *Food Research International*, **52**, 243–261.
- Chaplin M. 2014. *Water and Microwaves*. Available at: <http://www1.lsbu.ac.uk/water/microwave.html>.
- Cinquanta, L., Albanese, D., Cuccurullo, G., and Di Matteo, M. 2010. Effect on orange juice of batch pasteurization in an improved pilot-scale microwave oven. *Journal of Food Science*, **75**, 46–50.
- Condrón, R., Farrokh, C., Jordan, K., McClure, P., Ross, T., and Cerf, O. 2015. Guidelines for experimental design protocol and validation procedure for the measurement of heat resistance of microorganisms in milk. *International Journal of Food Microbiology*, **192**, 20–25.
- Corradini, M.G. and Peleg, M. 2006. Prediction of vitamins loss during non-isothermal heat processes and storage with non-linear kinetic models. *Trends in Food Science and Technology*, **17**, 24–34.
- De La Vega-Miranda, B., Santiesteban-López, N.A., López-Malo, A., and Sosa-Morales, M.E. 2012. Inactivation of *Salmonella* Typhimurium in fresh vegetables using water-assisted microwave heating. *Food Control*, **26**, 19–22.
- De Paula, C.M.D., Mariot, R.F., and Tondo, E.C. 2005. Thermal inactivation of *Salmonella enteritidis* by boiling and frying egg methods. *Journal of Food Safety*, **25**, 43–57.
- Dictionary of Food Science and Technology. 2005. International Food Information Service Editor. Blackwell Publishing, Oxford.
- Dipersio, P.A., Kendall, P.A., Yoon, Y., and Sofos, J.N. 2007. Influence of modified blanching treatments on inactivation of *Salmonella* during drying and storage of carrot slices. *Food Microbiology*, **24**, 500–507.
- Fellows, P.J. 2009. *Food Processing Technology – Principles and Practice*, 23rd edn. Woodhead Publishing Limited and CRC Press LCC, Cambridge, UK.
- Geeraerd, A.H., Herremans, C.H., Van Impe, J.F. 2000. Structural model requirements to describe microbial inactivation during mild heat treatment. *International Journal of Food Microbiology*, **59**, 185–209.
- Geeraerd, A.H., Valdramidis, V.P., and Van Impe, J.F. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102**, 95–105.
- Geveke, D.J. and Brunkhorst, C. 2008. Radio frequency electric fields inactivation of *Escherichia coli* in apple cider. *Journal of Food Engineering*, **85**, 215–221.
- Gomes, C.L., Pflanzner, S.B., de Felício, P.E., and Bolini, H.M.A. 2014. Temporal changes of tenderness and juiciness of beef strip loin steaks. *LWT – Food Science Technology*, **59**, 629–634.
- Goullieux, A. and Pain, J.-P. 2014. Ohmic heating, Chapter 22. In: *Emerging Technologies for Food Processing* (ed. Da Wen Sun), 2nd edn, pp. 399–426. Academic Press-Elsevier, San Diego, CA, USA.
- Ha, J.-W. and Kang, D.-H. 2013. Simultaneous near-infrared radiant heating and UV radiation for inactivating *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in powdered red pepper (*Capsicum annuum* L.). *Applied and Environmental Microbiology*, **79**, 6568–6575.
- Ha, J.-W. and Kang, D.-H. 2014a. Inactivation kinetics of *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and *Listeria monocytogenes* in ready-to-eat sliced ham by near-infrared heating at different radiation intensities. *Journal of Food Protection*, **77**, 1224–1228.
- Ha, J.-W. and Kang, D.-H. 2014b. Synergistic bactericidal effect of simultaneous near-infrared radiant heating and UV radiation against *Cronobacter sakazakii* in powdered infant formula. *Applied and Environmental Microbiology*, **80**, 1858–1863.
- Hamanaka, D., Uchino, T., Furuse, N., Han, W., and Tanaka, S.I. 2006. Effect of the wavelength of infrared heaters on the inactivation of bacterial spores at various water activities. *International Journal of Food Microbiology*, **108**, 281–285.
- Hamoud-Agha, M.M., Curet, S., Simonin, H., and Boillereaux, L. 2013. Microwave inactivation of *Escherichia coli* K12 CIP 54.117 in a gel medium: experimental and numerical study. *Journal of Food Engineering*, **116**, 315–323.

- Harris, L.J., Uesugi, A.R., Abd, S.J., McCarthy, K.L. 2012. Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. *Food Research International*, **45**, 1093–1098.
- Holdsworth S.D. 2009. Chapter 1. Principles of thermal processing: sterilization. In: *Engineering Aspects of Thermal Food Processing*. (Eds R. Simpson), Contemporary Food Engineering Series edn, pp. 3–11, CRC Press, Taylor and Francis Group, USA.
- Huang, L. 2009 Thermal inactivation of *Listeria monocytogenes* in ground beef under isothermal and dynamic temperature conditions. *Journal of Food Engineering*, **90**, 380–387.
- Huang, H., Brooks, M.S.-L., Huang, H.-J., and Chen, X.D. 2009. Inactivation kinetics of yeast cells during Infrared drying. *Drying Technology*, **27**, 1060–1068.
- Hulshager, H., Potel, J., and Niemann, E.G. 1981. Killing of bacteria with electric pulses of high-field strength. *Radiation and Environmental Biophysics*, **20**, 53–65.
- Jakób, A., Bryjak, J., Wójtowicz, H., Illeová, V., Annus, J., and Polakovič, M. 2010. Inactivation kinetics of food enzymes during ohmic heating. *Food Chemistry*, **123**, 369–376.
- Jiménez-Monreal, A.M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., and Murcia, M.A. 2009. Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, **74**, 97–103.
- Juneja, V.K., Eblen, B.S., and Marks, H.M. 2001. Modeling non-linear survival curves to calculate thermal inactivation of *Salmonella* in poultry of different fat levels. *International Journal of Food Microbiology*, **70**, 37–51.
- Juneja, V.K., Porto-Fett, A.C.S., Call, J.E., Marks, H.B., Tamplin, M.L., and Luchansky, J.B. 2010. Thermal inactivation of *Bacillus anthracis* Sterne in irradiated ground beef heated in a water bath or cooked on commercial grills. *Innovative Food Science and Emerging Technologies*, **11**, 123–129.
- ICMSF. 1996. *ICMSF: Microorganisms in Foods 5, Characteristics of Microbial Pathogens*. Blackie Academic and Professional, London.
- Kamonpatana, P., Mohamed, H.M.H., Shynkaryk, M., Heskitt, B., Yousef, A.E., Sastry, S.K. 2013. Mathematical modeling and microbiological verification of ohmic heating of a multicomponent mixture of particles in a continuous flow ohmic heater system with electric field parallel to flow. *Journal of Food Science*, **78**, 1721–1734.
- Konagaya, Y., Urakami, H., Hoshino, J., et al. 2009. Change of thermal inactivation of *Clostridium botulinum* spores during rice cooking. *Journal of Food Protection*, **72**, 2400–2406.
- Leadley, C. 2008. Novel commercial preservation methods. In: *Biodeterioration and Preservation* (ed. G. Tucker). Blackwell Publishing Ltd, Oxford, UK.
- Lee, S.-Y., Sagong, H.-G., Ryu, S., and Kang, D.-H. 2012. Effect of continuous ohmic heating to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in orange juice and tomato juice. *Journal of Applied Microbiology*, **112**, 723–731.
- Lee, S.-Y., Ryu, S., Kang, D.-H. 2013. Effect of frequency and waveform on inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium in salsa by ohmic heating. *Applied and Environmental Microbiology*, **79**, 10–17.
- Luchansky, J.B., Porto-Fett, A.C.S., Shoyer, B., Phebus, R.K., Thippareddi, H., and Call, J.E. 2009. Thermal inactivation of *Escherichia coli* O157:H7 in blade-tenderized beef steaks cooked on a commercial open-flame gas grill. *Journal of Food Protection*, **72**, 1404–1411.
- Mafart, P. 2005. Food engineering and predictive microbiology: on the necessity to combine biological and physical kinetics. *International Journal of Food Microbiology*, **100**, 239–251.
- Mafart, P. and Coroller, L. 2012. Sterilisation–pasteurisation values taking into account microbial non-linearities. In: *Progress on Quantitative Approaches of Thermal Food Processing* (eds Valdramidis, V.P. and Van Impe, J.F.), pp. 257–270. Nova Science Publishers, USA.
- Mafart, P., Couvert, O., Gaillard, S., and Leguerinel, I. 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology*, **72**, 107–113.

- Mafart, P., Leguérinel, I., Couvert, O., and Coroller, L. 2010. Quantification of spore resistance for assessment and optimization of heating processes: a never-ending story. *Food Microbiology*, **27**, 568–572.
- Michael, M., Phebus, R.K., Thippareddi, H., Subbiah, J., Birla, S.L., and Schmidt, K.A. 2014. Validation of radio-frequency dielectric heating system for destruction of *Cronobacter sakazakii* and *Salmonella* species in nonfat dry milk. *Journal of Dairy Science*, **97**, 7316–7324.
- Miller, N.F. and Wetterstrom, W. 2000. The beginnings of agriculture: the ancient Near East and North Africa. In: *The Cambridge World History of Food* (eds Kiple, K.F. and Ornelas, K.C.), pp. 1123–1139. Cambridge University Press, Cambridge, UK.
- Miller, F.A., Gil, M.M., Brandão, T.R.S., Teixeira, P., and Silva, C.L.M. 2009. Sigmoidal thermal inactivation kinetics of *Listeria innocua* in broth: influence of strain and growth phase. *Food Control*, **20**, 1151–1157.
- Park, S.H., Balasubramaniam, V.M., Sastry, S.K., and Lee, J. 2013. Pressure–ohmic–thermal sterilization: a feasible approach for the inactivation of *Bacillus amyloliquefaciens* and *Geobacillus stearothermophilus* spores. *Innovative Food Science and Emerging Technologies*, **19**, 115–123.
- Peleg, M. 1995. A model of microbial survival after exposure to pulsed electric-fields. *Journal of the Science of Food and Agriculture*, **67**, 93–99.
- Pereira, R.N. and Vicente, A.A. 2010. Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International*, **43**, 1936–1943.
- Periago, P.M., Van Zuijlen, A., Fernandez, P.S., et al. 2004. Estimation of the non-isothermal inactivation patterns of *Bacillus sporothermodurans* IC4 spores in soups from their isothermal survival data. *International Journal of Food Microbiology*, **95**, 205–218.
- Phungamngoen, C., Chiewchan, N., and Devahastin, S. 2013. Effects of various pretreatments and drying methods on *Salmonella* resistance and physical properties of cabbage. *Journal of Food Engineering*, **115**, 237–244.
- Pina-Pérez, M.C., Benlloch-Tinoco, M., Rodrigo, D., and Martinez, A. 2014. *Cronobacter sakazakii* inactivation by microwave processing. *Food and Bioprocess Technology*, **7**, 821–828.
- Pittia, P., Furlanetto, R., Maifreni, M., Tassan Mangina, F., and Dalla Rosa, M. 2008. Safe cooking optimisation by F-value computation in a semi-automatic oven. *Food Control*, **19**, 688–697.
- Rastogi, N.K. 2012. Recent trends and developments in infrared heating in food processing. *Critical Reviews in Food Science and Nutrition*, **52**, 737–760.
- Rinaldi, M., Dall'Asta, C., Meli, F., et al. 2013. Physicochemical and microbiological quality of sous-vide-processed carrots and Brussels sprouts. *Food and Bioprocess Technology*, **6**, 3076–3087.
- Rodríguez-Marval, M., Geornaras, I., Kendall, P.A., Scanga, J.A., Belk, K.E., and Sofos, J.N. 2009. Microwave oven heating for inactivation of *Listeria monocytogenes* on frankfurters before consumption. *Journal of Food Science*, **74**, 453–460.
- Roldán, M., Antequera, T., Martín, A., Isabel, A., and Ruiz, J. 2013. Effect of different temperature – time combinations on physicochemical, microbiological, textural and structural features of sous-vide cooked lamb loins. *Meat Science*, **93**, 572–578.
- Ruiz-Ojeda, L.M. and Peñas, F.J. 2013. Comparison study of conventional hot-water and microwave blanching on quality of green beans. *Innovative Food Science and Emerging Technologies*, **20**, 191–197.
- Sagong, H.-G., Park, S.-H., Choi, Y.-J., Ryu, S., and Kang, D.-H. 2011. Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in orange and tomato juice using ohmic heating. *Journal of Food Protection*, **74**, 899–904.
- Sandu, C. 1986. Infrared radiative drying in food engineering: a process analysis. *Biotechnology Progress*, **2**, 109–119.
- Sant'Ana, A. S., Rosenthal, A., and Massaguer, P.R. 2009. Heat resistance and the effects of continuous pasteurization on the inactivation of *Byssoschlamys fulva* ascospores in clarified apple juice. *Journal of Applied Microbiology*, **107**, 197–209.

- Schlisselberg, D.B., Kler, E., Kalily, E., Kisluk, G., Karniel, O., and Yaron, S. 2013. Inactivation of foodborne pathogens in ground beef by cooking with highly controlled radio frequency energy. *International Journal of Food Microbiology*, **160**, 219–226.
- Shen, C., Geornaras, I., Belk, K.E., Smith, G.C., and Sofos, J.N. 2011. Inactivation of *Escherichia coli* O157:H7 in moisture-enhanced nonintact beef by pan-broiling or roasting with various cooking appliances set at different temperatures. *Journal of Food Science*, **76**, 64–71.
- Skåra, T., Stormo, S.K., Skipnes, D., et al. 2014a. Estimation of surface temperature and thermal load in short-time heat treatment of surimi through reflectance spectroscopy and heat transfer modeling. *Journal of Food Engineering*, **120**, 75–80.
- Skåra, T., Valdramidis, V.P., Rosnes, J.T., Noriega, E., and Van Impe, J.F.M. 2014b. A novel model to assess the efficacy of steam surface pasteurization of cooked surimi gels inoculated with realistic levels of *Listeria innocua*. *Food Microbiology*, **44**, 64–70.
- Somavat, R., Mohamed, H.M.H., Chung, Y.-K., Yousef, A.E., and Sastry, S.K. 2012. Accelerated inactivation of *Geobacillus stearothermophilus* spores by ohmic heating. *Journal of Food Engineering*, **108**, 69–76.
- Somavat, R., Mohamed, H.M.H., and Sastry, S.K. 2013. Inactivation kinetics of *Bacillus coagulans* spores under ohmic and conventional heating. *LWT – Food Science and Technology*, **54**, 194–198.
- Steele, J.H. 2000. History, trends, and extent of pasteurization. *Journal of the American Veterinary Medical Association*, **217**, 175–178.
- Stoeckel, M., Abduh, S.B.M., Atamer, Z., and Hinrichs, J. 2014. Inactivation of *Bacillus* spores in batch vs continuous heating systems at sterilisation temperatures. *International Journal of Dairy Technology*, **67**, 334–341.
- Stumbo, C.R. 1965. *Thermobacteriology in Food Processing*. Academic Press, New York, USA.
- Sun, H., Kawamura, S., Himoto, J.-I., Itoh, K., Wada, T., and Kimura, T. 2008. Effects of ohmic heating on microbial counts and denaturation of proteins in milk. *Food Science and Technology Research*, **14**, 117–123.
- Tajchakavit, S., Ramaswamy, H.S., and Fustier, P. 1999. Enhanced destruction of spoilage microorganisms in apple juice during continuous flow microwave heating. *Food Research International*, **31**, 713–722.
- Tola, Y.B. and Ramaswamy, H.S. 2014. Thermal destruction kinetics of *Bacillus licheniformis* spores in carrot juice extract as influenced by pH, type of acidifying agent and heating method. *LWT – Food Science and Technology*, **56**, 131–137.
- Valdramidis, V.P., Bernaerts, K., Van Impe, J.F., and Geeraerd, A.H. 2005. An alternative approach to non-log-linear thermal microbial inactivation: modelling the number of log cycles reduction with respect to temperature. *Food Technology and Biotechnology*, **43**, 321–327.
- Valdramidis, V.P., Geeraerd, A.H., Bernaerts, K., and Van Impe, J.F. 2006. Microbial dynamics versus mathematical model dynamics: the case of microbial heat resistance induction. *Innovative Food Science and Emerging Technologies*, **7**, 80–87.
- Valdramidis, V.P., Geeraerd, A.H., Tiwari, B.K., Cullen, P.J., Kondjoyan, A., and Van Impe, J.F. 2011. Estimating the efficacy of mild heating processes taking into account microbial non-linearities: a case study on the thermisation of a food simulant. *Food Control*, **22**, 137–142.
- Valero, A., Cejudo, M., and García-Gimeno, R.M. (2014) Inactivation kinetics for *Salmonella* Enteritidis in potato omelet using microwave heating treatments. *Food Control*, **43**, 175–182.
- Vegara, S., Martí, N., Mena, P., Saura, D., and Valero, M. 2013. Effect of pasteurization process and storage on color and shelf-life of pomegranate juices. *LWT – Food Science and Technology*, **54**, 592–596.
- Velliou, E.G., Van Derlinden, E., Cappuyns, A.M., et al. 2011. Towards the quantification of the effect of acid treatment on the heat tolerance of *Escherichia coli* K12 at lethal temperatures. *Food Microbiology*, **28**, 702–711.

- Vishwanathan, K.H., Giwari, G.K., Hebbar, H.U. 2013. Infrared assisted dry-blanching and hybrid drying of carrot. *Food and Bioproducts Processing*, **91**, 89–94.
- Wainwright, K.E., Lagunas-Solar, M., Miller, M.A., *et al.* 2010. Radiofrequency-induced thermal inactivation of *Toxoplasma gondii* oocysts in water. *Zoonoses and Public Health*, **57**, 74–81.
- Wang, Y., Wig, T.D., Tang, J., and Hallberg, L.M. 2003. Dielectric properties of foods relevant to RF and microwave pasteurization and sterilization. *Journal of Food Engineering*, **57**, 257–268.
- Wang, B., Khir, R., Pan, Z., *et al.* 2014. Effective disinfection of rough rice using infrared radiation heating. *Journal of Food Protection*, **77**, 1538–1545.
- Witthuhn, M., Lücking, G., Atamer, Z., Ehling-Schulz, M., and Hinrichs, J. 2011. Thermal resistance of aerobic spore formers isolated from food products. *International Journal of Dairy Technology*, **64**, 486–493.
- Xiong, R., Xie, G., Edmondson, A.E., and Sheard, M.A. 1999. A mathematical model for bacterial inactivation. *International Journal of Food Microbiology*, **46**, 45–55.
- Yildiz-Turp, G., Sengun, I.Y., Kendirci, P., and Icier, F. 2013. Effect of ohmic treatment on quality characteristic of meat: a review. *Meat Science*, **93**, 441–448.
- Zeki, B. 2013. Frying baking roasting, Chapter 24. In: *Food Process Engineering and Technology*, 2nd edn, pp. 583–589. Academic Press – Elsevier, USA.

CHAPTER 6

Impact of refrigeration operations on the microbial ecology of foods[†]

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6.1 Introduction

The growth and survival of microorganisms in perishable foods, as with many other living organisms, is significantly affected by temperature. For each microorganism in a food product, it may exhibit three characteristic temperatures, i.e., minimum, optimum, and maximum temperatures ($T_{\min}$, T_{opt} , and $T_{\max}$) that define the range and rate that a microorganism can grow. Above $T_{\max}$ and below $T_{\min}$, a microorganism does not grow, but may survive or gradually die off. Between $T_{\min}$ and $T_{\max}$, a microorganism can actively grow and divide. The rate of microbial growth is dependent on the temperature relative to T_{opt} , the optimum growth temperature. At this temperature, the microbial growth reaches the highest rate. Below T_{opt} , the microbial growth rate increases with temperature, and it decreases at temperatures above T_{opt} . To preserve the quality and to extend the shelf life of a food product, it is often necessary to keep its temperature close to or even below $T_{\min}$ in order to decrease or stop the microbial growth.

Refrigeration is a general term for a physical process in which the temperature of an object, such as a food product, is decreased to an acceptable level, thus reducing the growth rate

of both spoilage and pathogenic microorganisms in food products. Because of low-temperature storage, the deterioration caused by many chemical and biochemical reactions, such as enzymatic reactions, is effectively slowed down, thus prolonging the shelf life of perishable food products. Since refrigeration removes thermal energy from the products, the temperature can be sufficiently decreased to cause a phase change in water, turning it from liquid to ice. For water under 1 atmosphere, the freezing point is 0°C. If a cooling process allows a product to chill to below 0°C, this process is a freezing process. Modern commercial freezing allows cooling products to −18°C or below, thus effectively preventing most microorganisms from growing and preserving the quality of the food in the frozen state. In many laboratory environments, biological samples are frozen to a lower storage temperature, such as −80°C, to preserve biological activities. For more critical applications, a cryogenic agent, such as liquid nitrogen, is used to keep samples at −196°C.

While refrigeration is a physical general term for removing heat from an object, chilling is a dynamic process that actually reduces the temperature of a product to the desired holding temperature just above the freezing point of

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water (Earle and Earle, 1983). After reaching the desired holding temperature, the product remains at this temperature in cold storage. Many fresh produce, raw and processed meats, and other products such as milk require cold storage to extend the shelf life. Unlike frozen storage, some microorganisms are still active in cold storage temperatures. The time and temperature of cold storage determines the shelf life of perishable goods.

6.2 Refrigeration as a unit operation

6.2.1 Basic principles of refrigeration

Refrigeration requires conversion of energy. A modern mechanical refrigeration system consists of four basic components, compressor, condenser, expansion valve, and evaporator (Figure 6.1). Flowing in the system is a refrigerant that is critical in the conversion of thermal energy. The whole refrigeration system can be basically divided into two sections,

the high-temperature/high-pressure (HTHP) section and the low-temperature/low-pressure (LTLP) section (Figure 6.1). Central to the HTHP section is the compressor, which receives LTLP vapor of the refrigerant and compresses it to form HTHP vapor. The HTHP refrigerant vapor is discharged to the condenser, in which the refrigerant is converted to HTHP liquid. The condensation of vapor releases heat from the refrigerant vapor, which is carried away from the condensers by mechanical fans. The HTHP refrigerant in the liquid form passes through the expansion valve, changing into low-pressure microdroplets at a lower temperature. The low-pressure refrigerant droplets evaporate in the evaporator, causing the refrigerant to change from the liquid form into the gas form. The conversion of the refrigerant from liquid to gas absorbs large amounts of latent heat from the environment, causing its temperature to drop. The evaporator is located in a refrigeration room, storage house, or freezing tunnel. Air circulation provided by mechanical fans is needed outside the evaporator to transfer the

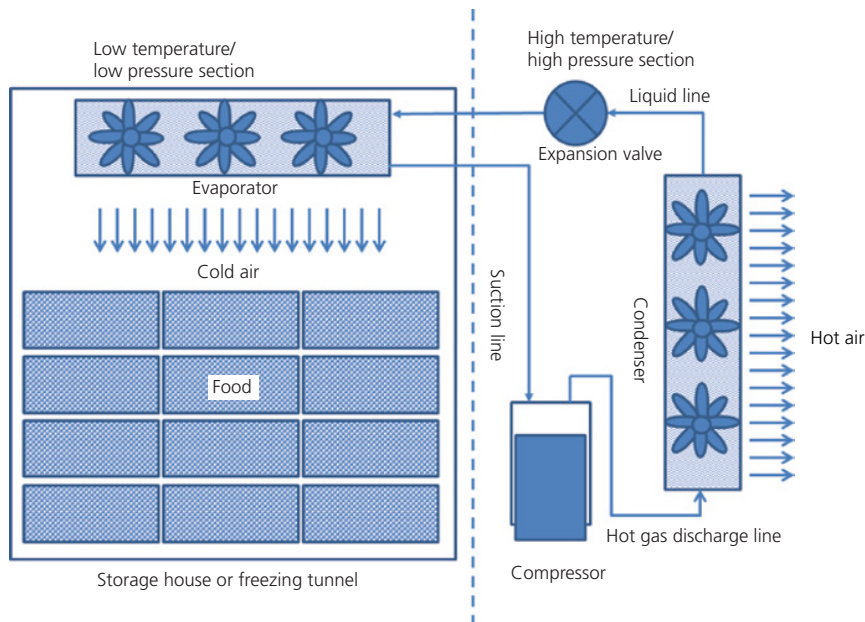


Figure 6.1 A basic refrigeration system.

energy from the evaporator to the surrounding environment. The LTLP refrigerant vapor is circulated through the suction line back to the compressor and completes a refrigeration cycle (Figure 6.1). Modern refrigeration systems may differ in size and complexity, but the basic principles remain the same. An air blast freezer uses high-speed air to quickly transfer the energy from the condenser to foods for rapid chilling and freezing. Forced air cooling is used in the storage house to chill and store perishable goods.

6.2.2 Changes in the microorganisms during freezing

Compared to refrigerated storage, freezing provides long-term preservation of perishable goods. While effective in preserving perishable goods, freezing, unfortunately, may also preserve microorganisms. As a result, many microorganisms can be found in frozen foods (Querol and Fleet, 2006; Gracey *et al.*, 1999; Erickson

and Hung, 1997; Dave and Ghaly, 2011). Freezing involves a change of phase in water and conversion of liquid water into ice. During freezing, ice crystals may form, aggregate, and grow in size in the foods. The formation of ice crystals, if large enough, may exert pressure on and pierce biological cells. The formation of ice crystals may also cause concentration of soluble solids, creating a microenvironment that is hostile to microorganisms. Therefore, the freezing process, if not done properly, may cause serious damage to the structure and biological integrity of biological materials (Van Venrooij *et al.*, 1975). As a result, microorganisms exposed to freezing may experience limited initial decrease in population in the initial stage of frozen storage due to inactivation or sublethal injury, after which the microbial population may stabilize during extended storage (Figure 6.2). The initial decline in population may be affected by the chilling rate, types of microorganisms, and chemical compositions of the food.

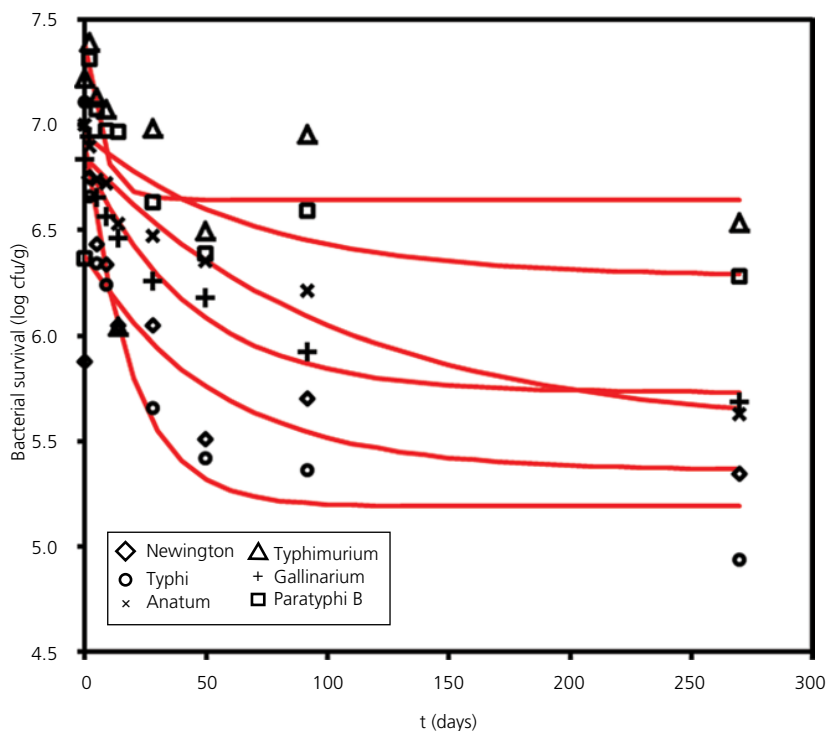


Figure 6.2 Survival of *Salmonella* spp. in chicken chow mein at -25.5°C (Jay *et al.*, 2005; original data source: Gunderson and Rose, 1948).

Table 6.1 Kinetic parameters of survival of *Salmonella* spp. in chicken chow mein at -25.5°C (Jay *et al.*, 2005; original data source: Gunderson and Rose, 1948).

Serovar	k (day^{-1})	N_0 (log cfu/g)	ΔN_m (log cfu/g)
<i>S. Newington</i>	1.88×10^{-2}	6.38	1.02
<i>S. Typhimurium</i>	1.48×10^{-2}	7.39	0.743
<i>S. Typhi</i>	5.20×10^{-2}	6.91	1.72
<i>S. Gallinarium</i>	2.88×10^{-2}	6.93	1.10
<i>S. Anatum</i>	9.68×10^{-3}	6.85	1.29
<i>S. Paratyphi B</i>	1.47×10^{-2}	6.95	0.671

The dynamic change of the initial decline and survival of microorganisms during frozen storage shown in Figure 6.2 can be described by the following mathematical equation:

$$N = (N_0 - \Delta N_m) + \Delta N_m e^{-kt} \quad (6.1)$$

where N_0 (log cfu/g) is the concentration of microorganisms before freezing, N (log cfu/g) is the concentration of microorganisms at storage time t (day), ΔN_m (log cfu/g) is the maximum reduction in the microbial population during frozen storage, and k (day^{-1}) is the rate of microbial inactivation. This equation suggests that the population of microorganisms would experience an exponential decline before it stabilizes. For microorganisms in Figure 6.2, the rate (k) and extent (ΔN_m) of inactivation are affected by the serovars of *Salmonella* (Table 6.1). Compared to other serovars, *S. Typhi*, having the largest k and ΔN_m , is more sensitive to freezing.

6.2.3 Changes in the microorganisms during refrigerated storage

Refrigerated storage refers to storing materials under low temperatures without freezing. In the food industry and at home, refrigerated storage is used to store mostly fresh produce, juice, milk and dairy products, raw and processed meats, and other perishable foods in order to preserve the freshness and extend the shelf life. Compared to frozen storage, the shelf life of products in refrigerated storage may be substantially shorter, but the products have much fresher quality attributes due to avoidance of the formation of

ice crystals. While most fresh perishable meats, fish, fruits, and vegetables can be at $1\text{--}4^{\circ}\text{C}$ for shelf life extension, some fruits and vegetables such as bananas and cucumbers must be stored around 10°C to prevent chilling injury (Cengel and Ghajar, 2011). Most of the microorganisms that can grow well at this refrigerated temperature range ($>0^{\circ}\text{C}$ but $<10^{\circ}\text{C}$) are psychrotrophs and mesophiles, and some of them are pathogenic. These microorganisms may be responsible for spoilage in and food-borne infections associated with foods under refrigerated storage. Table 6.2 lists some of the common psychrotrophs and mesophiles that are known to grow under refrigerated conditions, at home or in the commercial settings.

Table 6.2 Minimum growth temperatures of some common psychrotrophs and mesophiles in foods (FDA, 2011; Jay *et al.*, 2005; Hwang and Huang, 2014; Kraft, 1992).

Microorganism	Minimum temperature ($^{\circ}\text{C}$)
<i>Acetobacterium bakii</i>	1.0
<i>Aeromonas</i>	1
<i>Aeromonas hydrophila</i>	-0.5
<i>Aspergillus</i> (toxigenic fungi)	10
<i>Bacillus cereus</i>	1
<i>Bacillus psychrotolerans</i>	-2 to 1
<i>Brochothrix thermosphacta</i>	-0.8
<i>Clostridium botulinum</i> (Type A, and proteolytic Types B and F)	10
<i>C. botulinum</i> (Type E, and non-proteolytic Types B and F)	3.3
<i>C. perfringens</i>	10
<i>Enterococcus</i> spp.	0
<i>Escherichia coli</i> (pathogenic strains)	6.5
<i>Lactobacillus algidus</i>	0
<i>Lactobacillus alimentarius</i>	2
<i>Leuconostoc</i> sp.	2
<i>Listeria monocytogenes</i>	-0.4
<i>Penicillium</i> (toxigenic fungi)	<5
<i>Pseudomonas fluorescens</i>	$0\text{--}4$
<i>Salmonella</i> spp.	5.2
<i>Shigella</i> spp.	6.1
<i>Staphylococcus aureus</i>	7
<i>Vibrio cholera</i>	10
<i>Vibrio parahaemolyticus</i>	5
<i>Vibrio vulnificus</i>	8
<i>Yersinia enterocolitica</i>	-1.3

Compared with psychrotrophs, mesophiles such as *E. coli* O157:H7 in raw beef do not grow and may actually gradually die off if the temperatures are below the minimum growth temperature (Figure 6.3; Huang, 2010). However, the psychrotrophs such as *L. monocytogenes* in fresh cut cantaloupe can grow well under low refrigerated temperatures (Figure 6.4). The growth of microorganisms under refrigerated storage can

be described and simulated using the many models available in Chapter 2 of this book. Depending on microorganisms and food types, the growth of microorganisms in foods may or may not exhibit a lag phase. For *L. monocytogenes* in fresh-cut cantaloupe, for example, the growth does not exhibit a lag phase and can be described by a three-parameter logistic model (Fang *et al.*, 2013) (Equation 6.2).

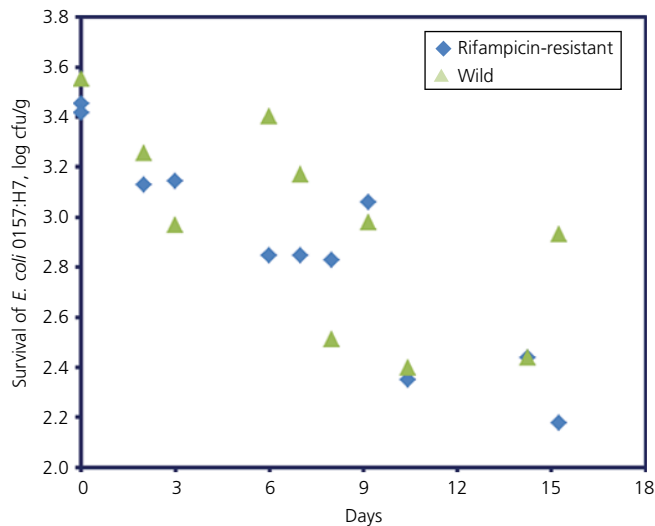


Figure 6.3 Survival of rifampicin-resistant and wild strains of *E. coli* O157:H7 in beef at 5°C (Huang, 2010, unpublished data).

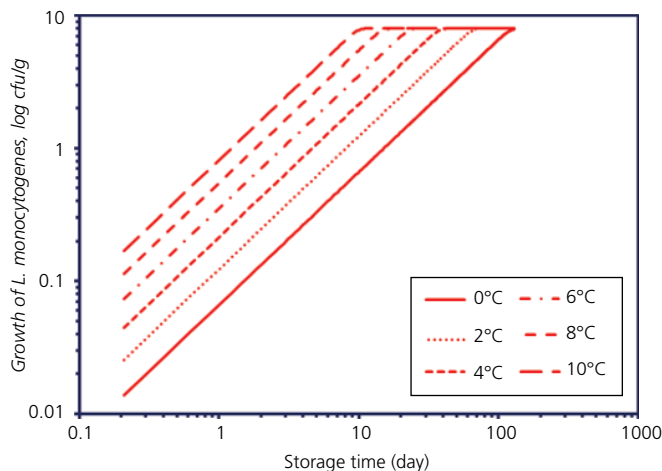


Figure 6.4 Growth of *L. monocytogenes* in fresh-cut cantaloupe stored between 0 and 10°C (based on Fang *et al.*, 2013; initial bacterial count = 1 cfu/g).

The effect of temperature ($^{\circ}\text{C}$) on the growth rate (h^{-1}) of *L. monocytogenes* in fresh-cut cantaloupe can be described by an Arrhenius-type model (Fang *et al.*, 2013) (Equation 6.3)

$$y(t) = y_0 + y_{\max} - \ln \left[e^{y_0} + (e^{y_{\max}} - e^{y_0}) e^{-\mu_{\max} t} \right] \quad (6.2)$$

where y , y_0 , and $y_{\max}$ are the natural logarithms of real time, initial, and maximum bacterial counts, and t is the storage time (h).

$$\mu_{\max} = 8.99 \times 10^{-3} (T + 273.15) e^{-\left(\frac{2.56 \times 10^3}{8.314(T + 273.15)} \right)^{14.9}} \left[1 - e^{-2.00 \times 10^{-1} (T - 46.6)} \right] \quad (6.3)$$

where $\mu_{\max}$ is the specific growth rate (h^{-1}) and T is the temperature ($^{\circ}\text{C}$). Figure 6.4 illustrates the growth of *L. monocytogenes* in fresh-cut cantaloupe at different refrigerated temperatures.

6.3 Dynamic effect of chilling on growth of *C. perfringens* during cooling

Chilling can be an important unit operation to prevent food-borne infections, and this is particularly true for controlling infections caused by *Clostridium perfringens*. *C. perfringens* is a spore-forming anaerobic bacterium ubiquitously distributed in the environment. Since *C. perfringens* is spore-forming, the bacteria may exist as spores in raw materials or ingredients used to make food products. During cooking under temperatures normally used to make processed meat and poultry products, such as roast beef, meat loaf, ham, and frankfurters, *C. perfringens* spores are not inactivated by heat. On the contrary, cooking at sub-boiling temperatures can activate the spores. Because of cooking, the air dissolved or entrapped in food materials is evacuated and escapes, thus creating an internal atmosphere that is low in or without oxygen and providing a condition for *C. perfringens* to grow. Once activated by heat, the spores may germinate,

outgrow, multiply, and, in the process, produce an enterotoxin that can cause acute abdominal cramp and diarrhea in consumers.

Once germinated, *C. perfringens* can grow very rapidly at temperatures suitable for its growth. The minimum and maximum growth temperatures of *C. perfringens* vegetative cells are around 10 and 52°C . The optimal growth temperature is between 43 and 47°C . At the optimal temperature, the generation time of *C. perfringens* can be as short as 7 min, making it one of the most rapidly growing food-borne pathogens known to food scientists (Craven, 1980). During cooling of meat products after cooking, the products must pass through a temperature zone most suitable for the growth of *C. perfringens*, causing the bacteria to multiply to a level potentially dangerous for human consumption. Therefore, *C. perfringens* is a common cafeteria bug in many parts of the world, and remains a serious public health and food safety hazard.

One of the most effective methods to prevent food-borne poisoning caused by *C. perfringens* is to control the temperature of meat products after cooking. After achieving the final cooking temperature, the products must be cooled at a rate to prevent its growth. For example, the compliance guidelines of the USDA Food Safety and Inspection Service (USDA-FSIS) (1999) require food manufacturers to cool uncured cooked meat and poultry products from 54.4 to 26.7°C in less than 1.5 h, and then from 26.7 to 4.4°C in less than 5 h. This 6.5 h process is designed to prevent food poisoning caused by an outgrowth of *C. perfringens* in uncured cooked meats. If a manufacturer fails to establish an effective cooling process that meets this requirement, a deviation has occurred. The manufacturer would need to demonstrate that the relative growth of *C. perfringens* is less than 1 log in the product. The manufacturer can demonstrate this by laboratory testing or using computer simulation. Therefore, refrigeration is a major unit operation to ensure proper cooling of cooked meat products for preventing the

growth of *C. perfringens*, and computer simulation may be used to analyze the dynamic growth of *C. perfringens* in cooked meats.

6.3.1 Mathematical modeling of growth of *C. perfringens* during cooling

Since *C. perfringens* is a spore-former and can exist in raw materials as spores, the growth of *C. perfringens* must go through the process of activation, germination, outgrowth, and multiplication. Therefore, the growth curve of *C. perfringens* in meat after cooking, which effectively eliminates its vegetative cells, always exhibits three phases, including lag, exponential, and stationary phases. For *C. perfringens*, the activation, germination, and outgrowth stages may be considered as part of the lag phase. Normally microbial growth curves can be described by many static growth models. Since cooling of processed and cooked meats is a dynamic process, it is more appropriate to directly use a set of differential equations to describe the growth of *C. perfringens*:

$$\frac{dN_L}{dt} = -a\mu N_L \quad (6.4)$$

$$\frac{dN_D}{dt} = a\mu N_L + \mu N_D \left(1 - \frac{N_L + N_D}{N_{\max}} \right) \quad (6.5)$$

In these equations, the subscripts *L* and *D* denote the cells of *C. perfringens* in the dormant state and the active dividing state. The cells in the dormant state are not engaged in active dividing activities, but may increase in individual size and fitness to grow before entering the active dividing state. Therefore, the cells in the dormant state are probably in the lag phase. Once a cell leaves the dormant state, it begins to divide actively, at a rate equal to the specific growth rate (μ , h^{-1}) at the temperature at which it grows. The number of cells at each state is represented by *N*, which may be the colony forming units (cfu) per unit weight of meat (cfu/g, for example), while $N_{\max}$ is the maximum

number of *C. perfringens* per unit weight. The coefficient *a* in Equations (6.4) and (6.5) is used to describe how fast the spores and cells leave the dormant state before entering the active dividing state.

Equation (6.4) describes the kinetics of dormant cells leaving the dormant state, following first-order kinetics. Equation (6.5) mathematically describes that the dormant cells, once leaving the dormant state, start to divide and increase exponentially in number until an equilibrium in cell population is established in the stationary phase. If proper microbiological procedures are used to enumerate the bacteria, the cells in both the dormant and active states will be recovered. Therefore, the total concentration of *C. perfringens* in the sample, at any given time, is the sum of both dormant and dividing cells:

$$N = N_L + N_D \quad (6.6)$$

Within the suitable temperature range, all three equations in Equations (6.4) to (6.6) can be used to describe the dynamic (transient) changes in the population of *C. perfringens* in cooked meat during cooling.

6.3.2 Effect of temperature on growth of *C. perfringens* during cooling – secondary model

The growth of *C. perfringens* is significantly affected by temperature. Cooling from a high temperature, *C. perfringens* is first exposed to a temperature range above the maximum growth temperature, during which the spores are activated but not killed by heat. After *C. perfringens* cells enter the temperature ranges suitable for growth, the growth rate and lag phase duration are continuously affected by temperature. If the temperatures are low enough, the growth of *C. perfringens* may be slowed down and the increase in the population may not be significant. For *C. perfringens*, Ratkowsky-type models can be used (Huang, 2003, 2004). Another model is the cardinal parameter model (Rosso *et al.*, 1993; Jaloustre *et al.*, 2011):

$$\mu = \frac{\mu_{\text{opt}} (T - T_{\text{max}})(T - T_{\text{min}})^2}{\left[(T_{\text{opt}} - T_{\text{min}})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\text{max}})(T_{\text{opt}} + T_{\text{min}} - 2T) \right] (T_{\text{opt}} - T_{\text{min}})} \quad (6.7)$$

where μ is the specific growth rate at temperature T (°C), T_{min} and T_{max} are the minimum and maximum growth temperatures (°C), and T_{opt} is the optimum growth temperature at which the bacterial growth rate (μ_{opt} , h⁻¹) reaches the optimum. In the cardinal parameter model, the key parameters pertinent to the growth of bacteria are clearly defined. During cooling, these cardinal parameters determine the rate at which *C. perfringens* grows.

6.3.3 Prediction of growth of *C. perfringens* during cooling – a finite element analysis

The cooling of cooked meat is a standard transient heat transfer process, with temperature changing with time and in space. For meat products, the temperature history at the slowest cooling point is needed to estimate the growth of *C. perfringens* during cooling. To obtain the temperature at the slowest cooling point, the most accurate method is to use a temperature sensor and a datalogger to monitor and record the temperature history for use in calculation. However, this is not always possible. This is where computer simulation becomes useful. Since cooling occurs after cooking, the distribution of temperature within the meat product immediately after cooking, which is the initial condition for cooling simulation, affects the prediction of the cooling temperature. Therefore, it is necessary to simulate the entire cooking and cooling process. In the following examples, the finite element method is used to simulate the temperature history during both cooking and cooling of a beef loaf.

6.3.4 Heat and mass transfer during heating and cooling

The temperature distribution and history of cooked meats during cooling is affected by complex heat and mass transfer phenomena that govern the transfer of thermal energy. For

a solid material, such as a meat loaf, the heat transfer is governed by Fourier's law of conduction. In a three-dimensional system, the heat transfer in a homogeneous solid material is governed by a partial differential equation (Incropera and DeWitt, 1996; Jiji, 2009; Lienhard and Lienhard, 2008):

$$\frac{\rho C_p}{k} \frac{\partial T}{\partial t} = \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \quad (6.8)$$

$$\alpha = \frac{k}{\rho C_p}$$

This equation describes an unsteady state heat conduction process, with x , y , and z being the coordinates of any location inside the solid material. The material's thermal properties, including thermal conductivity (k , W/m°C), density (ρ , kg/m³), and heat capacity (C_p , J/kg°C), affect the process of heat transfer during cooking and cooling. For most food products, the thermal properties do not change significantly in the range of temperature normally used for processing, and thus can be considered as constants. Another thermal property important to heat transfer is thermal diffusivity (α , m²/s), which determines the rate of heat conduction within the solid material.

For most cooking and cooling processes in food processing, a convective boundary condition exists on the surfaces, which defines the transfer of energy from the heating/cooling medium to the surfaces of cooked meat. The convective heat transfer on the boundary is governed by the following equation:

$$-k \frac{\partial T}{\partial n} = h(T - T_{\infty}) + \gamma m L_v \quad (6.9)$$

in which h is the surface heat transfer coefficient (W/m²°C), m is the moisture evaporation rate (kg/m²s), and L_v is the latent heat (J/kg) of water,

which carries the energy away from the surface as the water evaporates. In Equation (6.9), T_∞ is the ambient temperature ($^{\circ}\text{C}$) and is higher during cooking and lower during cooling than the surface temperature of meat. Within the temperature range of cooking and cooling, the moisture evaporation remains constant. This process is equivalent to the constant rate stage of dehydration.

The evaporation of moisture does not occur at low temperatures. According to Kondjoyan *et al.* (2013), most weight loss in cooked meat results from the water expelled from protein after denaturation and contraction during heating, and does not come directly from moisture evaporation. To more accurately account for the evaporative heat loss at high temperatures, a correction coefficient γ is added to the boundary condition (Equation (6.10)).

$$\gamma = \frac{1}{1 + e^{-0.1(T-100)}} \quad (6.10)$$

This correction coefficient is a function of temperature, and approaches 1.0 as the surface temperature rises to near 100°C . With this correction factor, the heat loss through moisture evaporation is negligible at low temperatures, but becomes more significant as the surface temperature is above 50°C . As the surface temperature approaches 100°C , the evaporative effect causes the surface to cool and prevents the surface temperature from increasing above 100°C during numerical simulation. This correction factor provides a simple empirical approach to account for the heat loss through moisture evaporation on the surface when the temperature is high during both cooking and cooling.

6.3.5 Finite element analysis

Because of the complex boundary and initial conditions, analytical solutions to Equation (6.8) are difficult to find, but can be easily solved using a numerical method, such as a finite element method (FEM). Finite element analysis

is a powerful tool for numerical analysis of heat transfer during cooking and cooling of meat products. In this section, a commercial finite element package (FlexPDE 6, Professional Version, PDE Solutions Inc., Spokane Valley, WA) is used to solve Equation (6.8). FlexPDE 6 can solve three-dimensional partial differential equations. The following example illustrates the application of FEM for a cooking process. The same methodology will be used to illustrate the application of FEM for cooling of meat after cooking.

The heating curve reported by Obuz *et al.* (2002) is used as an example to construct a heat transfer model, demonstrate the application of FEM, and validate its accuracy. In the work reported by Obuz *et al.* (2002), a finite difference method was used to simulate the heat transfer during cooking of cylindrically shaped beef roasts. The numerical simulation is reanalyzed using FEM and FlexPDE 6. Although FlexPDE 6 can solve three-dimensional heat transfer problems, for a cylindrically shaped object the heat transfer equation can be simplified to become a two-dimensional axisymmetric problem that can be more efficiently solved numerically by FEM. The

Table 6.3 Physical conditions and thermal properties used in development and validation of FEM analysis of heat transfer during cooking of cylindrical beef roasts.

Parameters	Value
Diameter (m) ^a	0.09
Height (m) ^a	0.19
Thermal conductivity (k , $\text{W/m}^{\circ}\text{C}$) ^b	0.476
Thermal diffusivity (α , m^2/s) ^b	1.3×10^{-7}
Surface heat transfer coefficient (h , $\text{W/m}^2\text{ }^{\circ}\text{C}$)	35
Rate of evaporation (m , $\text{kg/m}^2\text{s}$)	1.66×10^{-3}
Initial temperature (T , $^{\circ}\text{C}$) ^a	2
Heating temperature (T , $^{\circ}\text{C}$) ^a	163°
Heating time (t , s) ^a	1.5
Latent heat (L_v , J/kg) ^c	-2.5×10^{-3} $T + 2.5 \times 10^6$

^a Obuz *et al.* (2002).

^b Rahman (1996).

^c Amézquita *et al.* (2005b).

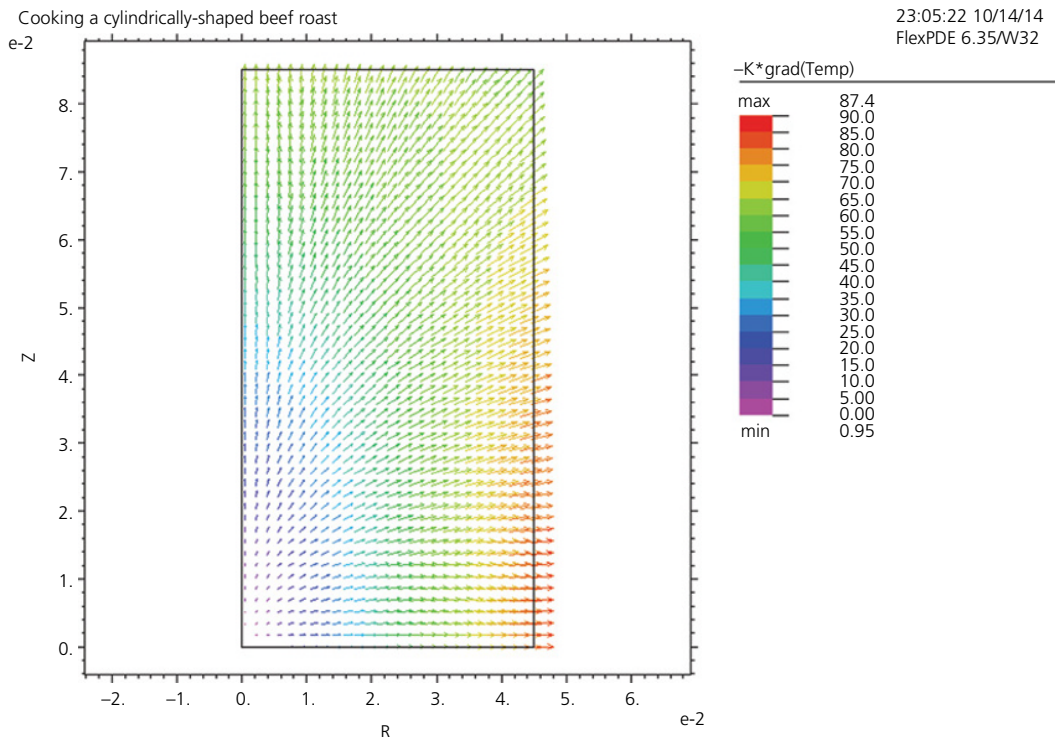
two-dimensional heat transfer for a cylinder can be expressed as

$$\frac{dT}{dt} = \alpha \left(\frac{d^2T}{dr^2} + \frac{1}{r^2} \frac{dT}{dr} \right) \quad (6.11)$$

where r is the radial position in a cylinder. The initial condition for Equation (6.8) is that the temperature in the meat roasts is uniformly distributed at time zero. The physical properties of the food remain constant throughout the heating and cooling process. The evaporation of moisture occurs on the surface only and the effect of internal moisture migration is negligible. Table 6.3 lists the thermal properties, initial conditions, and boundary conditions for FEM analysis. Figure 6.5 shows the geometry

used for simulation of the transient heat transfer process using FEM.

Different from the approach reported by Obuz *et al.* (2002), the evaporation of moisture from the surface is implemented using the simplified method with a correction factor for moisture evaporation (Equation (6.9)). The average rate of moisture evaporation is calculated when the surface temperature is above 60°C. With the simplified method for calculating the effect of moisture evaporation, the surface heat transfer is adjusted to 35 W/m²°C. With this approach, the simulation model provides a more accurate prediction of the temperature history at the geometric center (Figure 6.6). A similar approach was used by Huang and Sheen (2010) to simulate the same problem using a previous version of FlexPDE (Version 4). Different from the current



New Beef-Huamg-4Cool: Cycle= 560 Time= 23040. dt= 121.40 P3 Nodes= 1029 Cells= 274 RMS Err= 1.1e-5

Figure 6.5 Geometry used for finite element analysis of cylindrical beef roast. Shown in the figure is a quadrant of the axial cross-section of a beef roast (diameter = 0.09 m, height = 0.19 m). The arrows show the outflowing heat flux during cooling.

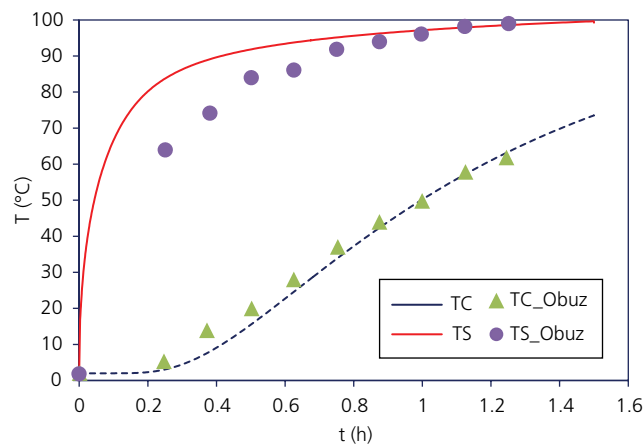


Figure 6.6 FEM simulation of heat transfer during cooking of cylindrical beef roasts (raw data taken from Obuz *et al.*, 2002). TC and TS: simulated temperature at the geometric center and on the surface; TC_Obuz and TS_Obuz: the temperature at the geometric center and on the surface taken from Fig. 2 of Obuz *et al.* (2002).

approach, the constant rate moisture evaluation was assumed to occur throughout the entire heating process. In the current approach, however, it is assumed that the moisture evaporation occurs only after the surface temperature is sufficiently high ($>60^{\circ}\text{C}$), with an adjustment following Equation (6.10). The temperature at the geometric center of the beef roasts obtained using this approach (Figure 6.6) is more accurate than that reported in Obuz *et al.* (2002).

6.3.6 Computer simulation of transient temperature changes during cooling

Once a heat transfer model is established and validated, FEM can be used to simulate the entire temperature history throughout the heating and cooling process. Since *C. perfringens* remains as spores during cooking, no growth would occur in the entire cooking process. *C. perfringens* would be activated by heat, germinate, outgrow, and multiply during cooling when the temperature becomes suitable for growth of this pathogen. The temperature profiles generated by FEM can be used to simulate the growth of *C. perfringens* during cooling. For FEM, the ordinary differential equations describing microbial growth (Equations (6.4) to (6.6)) can be incorporated into the heat transfer equation and solved

simultaneously with the temperature history curve. Amézquita *et al.* (2005a) developed and validated a numerical simulation model to predict the temperature profiles of large ready-to-eat meat products in small meat-processing facilities. This cooling heat transfer model was based on Matlab (Version 6.5, The Mathworks, Inc., Natick, MA), which is a sophisticated method for numerical analysis and requires substantial programming skills. FlexPDE 6, on the other hand, is a general purpose FEM package that is easy to program and can solve multiple differential equations simultaneously and, therefore, is used to simulate the bacterial growth during cooling.

The cooling of cooked meat products, such as beef roasts, is affected by the surface heat transfer coefficient (h) during cooling. According to Cengel and Ghajar (2011), the surface heat transfer coefficient (h) for meat products during cooling ranges from 10.6 to 35 $\text{W}/\text{m}^2\text{C}$. Ryland *et al.* (2006) studied heat transfer during cooling of cooked boneless ham, and reported an average surface heat transfer coefficient of 9.73 $\text{W}/\text{m}^2\text{C}$. The cooling is also affected by the temperature of the cooling medium used to take the thermal energy away from the surface of the cooked meat. In the study reported by Amézquita *et al.* (2005a), the

temperature of the cooling medium in small meat-processing facilities was $6.5 \pm 0.3^\circ\text{C}$.

According to the compliance guidelines of the USDA Food Safety and Inspection Service (USDA-FSIS, 1999), food manufacturers need to cool uncured cooked meat and poultry products from 54.4 to 26.7°C in less than 1.5 h, and then from 26.7 to 4.4°C in less than 5 h. In order to cool a product to a final temperature of 4.4°C , the temperature of the cooling medium must be at least 4.4°C or below. However, the minimum growth temperature of *C. perfringens* in cooked meat is around 10°C . Therefore, theoretically, the growth of *C. perfringens* should stop as the temperature drops below 10°C . As the temperature of a product approaches the minimum growth temperature for the pathogen, the growth activities of *C. perfringens* slow down.

The difference between the temperatures of the cooling medium and the product serves as the driving force for heat transfer during cooling. Under a constant cooling temperature, the temperature of the product will eventually equilibrate to the cooling temperature. The larger the driving force, the faster the temperature of the product decreases during cooling. The surface heat transfer coefficient, on the other hand, determines how fast that the heat is removed from the surface of the product and the time that the product equilibrates with the cooling medium. Together, both parameters are

the extrinsic factors contributing to the rate of cooling of a product.

FEM can be used to analyze the heat transfer process during cooling. In the following examples, FEM is used to simulate the temperature distributions in cylindrically shaped beef roasts of the same dimensions as shown in Figure 6.5. The heating conditions are identical to those listed in Table 3 and Figure 6.5. The total cooking time is 1.5 h and the total cooling time is 6.5 h, as recommended by the USDA-FSIS (1999). The temperature histories at three locations are simulated. All three locations are positioned on the axial cross-sectional area of the cylinder. The center of this cross-sectional area is the geometric center (GC), which is the first location and the slowest cooling point in the product. The second location, labeled as 0.5R, is positioned half-way between the GC and the surface. The third location, labeled as R, is located on the surface. A combination of different cooling conditions is simulated, including three cooling temperatures (0 , 5 , and 10°C) and seven surface heat transfer coefficients (5 , 10 , 15 , 20 , 25 , 30 , and $35 \text{ W/m}^2 \text{ }^\circ\text{C}$).

Figure 6.7 shows the simulated temperature histories of cooling processes with $h = 35 \text{ W/m}^2 \text{ }^\circ\text{C}$. With this h value, the end-point temperatures at all three locations can approach the cooling temperature (Table 6.2). With $h = 20 \text{ W/m}^2 \text{ }^\circ\text{C}$, the end-point temperatures at all three locations

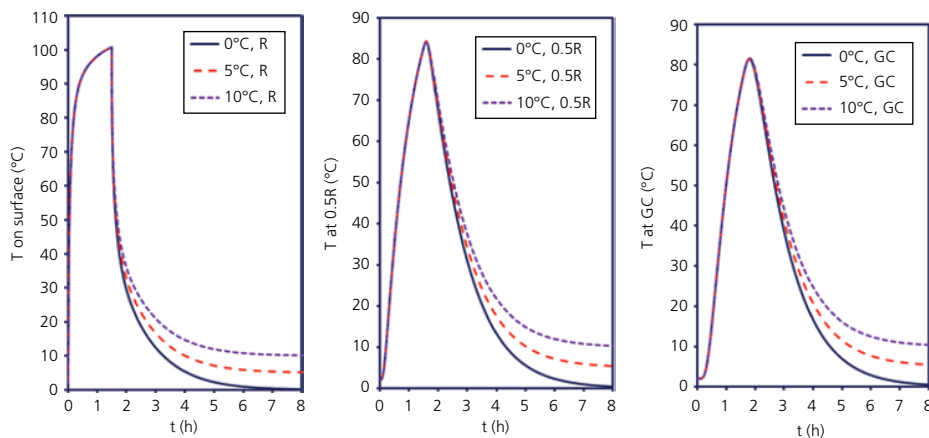


Figure 6.7 Simulated temperature histories at GC, 0.5R, and R with $h = 35 \text{ W/m}^2 \text{ }^\circ\text{C}$ and cooling temperatures = 0 , 5 , and 10°C .

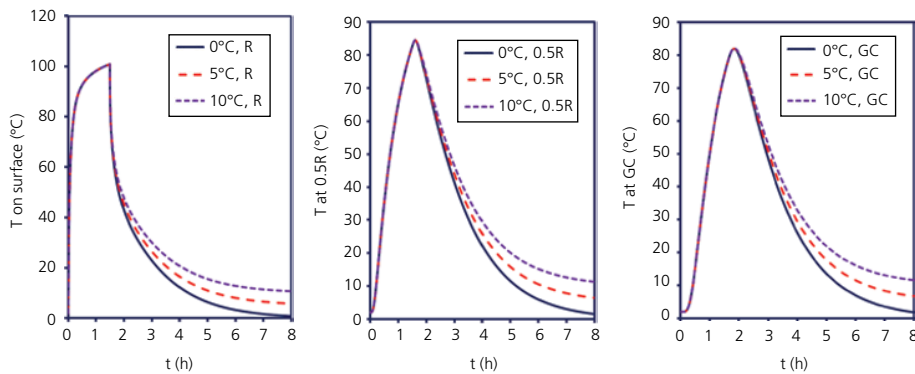


Figure 6.8 Simulated temperature histories at GC, 0.5R, and R with $h = 20 \text{ W/m}^2 \text{ } ^\circ\text{C}$ and cooling temperatures = 0, 5, and 10°C .

Table 6.4 End-point temperatures at the geometric center (GC), half-way between GC and surface (0.5R), and on the surface (R) under different cooling conditions of surface heat transfer coefficients (h).

$h \text{ (W/m}^2\text{ } ^\circ\text{C)}$	0°C			5°C			10°C		
	GC	0.5R	R	GC	0.5R	R	GC	0.5R	R
35	0.47	0.38	0.15	5.44	5.35	5.14	10.41	10.33	10.12
30	0.69	0.56	0.24	5.64	5.52	5.22	10.60	10.48	10.21
25	1.07	0.89	0.43	6.00	5.83	5.40	10.93	10.77	10.37
20	1.83	1.56	0.86	6.72	6.46	5.80	11.59	11.35	10.74
15	3.52	3.08	1.93	8.29	7.88	6.80	13.07	12.67	11.67
10	7.85	7.12	5.14	12.31	11.64	9.79	16.76	16.13	14.42

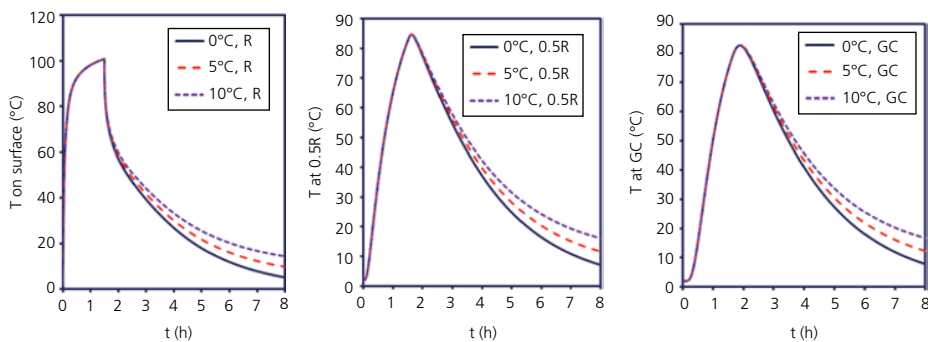


Figure 6.9 Simulated temperature histories at GC, 0.5R, and R with $h = 10 \text{ W/m}^2 \text{ } ^\circ\text{C}$ and cooling temperatures = 0, 5, and 10°C .

are within 2°C from the cooling temperature (Figure 6.8, Table 6.4). With $h = 10 \text{ W/m}^2 \text{ } ^\circ\text{C}$, the temperatures at GC are 7.85, 7.31, and 6.76°C higher than the respective cooling temperatures (0, 5, and 10°C) (Figure 6.9, Table 6.4). Apparently, any process with a cooling temperature of 5 or

10°C would fail to meet the performance guidelines set by the USDA-FSIS (1999; see Table 6.4). According to Table 6.4, the cooling processes with a cooling temperature of 0°C and $h > 15 \text{ W/m}^2 \text{ } ^\circ\text{C}$ may potentially meet the cooling performance guidelines.

6.3.7 Integration of heat transfer and growth kinetics to simulate dynamic growth of *C. perfringens* during cooling

Amézquita *et al.* (2005b) integrated the heat transfer model with the growth kinetics of *C. perfringens* to predict the dynamic growth of this microorganism in boneless ham during cooling. Huang and Sheen (2010) used FlexPDE 4 to simulate the effect of cooling conditions on the growth of *C. perfringens* in cooked beef roasts. For this chapter, the kinetic data recently obtained by Huang (2015) is used and Table 6.5 lists the kinetic parameters for beef.

Figure 6.10 shows the results of dynamic simulation of *C. perfringens* in cooked beef roasts under various cooling temperatures (0, 5, and 10°C) and a fixed surface heat transfer condition of 35 W/m² °C. The FEM simulation

results indicate that none of the cooling conditions used in Figure 6.10 would lead to a > 1 log cfu/g relative growth of *C. perfringens*, suggesting that, although some of cooling conditions may not meet the performance guidelines, the relative growth is all < 1 log cfu/g. Therefore, these products still meet the performance guidelines set by the USDA-FSIS (1999).

Table 6.5 Kinetic parameters used in simulation of dynamic growth of *C. perfringens* in cooked beef (Huang, 2015).

Parameters	Value
a	2.12×10^{-2}
μ_{opt} h ⁻¹	5.64
T_{opt} °C	45.5
T_{min} °C	10
N_{max} °C	52

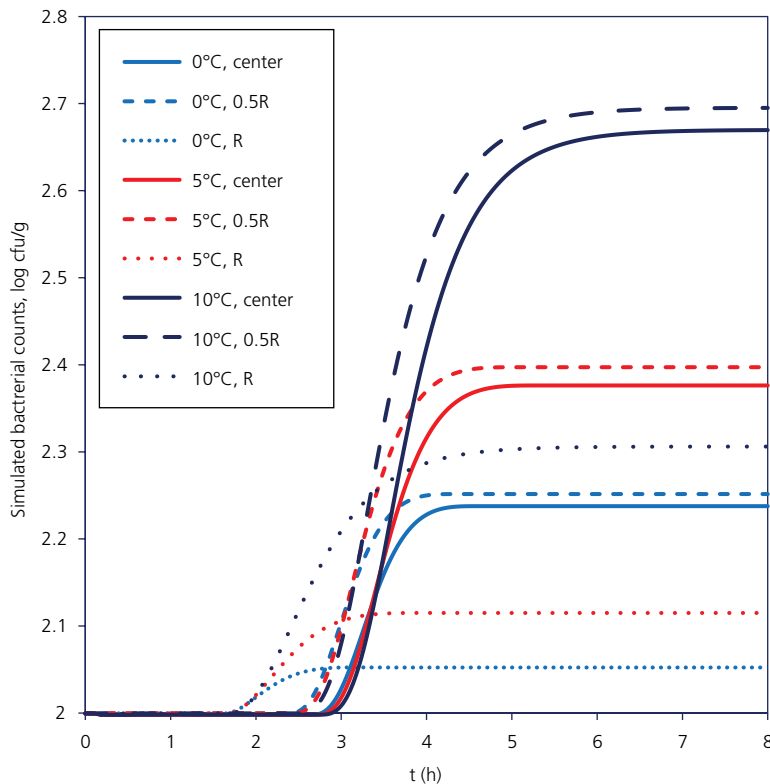


Figure 6.10 Dynamic simulation of growth of *C. perfringens* in beef roasts under different cooling temperatures and $h = 35 \text{ W/m}^2 \text{ } ^\circ\text{C}$.

It is noteworthy to point out that, although the GC of the beef roasts is the slowest cooling point throughout the cooling process (Figures 6.7 to 6.9 and Table 6.4), it is nevertheless not the fastest growing point for *C. perfringens* during cooling. The growth of *C. perfringens* at 0.5R is actually higher and faster than that at the geometric center. Figure 6.11 illustrates another example in which the growth at GC is not the fastest growing location in a beef roast. Under a cooling temperature of -5°C and a fixed h of $5\text{ W/m}^2\text{ }^{\circ}\text{C}$, the end-point temperature at all locations are way above 4.4°C and the relative growths are all $>1\text{ log cfu/g}$. Therefore, this particular cooling process does not follow the USDA-FSIS cooling guidelines. In this example, however, the simulation results show that *C. perfringens* grows faster and to a high level on the surface, while the geometric center becomes the slowest growing point for *C. perfringens*. If a product is not wrapped and exposed to air during cooling, this growth of *C. perfringens* on the surface may not be relevant, as *C. perfringens* is an anaerobic microorganism and the presence of air may prevent it from growing. However, if a

product is wrapped with a material that may prevent the permeation of oxygen, these simulation results may become relevant to the food industry and regulatory agencies, as it is always necessary to prevent the growth of *C. perfringens* at the fastest growth point from reaching the critical control limit set by the regulatory agencies.

Figure 6.12 further illustrates the dynamic effect of cooling conditions on the relative growth of *C. perfringens* at the end of a 6.5 h cooling. This figure shows that the combined effect of cooling temperature and surface heat transfer can effectively limit the growth of *C. perfringens* to a level below the regulatory limit. For example, for a cooling temperature of 10°C , the relative growth of *C. perfringens* can be effectively controlled to $<1\text{ log cfu/g}$ if the surface heat transfer coefficient is $>25\text{ W/m}^2\text{ }^{\circ}\text{C}$. For a cooling temperature of 5°C , the relative growth of *C. perfringens* can be effectively controlled to $<1\text{ log cfu/g}$ if the surface heat transfer coefficient h is $>\sim 17\text{ W/m}^2\text{ }^{\circ}\text{C}$. For a cooling temperature of 0°C , the relative growth of *C. perfringens* can be controlled

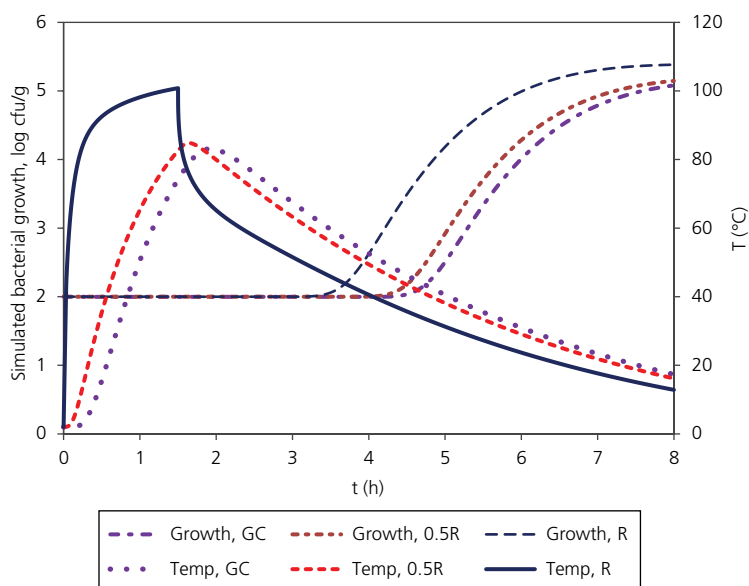


Figure 6.11 Dynamic simulation of growth of *C. perfringens* in beef roasts under a cooling temperature of -5°C and $h = 5\text{ W/m}^2\text{ }^{\circ}\text{C}$.

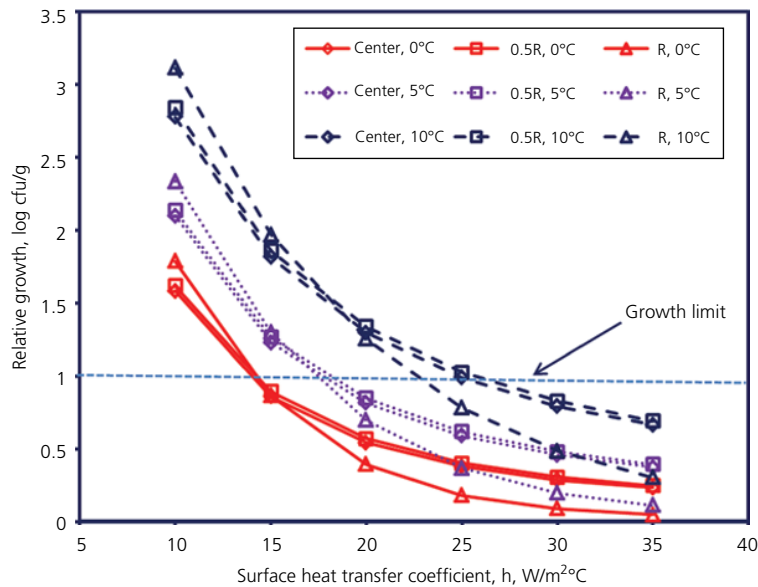


Figure 6.12 Simulated end-point relative growth under different cooling conditions. Smooth lines represent cooling temperatures (solid lines: 0°C; dotted lines: 5°C; broken lines: 10°C). Empty diamonds: geometric center; empty squares: 0.5R; empty triangles: surface (R).

to < 1 log cfu/g if $h > \sim 14$ W/m² °C. Again, the geometric center is not necessarily the fastest growing point for *C. perfringens*. The reason for this result is due to the difference in exposure to the time and temperature at each location, which determines the lag development and growth rate.

6.3.8 Conclusion and impact on growth of *C. perfringens* during cooling

The FEM simulation of the cooling process of cylindrically shaped beef roasts shows the effect of cooling rate and temperature on relative growth of *C. perfringens* during cooling. The simulation results suggest that it is possible to use a combination of cooling temperature and surface heat transfer to control the relative growth of *C. perfringens* to a level below the regulatory limit.

Refrigeration is an energy-intensive process. Reducing the cooling temperature, while it can contribute to faster cooling due to larger temperature gradients, may lead to increased energy consumption. Increasing the surface

heat transfer coefficient may be a more feasible approach. For a conventional refrigeration process, increasing the cooling air flow rate can effectively increase the surface heat transfer coefficient. On the other hand, a higher air flow rate may increase the moisture evaporation from the product surfaces, leading to extra weight loss, which is a concern for the food industry. However, increasing the humidity of the cooling air may reduce the weight loss caused by increased air flow. The design of a more effective refrigeration system, which not only ensures proper cooling of cooked meats to prevent the growth of *C. perfringens* but also reduces the energy consumption in the refrigeration process, is needed. This topic is beyond the scope of this chapter. The author would like to encourage the engineers in the food industry to consider the growth kinetics of *C. perfringens* when designing a cooling process for cooked meats. The author would also suggest adding more locations, such as the 0.5R position in the cylindrically shaped beef roasts, in addition to the geometric center, for evaluation of the

growth of *C. perfringens* during cooling. This may be important for risk assessments of *C. perfringens* in cooked meats during cooling.

References

- Amézquita, A., Wang, L., and Weller, C.L. 2005a. Finite element modeling and experimental validation of cooling rates of large ready-to-eat meat products in small meat-processing facilities. *Trans. ASAE*, **48**, 287–303.
- Amézquita, A., Weller, C.L., Wang, L., Thippareddi, H., and Burton, D.E. 2005b. Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. *International Journal of Food Microbiology*, **101**, 123–144.
- Cengel, Y.A. and Ghajar, A.J. 2011. Refrigeration and freezing of foods, Chapter 17. In: *Heat and Mass Transfer: Fundamentals and Applications*, 4th edn. McGraw-Hill Science/Engineering/Math, Columbus, OH, USA.
- Craven, S.E. 1980. Growth and sporulation of *Clostridium perfringens* in foods. *Food Technology*, **34**, 80–87, 90.
- Dave, D. and Ghaly, A.E. 2011. Meat spoilage mechanisms and preservation techniques: a critical review. *American Journal of Agricultural and Biological Sciences*, **6**, 486–510.
- Earle, R.L. and Earle, M.D. 1983. *Unit Operations in Food Processing*. The New Zealand Institute of Food Science and Technology, Inc. Available at: <http://www.nzifst.org.nz/unitoperations/>. Accessed on November 16, 2014.
- Erickson, M.C. and Hung, Y.C. 1997. *Quality in Frozen Food*, pp. 182. Chapman and Hall, New York, USA.
- Fang, T., Liu, Y., and Huang, L. 2013. Growth kinetics of *Listeria monocytogenes* and spoilage microorganisms in fresh-cut cantaloupe. *Food Microbiology*, **34**, 174–181.
- Food and Drug Administration (FDA). 2011. *Fish and Fishery Products – Hazards and Controls Guidance*, 4th edition. Food and Drug Administration, Rockville, MD, USA.
- Gracey, J.F., Collins, D.S., and Hey, R.J. 1999. *Meat Hygiene*, 10th edn, pp. 114. Harcourt Brace and Company, London, UK.
- Gunderson, M.F., and Rose, K.D. 1948. Survival of bacteria in precooked fresh-frozen food. *Food Research*, **13**, 254–263.
- Huang, L. 2003. Dynamic computer simulation of *C. perfringens* growth in cooked ground beef. *International Journal of Food Microbiology*, **87**, 217–227.
- Huang, L. 2004. Numerical analysis of the growth of *Clostridium perfringens* in cooked beef under isothermal and dynamic conditions. *Journal of Food Safety*, **24**, 53–70.
- Huang, L. 2010. Growth kinetics of *Escherichia coli* O157:H7 in mechanically-tenderized beef. *International Journal of Food Microbiology*, **140**, 40–48.
- Huang, L. 2015. Dynamic determination of kinetic parameters, computer simulation, and probabilistic analysis of growth of *Clostridium perfringens* in cooked beef during cooling. *International Journal of Food Microbiology*, **195**, 20–29.
- Huang, L. and Sheen, S. 2010. Cooling of cooked ready-to-eat meats and computer simulation. In: *Ready-to-Eat Foods: Microbial Concerns and Control Measures* (eds Hwang, C.A. and Huang, L.). CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.
- Hwang, C.A. and Huang, L. 2014. Chilled storage of foods: principles. In: *Encyclopedia of Food Microbiology*, vol. 1 (eds Batt, C.A. and Tortorello, M.L.), pp. 527–431. Elsevier Ltd., Academic Press.
- Incropera, F.P. and DeWitt, D.P. 1996. *Fundamentals of Heat and Mass Transfer*, 4th edn. John Wiley & Sons, Inc., New York, USA.
- Jaloustre, S., Cornu, M., Morelli, E., Noël, V., and Delignette-Muller, M.L. 2011. Bayesian modeling of *Clostridium perfringens* growth in beef-in-sauce products. *International Journal of Food Microbiology*, **28**, 311–320.
- Jay, J.M., Loessner, M.J., and Golden, D.A. 2005. *Modern Food Microbiology*, 7th edn, Food Science Text Series. Springer, New York, USA.
- Jiji, L.M. 2009. *Heat Transfer*, 3rd edn. Springer-Verlag, Berlin Heidelberg, Germany.

- Kraft, A.A. 1992. *Psychrotrophic Bacteria in Foods: Disease and Spoilage*. CRC Press, Boca Raton, FL, USA.
- Kondjoyan, A., Oillic, S., Portanguen, S., and Gros, J.B. 2013. Combined heat transfer and kinetic models to predict cooking loss during heat treatment of beef meat. *Meat Science*, **95**, 336–344.
- Lienhard, J.H., IV and Lienhard, J.H., V. 2008. *A Heat Transfer Textbook*, 3rd edn. Phlogiston Press, Cambridge, MA, USA.
- Obuz, E., Powell, T.H., and Dikeman, M.E. 2002. Simulation of cooking cylindrical beef roasts. *Leb. Wiss. Tech.*, **35**, 637–644.
- Querol, A. and Fleet, G.H. 2006. *Yeasts in Foods and Beverages*, pp. 7–8. Springer-Verlag, Berlin Heidelberg, Germany.
- Rahman, S. 1996. *Food Properties Handbook*. CRC Press, Boca Raton, FL, USA.
- Rosso, L., Lobry, J.R., and Flandrois, J.P. 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *Journal of Theoretical Biology*, **162**, 447–463.
- Ryland, K., Wang, L., Amézquita, A., and Weller, C. 2006. *Estimation of Heat Transfer Coefficients of Cooked Boneless Ham, RURALS: Review of Undergraduate Research in Agricultural and Life Sciences*, vol. 1: Issue 1, Article 1. Available at: <http://digitalcommons.unl.edu/rurals/vol1/iss1/1>.
- USDA Food Safety and Inspection Service (USDA-FSIS). 1999. Performance standards for the production of certain meat and poultry products. *Federal Register*, **64**, 732–749.
- Van Venrooij, G.E.P.M., Aertsen, A.M.H.J., Hax, W.M.A., Ververgaert, P.H.J.T., and Verhoeven, J.J. 1975. Freeze-etching: freezing velocity and crystal size at different locations in samples. *Cryobiology*, **12**, 46–61.

CHAPTER 7

Impact of dehydration and drying operations on the microbial ecology of foods

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7.1 Introduction

Moisture removal from solids is an integral part of food processing, with convective drying representing one of the most important techniques for preservation of biological products (McMinn and Magee, 1997). Next to the heat effect, the induced reduction of the water activity (a_w) contributes to the preservation of foods by avoiding microbial growth and chemical reactions causing deterioration (Rahman *et al.*, 2004).

Water can be found in foods in different ways according to the type of interaction between the food compounds and water molecules. The concept of water activity (a_w) is usually defined as the ratio of the water vapor pressure in the food (p) and the pure water vapor pressure (p_0) at a determined value of environmental pressure and temperature. Mathematically, this concept can be described as follows:

$$a_w = \frac{p}{p_0} \quad (7.1)$$

Water activity (a_w) is a critical parameter in the food industry since it is used to inform on the amount of water availability for biochemical and microbiological reactions.

Sorption curves or sorption isotherms in foods describe the relationship between a_w and the humidity percentage in the environment surrounding the food in equilibrium. A

phenomenon usually observed in food is the so-called hysteresis in which absorption and desorption processes follow different pathways, with higher values of humidity in the case of desorption. This means that the desorption process retains more water content than the absorption process at a specific a_w values. A lower hysteresis phenomenon is observed as temperature increases.

Sorption isotherms are important tools for the design and optimization of drying equipment, design of packages, predictions of quality, stability, shelf life, and for calculating moisture changes that may occur during storage. Several preservation processes have been developed in order to extend the shelf life of food products by lowering the availability of water for microorganisms and chemical reactions.

Isotherms can be divided into three different regions representing the three ways in which water is found in foods. The water in region A reflects bound water and the enthalpy of vaporization is considerably higher than that of pure water. The bound water includes structural water (H-bonded water) and monolayer water, which is attracted by the hydrophilic and polar groups of food components (polysaccharides, proteins, etc.). Bound water is unfreezable, it is not available for chemical reactions and can have a role as plasticizer. In region B, water molecules bind less firmly than in the first zone and are usually present in

small capillaries. The vaporization enthalpy is slightly higher than that of pure water. The properties of the water in region C are similar to those of the free water found in cavities, large capillaries, etc., where water weakly binds to food material.

Sorption curves are usually described by an empirical model in which different equations are fitted to observations. Although plenty of models are available, there is no model able to be applied to the broad range of humidity and temperature values. The Langmuir equation is one of the most important theoretical models describing sorption curves. This model describes the absorption of water vapor molecules in a monolayer and it assumes, among other conditions, that the surface is flat (homogenous), the sites where water molecules are absorbed are equivalent, and absorbed water molecules are immobile. This model treats the absorption phenomenon as a chemical reaction (Rizvi, 2005):

$$a_w \left(\frac{1}{X_e} - \frac{1}{X_m} \right) = \frac{1}{CX_m} \quad (7.2)$$

where X_m is the monolayer sorbate content, X_e is the equilibrium moisture content, and C is a constant.

According to Andrade *et al.* (2011), the most commonly used models in foods are the BET and GAB models. The BET model corresponds to the model proposed by Brunauer, Emmet, and Teller, which is an extension of Langmuir's kinetic model taking into account multilayer adsorption. This model is simple and enables the estimation of the amount of monolayer water and a rough value of the heat of wetting; however, the model application is limited to the a_w range between 0.05 and 0.45 (Andrade *et al.*, 2011):

$$\frac{a_w}{(1-a_w)X_e} = \frac{1}{X_m C} + \frac{a_w(C-1)}{X_m C} \quad (7.3)$$

where X_m is the moisture content when a monomolecular layer is absorbed and C is an empirical constant.

The GAB model proposed by Guggenheim–Anderson–de Boer is widely spread and is used even now as a standard tool to evaluate the internal surface area of porous materials. Although it shows simplicity and is easy to interpret, it is limited to values lower than 0.93 (Rizvi, 2005):

$$\frac{a_w}{X_e} = aa_w^2 + ba_w + c \quad (7.4)$$

where X_e is as in Equation 7.3, and:

$$a = \frac{k}{X_m} \left(\frac{1}{C} - 1 \right), \quad b = \frac{1}{X_m} \left(a - \frac{2}{C} \right), \quad c = \frac{1}{X_m C k} \quad (7.5)$$

The constants C and k are linked to the heat of sorption, the heat of condensation of water, and the temperature of the system; X_m is the moisture content.

In some ranges of a_w , sorption isotherms can be approximated to linear equations (Shigehisa *et al.*, 2015).

7.2 Modeling the drying process in food

The drying process involves heat transfer to food, the migration of moisture from the interior of food to the surface, and the evaporation of moisture from the surface to the external environment (i.e., air).

The drying process usually shows two well differentiated phases. The first one corresponds to the period in which the moisture removal occurs as if pure water were evaporated, showing a constant drying rate. Moisture moves from the interior of the food to the external surface (drying surface) at a constant rate. The duration of this phase greatly depends on the food structure. Foods where moisture movement is due to capillary and gravity forces exhibit a visible constant drying rate. In contrast, those foods in which moisture movement occurs due to diffusion do not show a constant drying rate (Rizvi, 2005).

The second step in the process shows a falling drying rate due to both the resistance by the internal food structure to the moisture moment within the food and a lower moisture content in the interior of the food. Thus, the moisture content migrating to the food surface becomes insufficient to restore the water content in the food surface (Rizvi, 2005).

These phases can be observed graphically in Figure 7.1, where after an initial step corresponding to the warming-up process (A–B), a constant drying rate period can be observed in the segment B–C. Point C determines the beginning of the falling drying rate period. This point corresponds to the critical moisture content. In the segment C–D, the first falling drying rate is observed due to the water evaporation rate in the surface being higher than the movement of the moisture to the surface. At point D, evaporation occurs in the interior of the food and after this point, mass transfer and heat transfer become longer and more complicated. Therefore, in the segment D–E, a second falling drying rate is observed, where point E corresponds to the moment when the vapor pressure in the food becomes equal to the vapor pressure in the surrounding air, and the drying process is finished. Point E is termed the equilibrium moisture content.

An analytical model describing drying in foods should consider heat transfer, mass transfer, and momentum transfer, the three within the food and the surrounding air. Heat energy is transferred due to the temperature gradient in

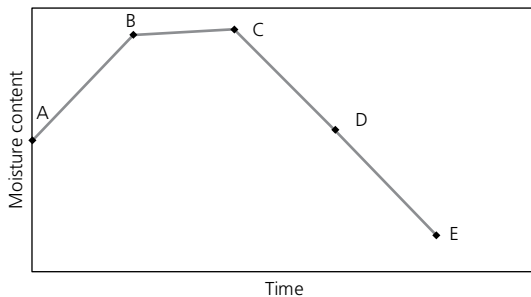


Figure 7.1 Representation of the typical phases of the drying process of foods.

the food, and the rate of heat transfer is proportional to the potential (temperature) difference and to the properties of the transfer system characterized by the heat transfer coefficient. The rate of mass transfer is proportional to the potential (pressure or concentration) difference and to the properties of the transfer system characterized by a mass transfer coefficient. The use of analytical models is advantageous in comparison to empirical ones (based on experimental data), given that they can be developed using less resources. As there are computational limitations in estimating certain model parameters, most analytical models are simplifications assuming that the main limiting factor is mass transfer and therefore they neglect heat transfer by conduction and radiation. Through the models, the drying rate and drying time can be estimated and also drying process parameters can be optimized.

The rate of drying is determined by the moisture content (X), food structure, the temperature of the food and the temperature, the (relative) humidity, and the velocity of the air in contact with the food. Also, it depends on the conditions of the dryer equipment. The drying rate (w) can be calculated according to the following equation:

$$w = -\frac{M_s}{A} \frac{dX}{dt} \quad (7.6)$$

where w ($\text{kg}/\text{m}^2 \text{ h}$) is the rate of water evaporation, A is the evaporation area (which may be different from the heat transfer area), and M_s is the mass on a dry basis. If A is not known, then the drying rate may be expressed in kg of water evaporated per hour.

As explained above, in the constant drying rate period, the drying process is governed by surface evaporation since the food surface is always saturated. Hence the drying rate can be calculated based on the heat transfer rate on the food surface as shown by:

$$w = \frac{\sum c}{\lambda_s} \quad (7.7)$$

where $\sum c$ represents the sum of heat fluxes due to convection, conduction, and/or radiation and λ_s is the latent heat of vaporization at the solid temperature.

In the case of a falling drying rate, calculations are more complex due to the fact that the rate strongly depends on the diffusion rate of water from the interior to the food surface. The falling drying rate is a function of X (i.e., moisture content) and should be calculated experimentally for a certain food under certain drying conditions. Nevertheless, there are some simple modeling alternatives such as assuming a linear equation for the falling drying rate:

$$N = aX + b \quad (7.8)$$

where a and b are regression parameters.

Assuming a liquid diffusion rate of water as the governing factor for the falling drying rate, Fick's second law of diffusion can be applied considering a constant diffusion rate. The differential equation for a one-dimension diffusion is shown by:

$$\frac{dX}{dt} = D_{eff} \left(\frac{d^2 X}{dr^2} + \frac{C}{r} \frac{dX}{dr} \right) \quad (7.9)$$

where C is a constant that determines the geometry of the solid (e.g., food) by assigning 0 for planar, 1 for cylindrical, and 2 for spherical geometries, D_{eff} is the effective diffusion constant and r is position (length). The initial and boundary conditions are

$$\begin{aligned} X(r, 0) &= X_i \text{ at } t=0 \\ X(0, t) &= X_e \text{ at } r=r_0 \text{ (at the surface)} \\ X(0, t) &= \text{finite at } r=0 \text{ (at the center)} \end{aligned}$$

In addition, the effective diffusion constant (D_{eff}) strongly depends on temperature, which can be adequately described by the Arrhenius equations as follows:

$$D_{eff} = D_{eff0} e^{\left(\frac{-E_a}{R_g T_{abs}} \right)} \quad (7.10)$$

where E_a is the activation energy, R_g is the gas constant and T_{abs} is the absolute temperature.

With regards to the drying time, it can be calculated from a simple equation, when the constant rate is assumed from the following formula:

$$t_c = \frac{M_s (X_1 - X_2)}{R_c} \quad (7.11)$$

where X_1 and X_2 are the initial and final moisture contents, respectively, R_c is the rate of drying in the constant rate period and M_s is the food mass on a dry basis.

A specific situation is when the whole drying curve is assumed to be a straight line. In this case, drying time can be simply calculated through

$$t_c = \frac{M_s (X_c - X_e)}{R_c} \ln \left[\frac{X_1 - X_e}{X_2 - X_e} \right] \quad (7.12)$$

Here, given that the drying time refers to the whole drying curve, X_c is the critical moisture content and X_e is the equilibrium moisture content.

7.3 Modeling microbial survival/inactivation in drying/dehydration processes

7.3.1 Overview

The development of predictive models allows the calculation of kinetic parameters to estimate the effect of certain processing and preservation methods. They also provide the tools to compare the impact of different process technologies on the reduction of microbial populations. Modeling survival and inactivation of food-borne pathogens has been widely studied since the 1920s, being one of the first achievements in predictive microbiology. Inactivation models firstly focused on the destruction of *Clostridium botulinum* spores in low-acid canned foods (Hersom and Hulland, 1980; Jay, 1992).

It has been generally accepted that the log reduction versus inactivation time of vegetative bacteria follows a linear relationship, based on the calculation of the time to achieve one decimal

log reduction (D value) and the increase in temperature that produces a reduction in the D value in a factor of 10 (z value). When dealing with dehydration processes, inactivation is mainly caused by a decrease in a_w and/or together with an increase in temperature. It is shown that – depending on the drying rate – dynamic effects have to be taken into account when modeling the survival during drying.

More recent studies intended to quantify microbial inactivation caused by the application of thermal and non-thermal processes, describing more complex modeling approaches in which deviation from linearity is observed. These deviations from first-order kinetics must be taken into account in order to avoid under- or overprocessing of food, which would result in the survival of pathogenic bacteria or the deterioration of nutritional or sensory attributes, respectively (Bermudez-Aguirre and Corradini, 2012).

One of the predictive microbiology tools for microbial inactivation is a freeware add-in for Microsoft Excel, so-called GInaFiT (the Geeraerd and Van Impe inactivation model fitting tool), which includes a large variety of primary inactivation models by using linear and non-linear regression approaches. The application, described by Geeraerd *et al.* (2005) comprises nine model types: (i) classical log-linear curves, (ii) curves displaying a so-called shoulder before a log-linear decrease is apparent, (iii) curves displaying a so-called tail after a log-linear decrease, (iv) survival curves displaying both shoulder and tailing behavior, (v) concave curves, (vi) convex curves, (vii) convex/concave curves followed by tailing, (viii) biphasic inactivation kinetics, and (ix) biphasic inactivation kinetics preceded by a shoulder.

7.3.2 Primary models at static conditions

7.3.2.1 Bigelow model (linear model) and Arrhenius model

This model was established to quantify microbial inactivation in the canning industry, assuming first-order kinetics. Apparently, cell death occurs

due to an inactivation of a critical enzyme and it is commonly stated that this inactivation follows first-order kinetics (although there might be some exceptions to this rule). The model (Higl *et al.*, 2007) has the following form:

$$S(t) = e^{-kt} \quad (7.13)$$

where $S(t)$ is the microbial population at time; t (cfu/g) and k is defined as the drying inactivation rate. Rearranging the equation, we obtain:

$$\ln N = \ln N_0 - kt \quad (7.14)$$

with N as the number of microorganisms, N_0 as the initial number of microorganisms, and k as the first-order rate constant. The above equation is then rearranged into

$$\log \frac{N}{N_0} = \log S(t) = -\frac{t}{D} \quad (7.15)$$

where $D = 2.303/K$, and it is defined as the decimal reduction time (min or s).

A drawback of this model is that it is mainly focused on thermal treatments rather than on novel preservation techniques.

The rate constant can be related to the temperature by the Arrhenius equation:

$$k = N_0 e^{(-Ea/RT)} \quad (7.16)$$

where Ea is the activation energy, R_g the universal gas constant, and T is the temperature in Kelvin degrees. The Arrhenius model has been used to describe the kinetic behaviour of *E. coli* as a function of temperature, pH, and a_w (Cerf *et al.*, 1996).

Another view to microbial inactivation has been discussed in a series of articles (Peleg and Cole, 1998, 2000; Peleg and Pechina 2000), although the basic principles can already be found in older literature (e.g., Vas and Prosz, 1957). It considers lethal events as probabilities, rather than deterministic events.

The alternative view thus looks upon survival curves as an expression of underlying statistical distributions of inactivation times.

7.3.2.2 Weibull model

The Weibull model has been used as a primary thermal inactivation model for vegetative bacteria (van Boekel, 2002). This model assumes non-linearity of semi-logarithmic survivor curves in the inactivation process through considering biological variation with respect to thermal inactivation, and is basically a statistical model of distribution of inactivation times. The model has two parameters, the scale parameter α (time) and the dimensionless shape parameter β . The logarithm of the scale parameter α linearly depends on temperature; however, this relationship is not so well established for parameter β . The shape parameter accounts for upward concavity of a survival curve ($\beta < 1$), a linear survival curve ($\beta = 1$), and downward concavity ($\beta > 1$). Therefore, if $\beta = 1$, no biological variation is assumed (each cell is equally susceptible to be destroyed).

In terms of a survival curve, the cumulative function is

$$\log S(t) = -\frac{1}{2.303} \left(\frac{t}{\alpha} \right)^\beta \quad (7.17)$$

The Weibull distribution function (which is closely related to the gamma, extreme value, and log-normal distributions) is widely used in reliability engineering to describe the time to failure in electronic and mechanical systems and is also appropriate for the analysis of survival data, i.e., the time to failure after the application of stress.

Despite the empirical nature of the Weibull model, a link can be made with physiological effects. Thus, $\beta < 1$ indicates that the remaining cells have the ability to adapt to the applied stress, whereas $\beta > 1$ indicates that the remaining cells become increasingly damaged.

This variation in parameters allows the Weibull model to be more flexible than the linear model based on D and z values. For instance, cell

behaviour may be quite different when it has been adapted to certain stress conditions in foods or when they have grown in “ideal” laboratory conditions.

Modifications of the Weibull model have been proposed by Mafart *et al.* (2002) for the calculation of heat treatment efficiency to describe non-linear survival curves of spores. Later on, Coroller *et al.* (2006) applied a general model based on two mixed Weibull distributions of cell resistance of vegetative bacteria subjected to an acidic stress (*Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium), which was proposed to describe the survival curves and the change in the pattern with the modifications of resistance of two assumed subpopulations.

7.3.2.3 Shoulder/tail models

Shoulder/tail models are based upon the existence of a shoulder or lag period (prior to inactivation) and a tail region (after the inactivation treatment). Stringer *et al.* (2000) reported a number of explanations for this deviation from linearity, like the variability in heating procedure, use of mixed cultures, clumping, or the protective effect of the food matrix or dead cells.

A linear approach was followed by Whiting (1993) for modeling inactivation of *L. monocytogenes* and *Salmonella* as a function of temperature and in the presence of NaCl, nitrites, and lactic acid:

$$\log N(t) = \begin{cases} \log N_0 & \text{when } 0 < t < t_L \\ \log N_0 - \left(\frac{1}{D} \right) (t - t_L) & \text{when } t > t_L \end{cases} \quad (7.18)$$

where N is the number of microorganisms surviving at time t , N_0 is the initial microbial load, t_L is the time prior to inactivation, and D is the D -value or time for decimal reduction of microorganisms.

Regarding the shoulder region, model fitting is more difficult since a high variability is associated with this parameter. Thus, survival is often described through the time required for a 4-log

reduction, T4D (Whiting, 1993). This value is calculated as the sum of $t_L + 4D$.

Non-linear approaches normally represent a shoulder/tailing function such as

$$\log \frac{N}{N_0} = -\frac{t^p}{D} \quad (7.19)$$

where p is lower than 1 in case of a concave curve, and higher than 1 in case of a convex (shoulder curve).

Another modeling approach, named as the Fermi equation, uses the logistic function, with the correspondent inactivation forms. For the

$$N = \left[(N_0 - N_{res}) e(-k_{max} t) \frac{e(-k_{max} t_L)}{1 + e((-k_{max} t_L) - 1)e(-k_{max} t)} + N_{res} \right] \quad (7.21)$$

where N is the number of microorganisms surviving at time t , N_0 is the initial microbial load, k_{max} is the maximum specific decay rate, t_L is the time prior to inactivation, and N_{res} is the residual population density.

Other inactivation models relied upon mechanistic processes in which predictions can be provided outside the range of the obtained experimental data. These models have been mainly developed for spore-forming bacteria and for high-temperature treatments (UHT, HTST sterilization). However, the primary drawback to mechanistic models lies on their complex development and successful performance.

7.3.3 Linear and non-linear inactivation kinetics at dynamic conditions

There are two different ways of adjusting predictive models at dynamic conditions. Traditionally, microbial kinetics is identified by a two-step modeling approach. In this way, kinetic parameters from a primary model are estimated for a given range of environmental conditions. These estimations are used for the construction of a secondary model, which is subsequently used for validation purposes at dynamic conditions. Despite the

quantification of sigmoidal decay curves, the following expression is used:

$$\log \frac{N}{N_0} = \log \left[\frac{1 + e^{-btL}}{1 + e^{b(t-t_L)}} \right] \quad (7.20)$$

where N is the number of microorganisms surviving at time t , N_0 is the initial microbial load, b is the maximum specific decay rate, and t_L is the time prior to inactivation.

Geeraerd *et al.* (2000) developed a shoulder/tail inactivation model considering the physiological state of cells and the residual population density (tail region):

relative ease and mathematical simplicity of this approach, in many cases modifications in the equation structure are needed to fit predictive models accurately at dynamic conditions (Valdramidis *et al.*, 2006a).

Performance of this approach requires the application of a one-step (or global) modeling identification procedure, i.e., parameters are estimated at once by studying the microbial population during the tested dynamic environment. It has already been shown that more precise estimates are obtained when compared with the classical two-step approach (Van Boekel, 1996).

The Bigelow model integrated in the reduced log-linear model without the tailing effect can be effectively used for estimation of microbial behavior at dynamic conditions:

$$\frac{d(N(t))}{dt} = -\frac{\ln 10}{AsymD_{ref}} \exp\left(\frac{\ln 10}{z}(T - T_{ref})\right) \left(\frac{1}{1 + C_c}\right) N \quad (7.22)$$

$$\frac{dC_c}{dt} = -\frac{\ln 10}{AsymD_{ref}} \exp\left(\frac{\ln 10}{z}(T - T_{ref})\right) \left(\frac{1}{1 + C_c}\right) C_c \quad (7.23)$$

where the explicit form of the Bigelow model can be written as

$$k_{max} = \frac{\ln 10}{AsymD_{ref}} \exp\left(\frac{\ln 10}{z}(T - T_{ref})\right) \quad (7.24)$$

and the constant physiological state (k_{phys}) is dependent on the critical component C_c :

$$k_{phys} = \frac{1}{1 + C_c} \quad (7.25)$$

where N is the microbial cell density (cfu/ml), C_c is related to the physiological state of the cells, and k_{max} is the inactivation rate (min^{-1}). The asymptotic decimal reduction time ($AsymD$ value) is the thermal parameter, together with the thermal resistance value (z value; i.e. the number of °C needed to produce a tenfold change in the $AsymD$ value).

For describing the dehydration processes, ramp rates can be defined by the Dabes-type equation (Valdramidis *et al.*, 2006b):

$$T = T_0 + T_{diff} \frac{(t + t_{crit}) - \sqrt{(t + t_{crit})^2 - 4(t_{crit} - B)t}}{2(t_{crit} - B)} \quad (7.26)$$

where T_0 (°C) represents the initial temperature, T_{diff} (°C) is the difference between the initial and the final temperature, B (min) is defined as a shaped parameter, and t_{crit} (min) is the time to reach a holding temperature.

Valdramidis *et al.* (2008) illustrated the application of a log-linear shoulder model to estimate the inactivation of *E. coli* K12 in an artificial medium at different ramp rates (0.15 °C/min, 0.43 °C/min, and 1.64 °C/min). Application of the dynamic model can be seen in Figure 7.2.

7.3.4 Secondary models

Evidence for an interacting effect between temperature and pH are referred in the studies of López *et al.* (1996) and Fernández *et al.* (1996) for *Geobacillus stearothermophilus* and *Clostridium sporogenes*. The model, subsequently developed by Mafart and L  gu  rinel (1998), considers an additive effect, on a log scale, as follows:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \frac{(pH - pH^*)^2}{z_{pH}^2} \quad (7.27)$$

where T^* is the reference temperature, pH^* is the pH of the maximum heat resistance (7.0 for spores), z_T is the commonly used thermal

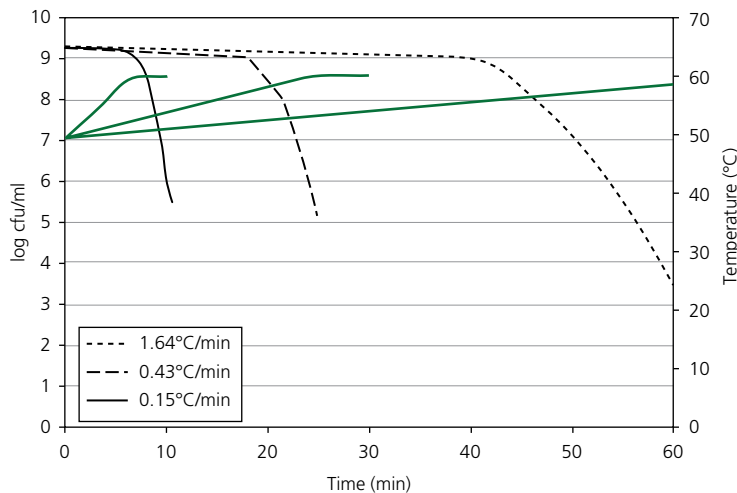


Figure 7.2 Dynamic model (log-linear shoulder) at different ramp rates representing inactivation of *E. coli* K12 in culture broth as a consequence of applying a heating process (adapted from Valdramidis *et al.*, 2008).

z value, z_{pH} is the distance from pH to pH^* , which leads to a tenfold reduction of the decimal reduction time, and D^* is the D value at T^* and pH^* .

A five-parameter model was proposed by Cerf *et al.* (1996) including an extension of Davey's model with the a_w term:

$$\ln k_{\max} = C_0 + \frac{C_1}{T} + C_2 pH + C_3 pH^2 + C_4 a_w^2 \quad (7.28)$$

where C_0 , C_1 , C_2 , C_3 , and C_4 are empirical coefficients without biological significance.

Gaillard *et al.* (1998) modified the Bigelow model to include an extension with the a_w term. The following relationship emerged:

$$\log D = \log D^* - \frac{T - T^*}{z_T} - \frac{(pH - pH^*)^2}{z_{pH^2}} - \frac{a_w - 1}{z_{aw}} \quad (7.29)$$

where z_{aw} is the distance of a_w from 1 which leads to a tenfold increase in the D value. In that work, an interaction between temperature and pH was noted for *B. cereus*. At pH 4.5 (mean z_T value = 10.48 °C) the thermal resistance of spores was less sensitive to variations of temperature than at pH 6.5 (mean z_T value = 8.78 °C). Interactions were also shown between a_w and temperature. At 85 and 95 °C a decrease of sensitivity of spores to pH variations was observed with decreasing a_w .

Published models for vegetative microorganisms are available, such as the study of Blackburn *et al.* (1997), who developed a secondary inactivation model for *Salmonella* Enteritidis and *E. coli* O157:H7. For both organisms, predicted z values of 4.6–7.0 °C were obtained depending on conditions, with larger z values at higher levels of NaCl. Optimum survival occurred between pH 5 and pH 7, and increasing acidity or alkalinity caused a decrease in the predicted D values. The effect of pH on thermal inactivation of *Salmonella* in broth medium was studied by

Mañas *et al.* (2003). They observed that the pH of the heating medium at which heat resistance was maximum was 6 for cells grown at 37 °C, but changed with growth temperature. The alkalization of the heating medium from pH 6 to 7.7 decreased the heat resistance of cells grown at 37 °C by a factor of 3.

Buchanan and Golden (1994, 1995) studied the effect and interactions of temperature (4–42 °C), lactic acid concentration (0–2%), pH (3.3–7.3), NaCl content (0.5–19.0%), and sodium nitrite concentration (0–200 µg/ml) on the survival of a three-strain mixture of *L. monocytogenes* in an aerobic and oxygen-reduced environment. Results obtained were similar in both cases and time to fourfold log reduction was inversely related to the lactic acid concentration, temperature, and NaCl content. The effect of sodium nitrite was pH-dependent.

Alternative secondary models for *L. monocytogenes* taking into account other factors such as the type of organic acid and the type of solute to reduce a_w values were subsequently developed (Fernández *et al.*, 2007; Miller *et al.*, 2009).

7.3.5 Survival/inactivation of microorganisms in low-moisture foods

Low-moisture foods are often complex mixtures of several food components. The range of particulate foods is large, including diverse products such as oil-in-water emulsions, reconstituted meat and fish products, and confectionary (e.g., chocolates containing fruits, nuts, and sugar granules). These components produce a more heterogeneous behavior of microorganisms in inactivation processes. Especially, oil components and fat matter of composite foods are reported to have a protective effect in microbial cells against preservatives and heat treatments (Wilson *et al.*, 2002).

These food types present a number of factors that should be taken into account for designing an inactivation treatment:

- Type of microorganisms to be controlled
- Number of microorganisms (i.e., initial microbial load)
- Exposure time
- Temperature
- Food matrix
 - Presence of organic material
 - pH
 - a_w
 - Biofilm formation
- Recovering conditions.

Survival and inactivation of food-borne pathogens have been reported in low- a_w composite foods. This low a_w allows pathogens such as *Salmonella* spp. to increase its thermal resistance and to survive to processing conditions that would normally be expected to destroy this pathogen (Barrile *et al.*, 1970; D'Aoust, 1977). Bayliss *et al.* (2004) studied the survival of *E. coli* O157:H7, O111:H₋ and O26:H11 in chocolate and confectionery products (Figure 7.3).

It was concluded that temperature is unlikely to be the only factor that affects the rate of decline of these bacteria. In addition to nutrient availability and the inhibitory effects of certain compounds in a food, growth and survival will also be influenced by a_w of the product. Indeed, survival was longer in chocolate, which was the product with the

lowest a_w (0.40–0.52), whereas death occurred more rapidly in the biscuit cream and mallow, which had higher a_w (0.73–0.79).

Predictive models must take into account possible changes of the temperature during the storage of low- a_w foods since they are exposed to temperature variations during storage, affecting both moisture transfer rate and their shelf-life.

7.4 Example of application/development of predictive microbiology models for describing microbial death during drying processes

7.4.1 *Listeria monocytogenes* inactivation as a function of temperature and a_w (Valdramidis *et al.*, 2006b)

7.4.1.1 Purpose and scope

The purpose of this study was the development of new secondary modeling structures that can describe the influence of temperature and a_w on the inactivation of *L. monocytogenes*, as a surface decontamination process of potatoes.

Heat treatment is a well-known decontamination process of foods, which entails moisture

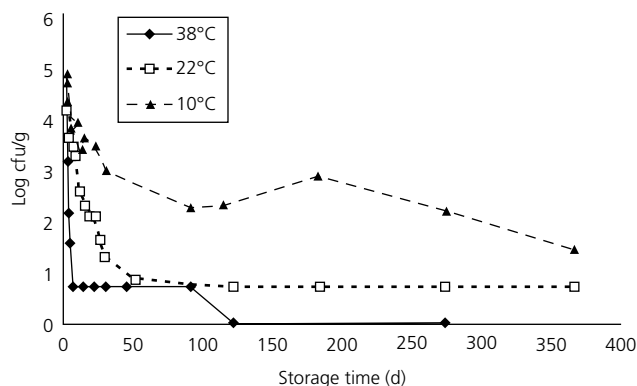


Figure 7.3 Fate of *Escherichia coli* O157:H7, O26:H11, and O111:H₋ in chocolate during storage at 10, 22, and 38 °C (adapted from Bayliss *et al.*, 2004).

removal. Next to the heat effect, the induced reduction of a_w contributes to preserve food safety, by avoiding microbial growth and chemical reactions causing deterioration.

7.4.1.2 Experimental design

Static experiments: a complete 32 factorial design (at the temperature range of 55–65 °C and a_w range of 0.71–0.99).

Dynamic experiments: two different heating rates, reaching the following final temperatures: 60, 75, 90, and 100 °C.

7.4.1.3 Conditions of food matrix or lab media

Static experiments: macerated potato. Experiments were initiated once the desired temperature and a_w were reached.

Dynamic experiments: potato skin-off discs.

7.4.1.4 Microorganism

Listeria monocytogenes Scott A.

7.4.1.5 Type of inactivation model

Primary model: dynamic sigmoidal-like model without tail.

Secondary model: response surface, Arrhenius and Bigelow type models, describing the inactivation rate k_{max} as a function of temperature and a_w . Based on their fitting capacity (R^2_{adj}), the Bigelow type model was finally selected.

7.4.1.6 Model shape/model parameters

It is worthy to mention that inactivation parameters of the integrated model (the primary model integrated with the Bigelow type model) were calculated in three different ways: (i) data from static experiments, (ii) data from static experiments plus the static data from dynamic experiments at 60 and 75 °C, and (iii) data from static experiments plus the static data from all dynamic experiments.

The integrated model is presented below according to the second (Equation (7.30)) and third (Equation (7.31)) batches of data, where k_{max} is the specific inactivation rate (min^{-1}), and T is temperature (°C).

$$k_{max}(T, a_w) = \frac{\ln 10}{3.67 \times 10^{-1}} \exp\left(\frac{\ln 10}{4.61}(T - 65)\right) \exp\left(\frac{\ln 10}{2.89 \times 10^{-1}}(a_w - 1)\right) \quad (7.30)$$

$$k_{max}(T, a_w) = \frac{\ln 10}{1.80} \exp\left(\frac{\ln 10}{7.11}(T - 60)\right) \exp\left(\frac{\ln 10}{2.31 \times 10^{-1}}(a_w - 1)\right) + 2.21 \times 10^{-1} \quad (7.31)$$

7.4.1.7 Validation

The performance of the integrated model was validated at 90 and 100 °C by confronting dynamic inactivation experiments with the values predicted by the developed integrated models.

7.4.1.8 Remarks

The phenomenon of heat resistance at decreased a_w values (low- a_w protection) was observed at temperatures between 55 and 65 °C, as corroborated by other authors, and was explained by the integrated models.

The validation of the integrated model by confrontation with dynamic inactivation data outlines the necessity of exploiting information from experiments. It was observed that the range of experimental data for estimating the inactivation parameters could influence the predictions of the model, rendering fail-dangerous or fail-safe predictions depending on the range of temperatures evaluated. In general, the second and third methods for estimating inactivation parameters (as described in a previous section) gave more accurate predictions, although they must be applied with caution at high temperatures.

7.4.2 *Salmonella* inactivation during drying of beef jerky (Yoon *et al.*, 2009)

7.4.2.1 Purpose and scope

The objective of this study was to model the effect of drying temperature in combination with predrying marination treatments on inactivation of *Salmonella* on beef jerky.

Due to several outbreaks and recalls, USDA/FSIS recommended through a jerky processing compliance guideline to apply any of the following processes: preheat meat slices in marinades to achieve an internal temperature of 71.1 °C, dipping the meat slices in 5% acetic acid for 10 min before marination, or drying the slices in the presence of moisture.

7.4.2.2 Experimental design

All samples were treated as follows:

- 1 Predrying treatment during 24 h at 4 °C: (a) no marination (C), (b) traditional marination (M), or (c) dipping into 5% acetic acid solution for 10 minutes followed by M (AM).
- 2 Drying: dehydration for 10 h at 52, 57, or 63 °C at an altitude of 1600 m. Every 2 hours, two samples were withdrawn for microbial analysis.

The study was repeated twice.

7.4.2.3 Conditions of food matrix or lab media

Beef slices of 0.6 cm thickness and 8.7 × 4 cm size were prepared by means of a food slicer. Slices were vacuum-packaged and stored at -18 °C until they were used (1 to 3 weeks). For inoculation, slices were left to thaw at 4 °C for 24 h, after which slices were inoculated by spreading a volume of 0.5 ml of *Salmonella* inoculum (10⁷ cfu/ml) on each flat surface using a flame-sterilized bent glass rod.

7.4.2.4 Microorganism

Salmonella enterica Typhimurium was used, specifically the strains ATCC14028, ATCC700408, and DT104 Copenhagen 2 (isolated from beef hide), SF530 UK1 (an isolate from a horse wound; provided by Dr J.W. Foster,

University of South Alabama, Mobile, AL, USA), and *Salmonella enterica* Agona 1 (an isolate from alfalfa sprouts; provided by Dr L.R. Beuchat, University of Georgia, Griffin, GA, USA).

7.4.2.5 Type of inactivation model

Primary model: Baranyi model.

Secondary model: polynomial model.

7.4.2.6 Model shape/model parameters

$$C: DR = 1.3179 - 0.03046 T \quad (R^2 = 0.762)$$

$$M: DR = 1.0202 - 0.0257 T \quad (R^2 = 0.524)$$

$$AM: DR = -0.0629 - 0.0220 T \quad (R^2 = 0.242)$$

where *DR* is the death rate (log cfu/cm² h) and *T* is temperature (°C).

7.4.2.7 Validation

Models developed were validated against data from Calicioglu *et al.* (2003), who studied *Salmonella* inactivation during drying and storage of beef jerky treated with marinades. Bias and accuracy factors were calculated to evaluate the performance of the models. Additionally, *R*² was also calculated as a measure of the strength of a linear relationship between observed and predicted cell counts (simple linear regression).

The models developed in this study for C and M showed good model performance, while the model for AM was acceptable.

7.4.2.8 Remarks

During dehydration, the AM-treated samples had higher DRs (-1.22 to -1.46 log cfu/cm² h) than C or M samples (-0.29 to -0.63 log cfu/cm² h) regardless of the drying temperature.

Although the models developed did not present good fitting indexes, validation indices as well as *R*² showed good or very good performance. Bias and accuracy factors were 1.03 and 1.06 (C), 1.01 and 1.05 (M), and 0.71 and 1.41 (AM), respectively, and *R*² was 0.965, 0.987, and 0.954 for C, M, and AM, respectively.

7.4.3 *Salmonella* inactivation during drying of sausages (Coroller et al., 2015)

7.4.3.1 Purpose and scope

The objective of this study was to develop a model describing the evolution of *Salmonella* during the fabrication processing of dried sausages and to optimize the food formulation to prevent pathogen presence at the end of the process. The formulations included: starter cultures type (“fast” or “moderate”) and two levels of sugar and salt contents.

Salmonella is known to be heat sensitive, which means that cooked products are secured provided that recontamination is prevented. However, processed products not thermally treated could cause real concern if manufactured with contaminated raw meat, such as the case of dry fermented sausages. In this type of product, only drying and addition of antibacterial ingredients or preservatives contribute to its safety.

7.4.3.2 Experimental design

A full factorial design with four factors and two levels was carried out, in order to investigate the effects of the starter cultures, sugar concentrations of dextrose and lactose, as well as salt concentration.

During fermentation and the drying process, sausages were sampled for microbiological and physicochemical analysis three times a day during fermentation and once a day during the drying stage.

7.4.3.3 Conditions of food matrix or lab media

Raw pork meat was obtained from a local store. The batter was prepared by first grinding the raw meat (pork lean meat 80%, derinded backfat 20%) to obtain particles of 6 mm diameter before blending the mixture with starter cultures (fast or moderate acidifying power at a level of 10^6 cfu/g), dextrose (5 or 8 g/kg), lactose (0 or 10 g/kg), salt (24 or 28 g/kg), black pepper (1.5 g/kg), and nitrate (0.3 g/kg).

After inoculation of the batter with *Salmonella*, it was stuffed into natural casings to obtain 450 g, and then fresh-made sausages were tied with strings before being dipped into a commercial *Penicillium* surface suspension and hung vertically in a temperature- and humidity-controlled incubator to undertake the ripening and drying process.

Temperature and relative humidity were set according to the average process conditions used in France by fermented dried sausages manufacturers and validated by a group of six industrial partners. During the fermentation step the temperature and the relative humidity evolved from 22 °C and 90–95%, respectively, to 16 °C and 82–90%, respectively, during 4 days. During the drying step (30 days), the temperature was maintained at 13–14°C and the relative humidity at 73–81%.

7.4.3.4 Microorganism

Salmonella enterica serovar Typhimurium ADQP305, from the Sym’Previus collection, isolated from brine and kindly provided by ADRIA Développement (Quimper, France), was used in this study.

The prepared batter was inoculated by a *Salmonella* suspension to reach a contamination level of 10^3 cfu/g.

7.4.3.5 Type of inactivation model and performance

For growth modeling: gamma model.

For inactivation modeling: double Weibull.

Transition from growth (observed during fermentation) and inactivation (observed during drying) was also modeled.

According to the type of starter used, the performance of the model was better using the “fast” starter compared to the “moderate” starter with a global RMSE of 0.228 and 0.984, respectively.

7.4.3.6 Validation

Performance of models was tested with an independent set of data. These data were collected during the manufacture process of

fermented dried sausages from previous works (Piaudel and Christeans, 2010a, 2010b; Zuliani and Christeans, 2009).

7.4.3.7 Remarks

Using this dynamic growth-inactivation model, the effect of the ingredients could be evaluated on the *Salmonella* behavior. The salt content (24 or 28 g/kg) had no direct effect on the growth nor on the inactivation of *Salmonella*. The salt content might have an effect on the starter behavior, and then on the pH and lactic acid evolutions, which have a direct impact on *Salmonella* behavior. The dextrose content directly enhanced the pathogen growth. Finally, the main ingredient impacting the growth and the inactivation of *Salmonella* was the type of starter, which allowed the growth and the resistance of this pathogen to reduce whatever the pH or acid lactic dynamic “fast” or “moderate” content due to the starter activity.

7.5 Conclusions

Microbial predictive models have been shown to be a powerful tool to tune the environmental conditions and food formulations to ensure that potential pathogens present in foods are inhibited during the drying process.

Although empirical models are used in most cases (e.g., polynomial, Weibull models), they are still valuable to predict the fate of microorganisms during a food processing step. Nevertheless, the application of predictive models should be taken with caution, and their assumptions and limitations should be perfectly understood.

In some cases, predictions of the drying effect on microorganisms in foods may be really challenging, especially in those cases where the food matrix/ingredients are complex or natural microflora is presented. Simplification is an extended approach for dealing with this issue, taking into account the main factors involved in the inhibition of the microorganism of interest.

Guidelines or Regulations on the Performance Criteria (PC) or Performance Objective (PO) for different pathogens in most industrial processes (e.g., drying) are still lacking. Predictive models would constitute a scientific support decision tool to set the different drying parameters having a role in the inhibition of pathogens, with the aim of achieving the target PC/PO.

References

- Andrade, R.D.P., Roberto, L.M., and Pérez, C.E.C. 2011. Modelos de isotermas de sorcion para alimentos: usos y limitaciones. *Vitae*, **18**, 325–334.
- Barrile, J.C., Cone, J.F., and Keeney, P.G. 1970. A study of *Salmonellae* survival in milk chocolate. *Manufacturing Confectioner*, **50**, 34–39.
- Bayliss, C.L., MacPhee, S., Robinson, A.J., Griffiths, R., Lilley, K., and Betts, R.P. 2004. Survival of *Escherichia coli* O157:H7, O111:H– and O26:H11 in artificially contaminated chocolate and confectionery products. *International Journal of Food Microbiology*, **96**, 35–48.
- Bermudez-Aguirre, D. and Corradini, M.G. 2012. Inactivation kinetics of *Salmonella* spp. under thermal and emerging treatments: a review. *Food Research International*, **45**, 700–712.
- Blackburn, C.W., Curtis, L.M., Humpheson, L., Billon, C., and McClure, P.J. 1997. Development of thermal inactivation models for *Salmonella* Enteritidis and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors. *International Journal of Food Microbiology*, **38**, 31–44.
- Buchanan, R.L. and Golden, M.H. 1994. Interaction of citric acid concentration and pH on the kinetics of *Listeria monocytogenes* inactivation. *Journal of Food Protection*, **57**, 567–570.
- Buchanan, R.L. and Golden, M.H. 1995. Model for the non-thermal inactivation of *Listeria monocytogenes* in a reduced oxygen environment. *Food Microbiology*, **12**, 203–212.
- Calicioglu, M., Sofos, J.N., Samelis, J., Kendall, P.A., and Smith, G.C. 2003. Effects of acid adaptation on inactivation of *Salmonella* during drying and

- storage of beef jerky treated with marinades. *International Journal of Food Microbiology*, **89**, 51–65.
- Cerf, O., Davey, K.R., and Sadoudi, A.K. 1996. Thermal inactivation of bacteria – a new predictive model for the combined effect of three environmental factors: temperature, pH and water activity. *Food Research International*, **29**, 219–226.
- Coroller, L., Leguerinel, I., Mettler, E., Savy, N., and Mafart, P. 2006. General model, based on two mixed Weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. *Applied Environmental Microbiology*, **72**, 6493–6502.
- Coroller, L., Jeuge, S., Couvert, O., Christeians, S., and Ellouze, M. 2015. Extending the gamma concept to non-thermal inactivation: a dynamic model to predict the fate of *Salmonella* during the dried sausages process. *Food Microbiology*, **45**, 266–275.
- D'Aoust, J.Y. 1977. *Salmonella* and the chocolate industry. A review. *Journal of Food Protection*, **40**, 718–727.
- Fernández, P.S., Ocio, M.J., Rodrigo, F., Rodrigo, M., and Martínez, A. 1996. Mathematical model for the combined effect of temperature and pH on the thermal resistance of *Bacillus stearothermophilus* and *Clostridium sporogenes* spores. *International Journal of Food Microbiology*, **32**, 225–233.
- Fernández, a L., Bernardo, a M., Condón, S., and Raso, J. 2007. Modelling thermal inactivation of *Listeria monocytogenes* in sucrose solutions of various water activities. *Food Microbiology*, **24**, 372–379. <http://doi.org/10.1016/j.fm.2006.07.017>.
- Gaillard, S., Lèguérinel, I., and Mafart, P. 1998. Model for combined effects of temperature, pH and water activity on thermal inactivation of *Bacillus cereus* spores. *Journal of Food Science*, **63**, 887–889.
- Geeraerd, A.H., Herremans, C.H., and Van Impe, J.F. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, **59**, 185–209.
- Geeraerd, A.H., Valdramidis, V.P., and Van Impe, J.F. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102**, 95–105.
- Hersom, A.C. and Hulland, E.D. 1980. Principles of thermal processing. In: *Canned Foods. Thermal Processing and Microbiology* (eds Hersom, A.C. and Hulland, E.D.), pp. 177–207. Churchill Livingstone, New York, USA.
- Higl, B., Kurtmann, L., Carlsen, C.U., Ratjen, J., Forst, P., Skibsted, L.H., Kulozik, U., and Risbo, J. 2007. Impact of water activity, temperature, and physical state on the storage stability of *Lactobacillus paracasei* spp. *paracasei* freeze-dried in a lactose matrix. *Biotechnology Progress*, **23**, 794–800.
- Jay, J.M. 1992. *Modern Food Microbiology*, 4th edn. Van Nostrand Reinhold, New York, USA.
- López, M., González, I., Condón, S., and Bernardo, A. 1996. Effect of pH heating medium on the thermal resistance of *Bacillus stearothermophilus* spores. *International Journal of Food Microbiology*, **28**, 405–410.
- Mafart, P. and Lèguérinel, I. 1998. Modeling combined effects of temperature and pH on heat resistance of spores by a linear-Bigelow equation. *Journal of Food Science*, **63**, 6–8.
- Mafart, P., Couvert, O., Gaillard, S., and Leguérinel, I. 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology*, **72**, 107–113.
- Mañas, P., Pagan, R., Raso, J., and Condón, S. 2003. Predicting thermal inactivation in media of different pH of *Salmonella* grown at different temperatures. *International Journal of Food Microbiology*, **87**, 45–53.
- McMinn, W.A.M. and Magee, T.R.A. 1997. Physical characteristics of dehydrated potatoes – Part II. *Journal of Food Engineering*, **33**, 49–55.
- Miller, F.A., Ramos, B., Gil, M.M., Brandao, T.R.S., Teixeira, P., and Silva, C.L.M. 2009. Influence of pH, type of acid and recovery media on the thermal inactivation of *Listeria innocua*. *International Journal of Food Microbiology*, **133**, 121–128.

- Peleg, M. and Cole, M.B. 1998. Reinterpretation of microbial survival curves. *Critical Review of Food Science and Nutrition*, **38**, 353–380.
- Peleg, M. and Cole, M.B. 2000. Estimating the survival of *Clostridium botulinum* spores during heat treatments. *Journal of Food Protection*, **63**, 190–195.
- Peleg, M. and Penchina, C.M. 2000. Modeling microbial survival during exposure to a lethal agent with varying intensity. *Critical Review of Food Science and Nutrition*, **40**, 159–172.
- Piaudel, G. and Christieans, S. 2010a. *Impact de la flore de compétition endogène sur le comportement des salmonelles dans le saucisson sec*. Rapport d'étude Financée par INAPORC. ADIV.
- Piaudel, G. and Christieans, S. 2010b. *Comportement des salmonelles au cours du procédé de fabrication et de la conservation des salaisons sèches de différents calibres*. Rapport d'étude Financée par Aprivis. ADIV.
- Rahman, M.S., Guizani, N.G., and Al-Ruzeiki, M.H. 2004. D- and Z-values of microflora in tuna mince during moist- and dryheating. *Lebensmittel-Wissenschaft und -Technologie*, **37**, 93–98.
- Reichert, O. 1994. Modeling the destruction of *Escherichia coli* on the base of reaction kinetics. *International Journal of Food Microbiology*, **23**, 449–465. doi: 10.1016/0168-1605(94)90169-4.
- Rizvi, S.S.H. 2005. *Engineering Properties of Foods*, 3rd edn (eds Rao, M.A., Syed Rizvi, S.H., and Datta, A.K.), p. 768. CRC Press, Boca Raton, FL, USA.
- Shigehisa, T., Inoue, T., and Kumagai, H. 2015. Mathematical model of water sorption isotherms of UBC. *Fuel Processing Technology*, **131**, 133–141.
- Stringer, S.C., George, S.M., and Peck, M.W. 2000. Thermal inactivation of *Escherichia coli* O157:H7. *Journal of Applied Microbiology*, **88**, 79S–89S.
- Valdramidis, V.P., Geeraerd, A.H., Bernaerts, K., and Van Impe, J.F. 2006a. Microbial dynamics versus mathematical model dynamics: the case of microbial heat resistance induction. *Innovative Food Science of Emerging Technology*, **7**, 80–87.
- Valdramidis, V.P., Geeraerd, A.H., Gaze, J.E., Kondjoyan, A., Boyd, A.R., Shaw, H.L., and Van Impe, J.F. 2006b. Quantitative description of *Listeria monocytogenes* inactivation kinetics with temperature and water activity as the influencing factors; model prediction and methodological validation on dynamic data. *Journal of Food Engineering*, **76**, 79–88.
- Valdramidis, V.P., Geeraerd, A.H., Bernaerts, K., and Van Impe, J.F. 2008. Identification of non-linear microbial inactivation kinetics under dynamic conditions. *International Journal of Food Microbiology*, **128**, 146–152.
- Van Boekel, M. 1996. Statistical aspects of kinetic modeling for food science problems. *Journal of Food Science*, **61**, 477–485.
- Van Boekel, M. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, **74**, 139–159.
- Vas, K. and Prosz, G. 1957. Observations on the destruction of spores of *Bacillus cereus*. *Journal of Applied Bacteriology*, **21**, 431–441.
- Whiting, R.C. 1993. Modeling bacterial survival in unfavourable environments. *Journal of Industrial Microbiology*, **12**, 240–246.
- Wilson, P.D.G., Brocklehurst, T.F., Arino, S., Thuault, D., et al. 2002. Modelling microbial growth in structured foods: towards a unified approach. *International Journal of Food Microbiology*, **73**, 275–289.
- Yoon, Y., Geornaras, I., Kendall, P.A. and Sofos, J.N. 2009. Modeling the effect of marination and temperature on *Salmonella* inactivation during drying of beef jerky. *Journal of Food Science*, **79**, M165–M171.
- Zuliani, V. and Christieans, S. 2009. *Comportement des salmonelles au cours du procédé de fabrication et de la conservation des saucissons secs de ménage*. Rapport d'étude Financée par Aprivis et INAPORC. IFIP, ADIV.

CHAPTER 8

Impact of irradiation on the microbial ecology of foods

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8.1 Introduction

Irradiation is a process of exposing a food item to certain types of radiation energy to induce desirable changes. Radiation is defined as energy moving through space in invisible waves. Radiant energy has different wavelengths and degrees of power (Josephson and Peterson, 2000). Figure 8.1 depicts the electromagnetic spectrum. The spectrum has two major divisions including non-ionizing radiation and ionizing radiation.

Radiation that has energy to move atoms in a molecule around or cause them to vibrate, but not enough to remove electrons, is referred to as “non-ionizing radiation”. Near ultraviolet (UV) light, visible light, infrared, microwave, and radio waves are all examples of non-ionizing radiation.

Radiation that has enough energy to remove tightly bound electrons from atoms, thus creating ions, is called “ionizing radiation”. It has a higher frequency and shorter wavelength than non-ionizing radiation. Far UV light, X-rays, and gamma rays are regarded as ionizing radiation.

8.2 Ionizing radiation

The ionizing radiation of interest in food preservation is known as “irradiation”. Irradiation is capable of destroying harmful bacteria, viruses,

or insects that might be present in the food (Sommers and Fan, 2006). In comparison with other food processing techniques, food irradiation is relatively well studied. Although the safety and benefits of food irradiation have been thoroughly documented, the commercial application of the process has been prevented due to some misconception by the general public on its safety (ICGFI, 1999). The use of irradiation has been approved for different types of foods in over 55 countries worldwide (Farkas and Mohacsi-Farkas, 2011). The list of irradiated products is limited to spices, herbs, seasonings, some fresh or dried fruits and vegetables, seafood, meat and meat products, poultry and egg products (CFS, 2009).

The radiation dose (D) is the quantity of radiation energy absorbed by the food as it passes through the radiation field during processing. It is measured using a unit called the Gy (ICGFI, 1999). Irradiation can be used to sterilize food products at levels above 10 kGy. In the range of 1–10 kGy it can be used to pasteurize food by eliminating a significant number of microorganisms including those of public health significance. In some products it can be used as an insect disinfestation treatment (<1 kGy) and a sprout inhibitor in potatoes and onions (<0.5 kGy). It can also delay ripening of certain fruits (<0.3 kGy) and eliminate *trichinosis* in pork (<1 kGy) (Keener, 1998).

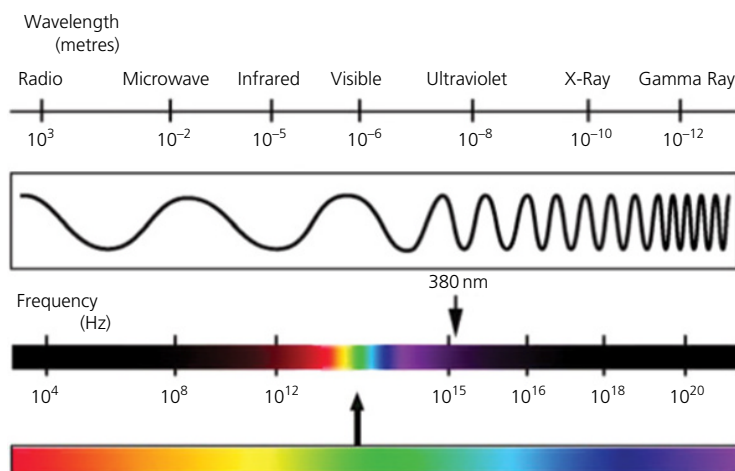


Figure 8.1 Electromagnetic spectrum (from <http://www.viewzone2.com/dna.html>).

The food irradiation process uses three types of ionizing radiation sources including Cobalt-60 and Cesium-137 gamma sources, electron beam generators, and X-ray accelerators. Cobalt-60 or Cesium-137 emits ionizing radiation in the form of intense gamma rays. X-ray accelerator uses an electron beam to target electrons on a metal plate. Some of the energy is absorbed and the rest is converted into X-rays. Electron beam generators produce e-beams accelerated to 99% of the speed of light and generate energies of up to 10 MeV. The electromagnetic radiations of the first two types of sources have good penetration ability, while accelerated electrons have low penetrability (Farkas and Mohacsi-Farkas, 2011).

8.2.1 Impact of ionizing radiation on food-borne microorganisms

8.2.1.1 Inactivation mechanism

Except for different penetration, the effects of electromagnetic ionizing radiations and electrons are the same in food irradiation (Farkas, 2006). The primary effect of food irradiation is produced by energetic electrons. When ionizing radiation is passing through an item, it may result in ionization (removal of an electron), dissociation (loss of a hydrogen atom) and excitation

(raising the energy of a molecule to a higher energy level) types of chemical reactions in irradiated foods (CFS, 2009). The major product of an ionization reaction is reactive free radicals produced as a result of the primary effect. The secondary effects will occur when the free radicals undergo reactions such as recombination, electron capture, or dimerization (Alper, 1977). The presence of oxygen and water also have a profound effect on the radiolytic process.

The ionizing radiation generates reactive oxygen species, e.g., hydroxyl radicals and hydrogen peroxide, which result in oxidative stress in biological cells and damage cell components such as proteins, lipids, and deoxyribonucleic acid (DNA), leading to cell death (Asad *et al.*, 2004). Irradiation-induced cell death is primarily through extensive DNA damage. DNA is very sensitive to irradiation and is regarded as the main target of both ionizing and non-ionizing radiation. Irradiation of DNA causes base damage, breaking DNA strands, and cross-linking. All of these result in cell death (Alper, 1977).

The ability of microorganisms to survive upon irradiation depends on the damage produced in the cell, the number, nature, and duration of irradiation-induced chemical compounds, and

the inherent ability of cells to withstand ionizing radiation. pH, temperature, and chemical composition of the food in which microorganisms are suspended have also an important influence on the survival of microorganisms upon irradiation (Monk *et al.*, 1995).

There is an increasing public awareness of the health threat posed by food-borne pathogens. *Escherichia coli* O157:H7, *Salmonella*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Vibrio* are of primary concern from a public health standpoint. *Salmonella* and *C. jejuni* are usually associated with poultry. *E. coli* O157:H7 has also been linked to major food-borne disease outbreaks through meat and dairy products, apple juice, water, and vegetables (Moterjani *et al.*, 2014). *Listeria monocytogenes* has been associated with dairy products, processed meats, and other foods having a relatively long shelf life under refrigeration. *Vibrio* spp. have been the causative agents in world cholera pandemics. These pathogens are sensitive to low levels of ionizing radiation (ICGFI, 1999).

8.2.1.2 Modeling inactivation kinetics

Development of mathematical equations describing the behavior of microorganisms under different environmental conditions (physical, chemical, competitive) is the goal of predictive microbiology. In other words, predictive microbiology is related to the quantitative microbial ecology of foods. Mathematical models allow the description and prediction of microbial behavior under specific environmental conditions. Models can be classified as kinetic and probability models, empirical and mechanistic models, or primary and secondary models (Fakruddin, 2011). Kinetic models are considered with the rates of response (growth or death) (e.g., log-linear, Gompertz, square root models, etc.). Probability models indicate the probability of growth. Empirical models usually take the form of first or second degree polynomials and are used in curve fitting (e.g., the quadratic response surface model of Gibson *et al.*, 1988). Mechanistic or deterministic models are developed from the theoretical bases

and permit interpretation of the response in terms of known phenomena and processes (McMeekin *et al.*, 1993; Draper, 1988; Whiting and Buchanan, 1993). Primary models measure the response of the microorganism with time to a specific condition (e.g., growth, growth-decline, D values or thermal inactivation, inactivation-survival models). Secondary models describe how the factors of an actual growth environment affect the growth parameters (response surface, Arrhenius models, etc.) (Baranyi, 2003).

Mathematical models that allow predictions to be made of microorganism growth or decline represent a great benefit for the food irradiation process for assessment of the safety of foods (Tomac *et al.*, 2013).

The first-order inactivation model (log-linear model) satisfactorily describes the microorganisms response to irradiation treatment and estimates the D_{10} value. The D_{10} value is the irradiation dose necessary to reduce the number of particular microorganisms by one logarithmic unit. It provides a basis for accurate estimation of inactivation doses (Adu-Gyamfi *et al.*, 2012).

The first-order inactivation model assumes that the time rate of decrease in population (inactivation rate) (dN/dt) changes linearly with respect to population, N , and the dose rate (dD/dt) (Miller, 2005), such that

$$\frac{dN}{dt} = -kN \left(\frac{dD}{dt} \right) \quad (8.1)$$

After eliminating the time variable and integrating gives

$$N = N_0 e^{-kD} = N_0 e^{-(kD/2.3)} \quad (8.2)$$

where k is the first-order inactivation constant in units of kg/y , N_0 is the initial population at the start of irradiation, and D is the accumulated dose (kGy). The D_{10} value showing the resistivity of a microorganism to irradiation can be defined as

$$D_{10} = \frac{2.3}{k} \quad (8.3)$$

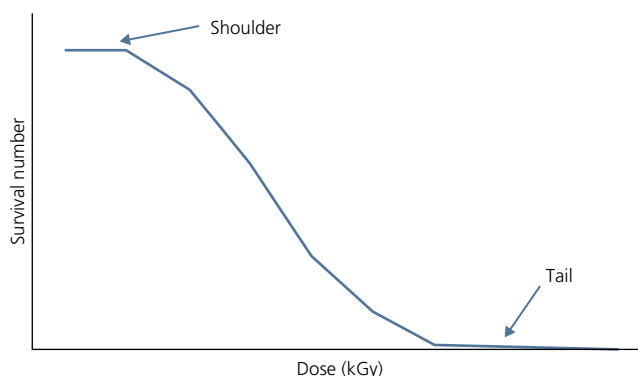


Figure 8.2 The dose inactivation curve of a typical bacterial population.

Equation (8.2) can also be expressed in logarithmic form (Tomac *et al.*, 2013), such that

$$\frac{\log N}{\log N_0} = -kD \quad (8.4)$$

In this case, the value of D_{10} is determined by calculating the reciprocal of the slope ($1/k$).

The first-order model was able to reasonably predict microbial inactivation when the irradiation dose was within certain limits. However, at low irradiation dose levels, a shoulder can be observed for some microorganisms (see Figure 8.2). The value of the first-order inactivation constant is low in the low-dose range, indicating that the organism has an effective DNA repair system. The first-order inactivation constant increased with dose and remained constant within a certain dose range. For these types of inactivation curves, two parameter models can be used to take account of the shoulder effect (Miller, 2005).

The radiation resistance (D_{10} value) of pathogenic bacteria exhibit differences in various media. It also varies between species. The order of radiation resistances of the common vegetative food-borne pathogens inoculated into raw meat or poultry products is found to be *Salmonella* spp. (~ 0.6 kGy) > *Staphylococcus aureus* (~ 0.5 kGy) > *Listeria monocytogenes* (~ 0.4 kGy) > *Escherichia coli* O157:H7 (~ 0.3 kGy) > *Yersinia enterocolitica*

(~ 0.2 kGy) > *Campylobacter* spp. (< 0.2 kGy) (Sommers, 2004). The radiation D_{10} values and the order of radiation resistances of the various food-borne pathogens inoculated on to complex ready-to-eat food products such as beef and vegetarian cheeseburgers and frankfurters are shown to be similar to those obtained in raw meat and poultry products. However, the D_{10} values of pathogens decrease three- to fourfold when inoculated on to leafy green vegetables such as iceberg, Boston, green leaf, and red leaf lettuce (Sommers, 2004).

8.2.1.3 Applications of ionizing irradiation

Ionizing radiation can be applied for the reduction of pathogenic microorganisms in food materials, decontamination of food surfaces, extension of the shelf life of certain foods, disinfestation of grains, and elimination of undesirable or toxic materials (CFS, 2009). These applications can also be organized according to the range of delivered dose (Miller, 2005). Low-dose applications (< 1 kGy) are concerned with inhibition of sprouting, delaying of maturation, parasite disinfection, and insect disinfestations. Medium-dose applications (1–10 kGy) are used for the control of food-borne pathogens and extension of the shelf life by retarding spoilage. High-dose applications (> 10 kGy) are associated with radiation sterilization of foods (Miller, 2005). Kume *et al.*

(2009) investigated the status of food irradiation in the world in 2005. They claimed that the irradiation was usually employed for disinfection of spices and vegetables (which comprise 46% of irradiated foods), disinfestation of grains and fruits (20%), disinfection of meat and fish (8%), and sprout inhibition of garlic and potatoes (22%).

In this section, a few representative examples of recent disinfection applications will be discussed. It has been shown that *Salmonella* contaminates the surface of vegetables and enters into the edible parts of different varieties of plants like lettuce, tomatoes, radish sprouts, bean sprouts, and barley (Tahergorabi *et al.*, 2012). James *et al.* (2010) studied the effect of e-beam on *S. Montevideo* inactivation in tomatoes. They observed significant reductions in the number of microorganisms subjected to >1.5 kGy irradiation. D_{10} values for *S. Montevideo* in tomatoes were 1.07 and 1.5 kGy. In another study, Mahmoud *et al.* (2010) investigated the effects of X-ray at different doses (0.1–2.0 kGy) on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Shigella flexneri* inoculated on spinach leaves. They observed more than 5 log cfu/leaf with 2.0 kGy X-ray for all tested pathogens. Mahmoud (2009) also studied the effect of X-ray treatments on inoculated *Escherichia coli* O157:H7, *Salmonella enterica*, *Shigella flexneri*, and *Vibrio parahaemolyticus* in ready-to-eat shrimp and achieved more than a 6 log cfu reduction of *E. coli* O157:H7, *S. enterica*, *S. flexneri*, and *V. parahaemolyticus* with 2.0, 4.0, 3.0, and 3.0 kGy X-ray, respectively. Jia *et al.* (2013) tested the effectiveness of gamma and e-beam irradiation for the inactivation of *Staphylococcus aureus*, *Salmonella enteritidis*, and *Listeria innocua* inoculated on steamed tofu rolls. The results of their study showed that gamma irradiation yielded D_{10} values of 0.20, 0.24, and 0.22 kGy for *S. aureus*, *S. Enteritidis*, and *L. innocua*, respectively. The respective D_{10} values for e-beam irradiation were 0.31, 0.35, and 0.27 kGy. In a recent study, Kundu *et al.* (2014) determined the radiation sensitivity of *E. coli* O157:H7, non-O157

(VTEC) *E. Coli*, and *Salmonella* strains on beef surfaces to 1 kGy e-beam treatment. *Salmonella* serovars were most resistant to 1 kGy treatment, showing a reduction of ≤ 1.9 log cfu/g. This treatment reduced the viability of two groups of non-O157 *E. coli* mixtures by ≤ 4.5 and ≤ 3.9 log cfu/g. Log reductions of ≤ 4.0 log cfu/g were observed for *E. coli* O157:H7 cocktails.

8.3 Non-ionizing radiation

Non-ionizing radiation is the term used to describe the part of the electromagnetic spectrum covering two main regions, namely, optical radiation (ultraviolet (UV), visible and infrared (IR)) and electromagnetic fields (EMFs) (extremely low frequency (ELF), microwaves (MWs), and radio frequencies (RFs)). Optical radiation, i.e., another term for light, covers ultraviolet (UV) radiation, visible light, and infrared radiation (IR) regions. Electromagnetic fields (EMFs) arise whenever electrical energy is used. For example, ELF radiation at 60 Hz is produced by power lines, electrical wiring, and electrical equipment. Radio frequencies have wavelengths of between 1 and 100 m and frequencies in the range of 1 to 100 MHz. Microwaves have wavelengths starting from 100th of a meter and have frequencies of about 2.5 GHz (Figure 8.1).

In a radio frequency radiation system the RF generator creates an alternating electric field between two electrodes. The material to be heated is conveyed between the electrodes, causing polar water molecules in the material to continuously reorient to face opposite electrodes. Friction resulting from this molecular movement causes the food material to rapidly heat volumetrically (<http://www.radiofrequency.com/rftech.html>). The most common commercial RF frequencies are 13, 27, and 40 MHz. RF energy has an ability to penetrate into foods with a depth of more than 5 cm (Ramaswamy and Tang, 2008).

Microwave radiation, which uses electromagnetic energy in the frequency range of 300 to 3000 MHz can be employed successfully to

heat many dielectric materials including foods. The US Federal Communication Commission (FCC) allocates 915 and 2450 MHz bands for microwave heating applications (<http://microwaveheating.wsu.edu/factsheet/index.html>). Microwaves interact with polar water molecules and charged ions. The friction resulting from molecule alignment and migration of charged ions in a rapidly alternating electromagnetic field generates heat within foods. In other words, the dominant mechanism for dielectric heating is dipolar loss. When a material containing permanent dipoles is subject to a varying electromagnetic field, the dipoles are unable to follow the rapid reversals in the field. As a result of this phase lag, power is dissipated as heat in the material (Bradshaw *et al.*, 1998). Microwaves can penetrate up to 3 cm in packaged foods (Ramaswamy and Tang, 2008).

Infrared (IR) is electromagnetic radiation with longer wavelengths than those of visible light. The wavelength of the peak of the infrared radiation ranges from 780 nm to 1 mm. This range of wavelengths corresponds to a frequency range of approximately 430 THz down to 300 GHz. The infrared range is divided into three regions: short wave or near infrared range (780–1400 nm), medium infrared range (1400–3000 nm), far infrared range (>3000 nm). Near IR is the most used wavelength for industrial heating applications because of the higher temperatures produced (Staack, 2008). However, FIR radiation could be advantageous for food processing because most food components absorb energy in the FIR region (Krishnamurthy *et al.*, 2008a). Infrared energy is emitted or absorbed by molecules when they change their rotational-vibrational movements. Infrared energy causes vibrational modes in a molecule through a change in the dipole moment. When the molecule returns into the normal state, the absorbed energy is transferred into heat. In food products, water is the molecule most affected by IR radiation. Other molecules affected by IR are proteins, lipids, and carbohydrates containing polar groups (-NH, -CO, -OH, C=C) (Staack, 2008).

The wavelength for UV light ranges from 100 to 400 nm. This spectral band can be subdivided into UV-C (180–280 nm), UV-B (280–315 nm), UV-A (315–400 nm), and the vacuum UV range (100–200 nm). Low- and medium-pressure mercury lamps have been the main sources of radiation in most UV-based disinfection systems. The UV-C range is called the germicidal range, since it effectively inactivates bacteria and viruses (Koutchma *et al.*, 2009). Absorption of non-ionizing radiation leads to electronic excitation of atoms and molecules. An atom and most ions consist of electrons orbiting a nucleus of protons and neutrons. When electrons having a unique energy state make a transition from a higher energy to a lower energy, a discrete amount of energy is released as photons of light. Each element emits a unique spectrum of light. UV light is emitted when the difference between energy levels is appropriate (Koutchma *et al.*, 2009).

8.3.1 Impact of non-ionizing radiation on food-borne microorganisms

8.3.1.1 Inactivation mechanism

The microbial destruction in MW and RF radiation is usually attributed to the thermal effect. In other words, microbial inactivation kinetics for these technologies are the same as the inactivation kinetics of conventional thermal processing (IFT, 2000). However, a number of microbiological studies involving MW irradiation showed that the cell death was due to not only heat but also nonthermal (athermal) effects of the microwave electric field. Dreyfuss and Chipley (1980), Singh *et al.* (1994), Banik *et al.* (2002a, 2002b, 2003) tried to demonstrate the existence of nonthermal effects of microwave irradiation. They revealed that “microwaves athermally induced different biological effects by changing the structures by differentially partitioning the ions, altering the rate and/or direction of biochemical reactions” (Banik *et al.*, 2003). The nonthermal effect of RF and MW radiation is attributed to four different mechanisms including selective heating

of microorganisms, electroporation, cell membrane rupture and cell lysis due to electromagnetic energy coupling (Kozempel *et al.*, 1998). However, the studies reporting non-thermal effects have been shown to be inconclusive, and only thermal effects are supposed to exist (IFT, 2000). In fact, Shazman *et al.* (2007) did not detect any athermal (nonthermal) effects due to microwave radiation in a number of chemical, biochemical, and microbiological systems. Additionally, Geveke *et al.* (2002) showed that RF energy at 18 MHz and 0.5 kV/cm does not have any nonthermal effect on *E. coli* K12, *L. innocua*, and yeast in liquids. They claimed that the low-temperature effects of RF were due to heat. It was also indicated that the electric field strength was not enough to cause any rupture in the cell membrane of microorganisms to generate a nonthermal effect.

The infrared power level, temperature of food sample, peak wavelength and bandwidth of IR heating, sample depth, types of microorganisms (spores are more resistant than vegetative cells), moisture content, and the physiological phase of the microorganisms (exponential cells are more sensitive than stationary phase cells) are the important factors for the efficiency of microbial inactivation by IR radiation (Krishnamurthy *et al.*, 2008a). The inactivation mechanisms of microorganisms by IR radiation may include both DNA damage, which is similar to that of UV irradiation, and the thermal effect, which is the same as in MW heating (Hamanaka *et al.*, 2000). Thermal inactivation can damage DNA, RNA, ribosome, the cell envelope, and proteins in microbial cells. However, Sawai *et al.* (1995) demonstrated that RNA, protein, and cell wall damage were more pronounced in *E. coli* cells subjected to far-infrared (FIR) radiation. They claimed that FIR radiation damages RNA polymerase and ribosome in *E. coli*. Though similar changes in the sensitivity are obtained in the case of thermal conductive heating, the pasteurization effect of FIR radiation is much greater than that of conductive heating. Krishnamurthy (2006) also showed

cell wall damage, condensation of cytoplasm, and cellular content leakage in infrared heat-treated *S. aureus* cells by using transmission electron microscopy (TEM) and FTIR spectroscopy techniques.

The inactivation mechanism of UV irradiation is related to the absorption of UV photons by DNA or RNA pyrimidine bases. DNA of most living organisms is double stranded, including adenine in one strand and thymine in the other, and, linked by one hydrogen bond, guanine is paired with cytosine by one hydrogen bond (Bank, 1990; Bintsis, 2000; Cadet *et al.*, 2005; Miller *et al.*, 1999; Tornaletti, 2005). The purine and pyrimidine combinations are called base pairs. When UV light of a germicidal wavelength is absorbed by the pyrimidine bases, the hydrogen bond is ruptured (Cieminis *et al.*, 1987; Tornaletti, 2005). New bonds between adjacent nucleotides are structured with the help of high energy of electromagnetic wavelengths found in the UV-C region. This phenomena creates double molecules or dimers (Tornaletti, 2005), shown in Figure 8.3. Dimerization of adjacent pyrimidine molecules is the most common photochemical damage. However, in contrast, cytosine–cytosine, cytosine–thymine, and uracil dimerizations are also identified. Hence, the cell replication is interrupted by formation of numerous dimers in the DNA and RNA of the microbial structure with the effect of other types of damage such as cross-linking of nucleic acids and proteins resulting in cell death (Tornaletti, 2005). In conclusion, the incident light causes a pyrimidine dimer formation on the same DNA strand between two adjacent nucleotides, leading to inhibition of transcription and replication and eventually death of the cell (Bolton and Linden, 2003; Koutchma, 2009).

8.3.1.2 Modeling inactivation kinetics

The classical approach is commonly used to model thermal microbial inactivation (Chick, 1908). The local change in microbial population (N) is modeled as a first-order decay reaction (similar to Equation (8.1)):

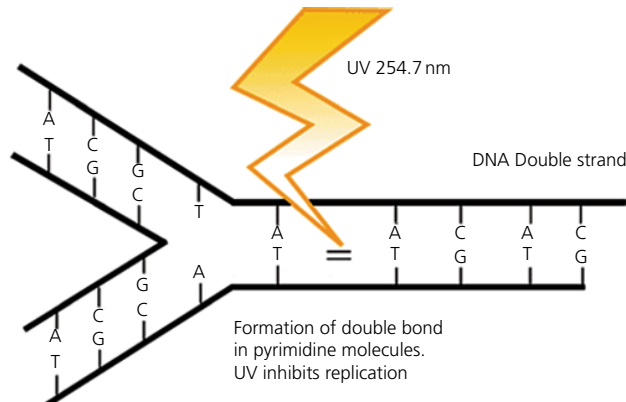


Figure 8.3 Effect of UV-C light on DNA double strand (from <http://www.aquafineuv.com/UVTechnology/UVScience.aspx>).

$$\frac{dN}{dt} = -kN \quad (8.5)$$

The death of microorganisms can be predicted from a logarithmic form of Equation (8.5) that is known as a log-linear model (Unluturk *et al.*, 2010):

$$\log_{10} \left(\frac{N}{N_0} \right) = -kt \quad (8.6)$$

where N_0 and N are the number of untreated and treated microorganisms (cfu/ml or cfu/cm²), t is the time, and k (min⁻¹) is the inactivation rate constant. Because k is a function of temperature, it is often described with the Arrhenius equation (Huang *et al.*, 2009).

However, thermal inactivation models for bacteria have shown deviation from first-order kinetics (van Boekel, 2009). Survival curves can have tail and/or shoulder regions (Figure 8.2). These types of non-log-linear sigmoidal shape survival curves are very common in conventional isothermal treatment. It has been shown that a non-log-linear survivor curve can be observed when a mild heat treatment is applied. Geeraerd *et al.* (2000) thoroughly analysed modeling approaches to describe microbial inactivation during mild heat treatment. They formulated structural model requirements and illustrated the different model performances by using a range of

experimental data of *Bacillus cereus*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Lactobacillus sake*.

However, MW is considered as a non-isothermal process. Valero *et al.* (2014) studied the effect of MW heating on the inactivation of *Salmonella enterica* serovar Enteritidis in a potato omelet. They built a log-linear + shoulder inactivation model as a function of MW power levels and considered the effect of temperature during MW heating by developing non-isothermal Weibull models. The log-linear + shoulder model takes into account the resistance period of the microbial population at the beginning of the treatment (Valero *et al.*, 2014). It uses the following equation:

$$N = N_0 e^{-k_{\max} t} \frac{e^{k_{\max} SI}}{1 + (e^{k_{\max} SI} - 1) e^{-k_{\max} t}} \quad (8.7)$$

where N_0 and N are the initial concentration (cfu/g) and the cell concentration (cfu/ml), respectively, after a treatment time t (s), $k_{\max}$ is the maximum inactivation rate (s⁻¹) and SI is the shoulder length (s).

The Weibull distribution is defined as a simple kinetic model used to describe microbial inactivation by thermal and nonthermal treatment methods. It is a simple model and usually used to describe the concavity or convexity of non-linear survival curves as a function

of inactivation time. This model has been well described by other authors (e.g., Bialka *et al.*, 2008; Buzrul and Alpas, 2007; Buzrul *et al.*, 2005; Van Boekel, 2002; Peleg and Normand, 2004) and uses the following equation:

$$\log N(t) = -bt^n \quad (8.8)$$

where $N(t)$ is the instantaneous survival ratio and b and n are the temperature dependent rate and shape parameters, respectively. The non-isothermal Weibull model is used to describe the effect of temperature on the inactivation rate of *Salmonella enterica* in a potato omelet (Valero *et al.*, 2014). The non-isothermal model is developed using the Weibull model and the model developed by Mattick *et al.* (2001). Thus the following survival rate equation is obtained:

$$\frac{d \log N(t)}{dt} = -b[T(t)]^n [N(t)]^{\left\{ \frac{\log N(t)}{b[T(t)]} \right\}^{(n[T(t)]-1)/n[T(t)]}} \quad (8.9)$$

Bennloch-Tinoco *et al.* (2014) investigated the inactivation of *Listeria monocytogenes* in a kiwifruit puree by conventional and microwave heating. They showed that the survival behavior of *Listeria monocytogenes* under MW processing was properly fitted to first-order kinetics.

First-order kinetics (Equation (8.5)) and exponential reduction is observed in the population of vegetative cells when treated with infrared radiation. However, shoulder and tailing effects are reported for bacterial spores exposed to IR heating. For example, Hamanaka *et al.* (2000) and Daisuke *et al.* (2001) proclaimed shoulder and tailing effects for *Bacillus subtilis* spores exposed to infrared heating. Sawai *et al.* (2003, 2006) indicated that inactivation of *Escherichia coli* due to both IR heating and thermal conductive heating followed first-order reaction kinetics (Equations (8.5) and (8.6)).

The inactivation curve of microorganisms exposed to UV irradiation has a sigmoidal shape that is composed of an initial plateau

region, linear part, and tailing phase. The behavior of the survival curve is explained by a multihit phenomena, i.e., inactivation efficiency decreases by the effect of the multiple hit. In this phenomenon, multiple UV contacts a single cell or a single UV light affects a group of cells. Thus, less-resistant cells are eliminated first, and then more resistant cells are left to form a tail in the survival curve. When there is a resistivity to inactivation of the target microorganism at the beginning of UV irradiation, this behavior is represented as a “shoulder” effect in the non-linear inactivation curve (Figure 8.3) (Benabbou *et al.*, 2007; Bermudez-Aguirre *et al.*, 2009; Marugán *et al.*, 2008). If the inactivation rate slows down at the end of the inactivation process, this part of the inactivation curve is named the “tailing” region (Bialka *et al.*, 2008; Buzrul and Alpas, 2007; Fernández *et al.*, 2007; Marugán *et al.*, 2008; Van Boekel, 2002). Tailing of inactivation curve occurs by the shadowing effect of high concentration of non-microbial solid particles or improper mixing of liquid food (Marugán *et al.*, 2008). Another reason for the tailing effect might be the increase in resistance and the survival rate of a microbial population in disinfected liquid food. Also, agglomeration of the death population is likely to prevent UV penetration to living cells due to improper mixing.

The survival curve of microorganisms can be described by using both linear and non-linear models. Besides the log-linear model (Equation (8.5)), the non-linear behavior of microbial inactivation is described by using some empirical models (e.g., the Weibull model (Equation (8.8)), the modified Chick–Watson model, the Hom model, etc.) (Atilgan, 2013). The Weibull model is explained in previous paragraphs of this section.

The Chick–Watson equation is an empirical disinfection model considering the inactivation rate of a target microorganism changing as an exponential function of the disinfectant agent concentration (Chick, 1908; Watson, 1908). Integrated as a function of time, the model can be represented as

$$\left(\frac{N}{N_0}\right) = \exp(-kC^n t) \quad (8.10)$$

where k represents the Chick–Watson coefficient of specific lethality (min^{-1}), C is the concentration of the disinfectant (mg/l), n is the dilution coefficient, and t is the disinfection time (min). At low concentrations of microbial population exposed to a long treatment period, the rate of inactivation stays constant until the end of the inactivation process (Huang *et al.*, 2009; Marugán *et al.*, 2008). In order to describe the outward convexity or inward concavity in the inactivation curve, the Chick–Watson model could be modified by introducing two parameters in Equation (8.10) (Cho *et al.*, 2003; Marugán *et al.*, 2008):

$$\log \frac{N}{N_0} = -k_1 [1 - \exp(-k_2 t)] \quad (8.11)$$

where k_1 is the inactivation rate constant (min^{-1}) for the log-linear inactivation part of the curve and k_2 is the first-order UV decay constant (min^{-1}), representing the tailing effect of the decelerated inactivation.

The Hom model incorporates the parameter h . The expression is

$$\log \left(\frac{N}{N_0}\right) = -k' t^h \quad (8.12)$$

where k' is the inactivation rate constant of the Hom model (min^{-1}) and h represents the UV penetration rate constant that describes the tailing or shoulder effect of the inactivation rate. When $h=1$, this equation simplifies to the log-linear equation. For the case $h<1$, the equation permits the fitting of the tailing (inward concavity) part of the inactivation curve, while for $h>1$, the shoulder (outward convexity) part is predicted (Haas and Joffe, 1994; Lee and Nam, 2002; Marugán *et al.*, 2008). A small k' value (inactivation rate constant) indicates that the microorganism shows a larger resistivity to treatment.

8.3.1.3 Applications of non-ionizing irradiation

There have been a number of reviews and studies in the area of MW and RF radiation, which include publications by Chandrasekaran *et al.* (2013), Banik *et al.* (2003), Ramaswamy and Tang (2008), Marra *et al.* (2009), Piyasena *et al.* (2003), and Wang *et al.* (2003) who considered the major technological aspects and applications of MW and RF treatment in food processing.

Radio frequency has been applied for cooking, heating, drying, post-baking, thawing of frozen food products, and has been used in meat processing (Piyasena *et al.*, 2003; Marra *et al.*, 2009). For example, Byrne *et al.* (2010) performed a study to investigate the effect of RF radiation on the inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spore cocktails in pork luncheon meat. The results of their study showed a reduction in *B. cereus* vegetative cells and spores of 5.4 and 1.8 $\log_{10}$ cfu/g, respectively, while the corresponding reduction for *C. perfringens* vegetative cells and spores were 6.8 and 4.1 $\log_{10}$ cfu/g, respectively.

RF radiation has been also considered as an alternative method for the pasteurization of liquid foods (Piyasena and Dussault, 2003; Awuah *et al.*, 2002, 2005). Geveke and Brunkhorst (2004, 2008) and Geveke *et al.* (2007) assessed the effect of RF treatment on microbial inactivation in a number of different beverages. They showed that RF treatment has a potential in inactivation of *E. coli* K12 by up to 3, 3.3, and 4.8 $\log$ cfu/ml in apple juice, orange juice, and apple cider, respectively. More recently, Jeong and Kang (2014) investigated the effect of RF heating on the inactivation of food-borne pathogens (*Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium) in red and black pepper. They reported more than 7 $\log$ cfu/g reduction in the number of *E. coli* O157:H7 and *S. Typhimurium* under studied conditions by means of an RF system with 27.12 MHz. The authors proposed to use RF heating to control pathogens and reduce moisture levels in spices.

MW heating can be used for cooking, blanching, drying, pasteurization, and sterilization of food materials and MW radiation can be easily combined with other conventional methods. That is why it has vast applications in the field of food processing. These applications have been extensively reviewed by Chandrasekaran *et al.* (2013) and Salazar-Gonzalez *et al.* (2012). In a recent study, microwave pasteurization was used for the inactivation of *Salmonella* Typhimurium in the yolk of shell eggs (Shenga *et al.*, 2010). In another study, De La Vega-Miranda *et al.* (2012) demonstrated that water-assisted microwave treatment has an effect against *Salmonella* Typhimurium which resulted in the reduction of 4–5 log cycles of microbial population in fresh jalapeno peppers and coriander foliage. Guilian *et al.* (2010) applied microwave processing to a cream of asparagus and achieved a twofold reduction in the number of *Alicyclobacillus acidoterrestris*. More recently, the US Food and Drug Administration has approved the microwave sterilization process for mashed potatoes in trays and salmon fillet in sauce in pouches (Brody, 2012).

Infrared radiation has been applied in drying, baking, roasting, blanching, pasteurization, and sterilization of food products. It can be employed for inactivation of bacteria, spores, yeast, and mold in both liquid and solid foods (Krishnamurthy *et al.*, 2008a). The studies pertinent to pathogen inactivation by IR radiation in different types of food materials such as liquid, solid, and non-food materials is summarized by Krishnamurthy *et al.* (2008a, 2009) and Rastogi (2012). Krishnamurthy *et al.* (2008b) demonstrated the potential of IR heating in milk processing for effective inactivation of *Staphylococcus aureus*. Depending upon the treatment conditions, the population was reduced from 0.10 to 8.41 log₁₀ cfu/ml. Erdogdu and Ekiz (2011, 2013) applied far infrared (FIR) and UV-C radiation for the surface pasteurization of black pepper seeds and cumin seeds. They reported complete elimination of the

microorganisms (total mold and yeast, *E. coli*, and *B. cereus*) in the black pepper seed samples after 4.7 and 3.5 min FIR treatment at 300 and 350°C. UVC treatment after FIR application did not exhibit any significant additional reduction in the total mesophilic aerobic bacteria (TMAB) count. However, when FIR is combined with UV, it is stated that TMAB of the cumin seeds is decreased to the target level of 10⁴ cfu/g after 1.57, 2.8, and 4.8 min FIR treatment at 300, 250, and 200°C, respectively, following a 2 h UVC treatment. More recently, Huang and Sites (2012) achieved maximum of 1.6 log reductions in the number of *Listeria monocytogenes* inoculated on the surface of ready-to-eat (RTE) chicken meats by near infrared (NIR). They found the NIR surface pasteurization process more effective than the hot water immersion process to eliminate *Listeria monocytogenes*.

UV-C irradiation between 200 and 280 nm has been widely used for disinfection of transparent, semi-transparent liquid foods in addition to solid food surfaces due to its germicidal effect (Bolton and Linden, 2003; Koutchma *et al.*, 2009). The Food and Drug Administration (FDA) has allowed the use of ultraviolet irradiation to eliminate food-borne pathogens and other microorganisms in juice products since November 29, 2000. Unluturk *et al.* (2004) studied the effects of UV light efficiency on the destruction of *Escherichia coli* K-12 bacteria in the model fluids using laminar and turbulent flow UV systems in terms of the single factor, i.e., UV absorbance. Ngadi *et al.* (2003) evaluated the effect of pH, depth of food medium, and UV dose on *E. coli* O157:H7 reduction in egg white and apple juice exposed to UV for 0–16 minutes at 0.315 mW/cm². There are also many studies recently published and cited in the literature about the efficacy of UV-C light for the reduction of different microorganisms by UV irradiation (Franz *et al.*, 2009; Char *et al.*, 2010; Unluturk *et al.*, 2010; Oteiza *et al.*, 2010; Ukuku and Geveke, 2010; Choudhary *et al.*, 2011; Bandla *et al.*, 2012; Gabriel, 2012; Geveke and Torres, 2012; Ochoa-Velasco and Guerrero-Beltrán, 2012).

In summary, the UV disinfection method is mainly applied in the treatment of spent process water in the food and beverage industries (Chmiel *et al.*, 2002; Hassen *et al.*, 2000), treatment of drinking water (Lehtola *et al.*, 2004), pasteurization of milk and clarified fruit juices (Bandla *et al.*, 2012; Choudhary *et al.*, 2011; Koutchma *et al.*, 2004; Matak *et al.*, 2005; Geveke and Torres, 2012). It has also been applied in tropical fruit juice production such as pitaya, mango, guava and pineapple, and mango and pineapple juices (Keyser *et al.*, 2008; Ochoa-Velasco and Guerrero-Beltrán, 2012), liquid egg products (Unluturk *et al.*, 2008, 2010), and grape juice processing (Unluturk and Atilgan, 2014; Guerrero-Beltrán *et al.*, 2009). UV-C light treatment has also been used in the food industry for different purposes, including air sanitation in meat and vegetable processing, and reduction of pathogen microorganisms in red meat, poultry, and fish processing (Liltved and Landfald, 2000; Wong *et al.*, 1998).

References

- Adu-Gyamfi, A., Torgby-Tetteh, W., and Appiah, V. 2012. Microbiological quality of chicken sold in Accra and determination of D10-value of *E. coli*. *Food and Nutrition Sciences*, **3**, 693–698. Available at: <http://dx.doi.org/10.4236/fns.2012.35094>.
- Alper, T. 1977. The role of membrane damage in radiation induced cell death. *Advances in Experimental Medicine and Biology*, **84**, 139–165.
- Asad, N.R., Asad, L.M.B.O., de Almedia, C.E.B., Felsenszwalb, I., Cabnel-Neto, J.B., and Leitao, A.C. 2004. Several pathways of hydrogen peroxide action that damage the *E. coli* genome. *Genetics and Molecular Biology*, **27**, 291–303.
- Atilgan, M.R. 2013. Design of a continuous flow UV reactor for opaque liquid foods by using computational fluid dynamics (CFD). PhD Thesis, Department of Food Engineering, Izmir Institute of Technology.
- Awuah, G.B., Ramaswamy, H.S., and Piyasena, P. 2002. Radio frequency (RF) heating of starch solutions under continuous flow conditions: effect of system and product parameters on temperature change across the applicator tube. *Journal of Food Process Engineering*, **25**, 201–223.
- Awuah, G.B., Ramaswamy, H.S., Economides, A., and Mallikarjuna, K. 2005. Inactivation of *Escherichia coli* K-12 and *Listeria innocua* in milk using radio frequency (RF) heating. *Innovative Food Science and Emerging Technologies*, **6**, 396–402.
- Bandla, S., Choudhary, R., Watson, D.G., and Haddock, J. 2012. UV-C treatment of soymilk in coiled tube UV reactors for inactivation of *Escherichia coli* W1485 and *Bacillus cereus* endospores. *LWT*, **46**, 71–76.
- Bank, S., Bandopadhyay, S., Ganguly, S., and Dan, D. 2002a. Effect of inoculation of microwave irradiated methanogenic bacteria for improvement of biomethanation from jute caddis. In: *Proceedings of the National Symposium on Frontier Studies in Microbes and Host Microbe-Interactions*, Feb. 2–3, 2002, pp. 55–56.
- Bank, S., Bandopadhyay, S., Ganguly, S., and Dan, D. 2002b. Application of microwave technology on methanogenic bacteria for improvement of its efficiency and endeavour to increase yield of biogas. In: *Proceedings of the Ninth West Bengal State Science and Technology Congress*. Feb. 28–Mar. 2, 2002, p. KJ.18.
- Bank, S., Bandopadhyay, S., and Ganguly, S. 2003. Bioeffects of microwave—a brief review. *Biore-source Technology*, **87**, 155–159.
- Bank, H.L., Schmehl, J.L., and Dratch, R.J. 1990. Bacteriocidal effectiveness of modulated UV light. *Applied and Environmental Microbiology*, **56**, 3888–3889.
- Baranyi, J. 2003. Predictive modelling to control microbial hazards in the food processing industry. Available at: http://smas.chemeng.ntua.gr/files/webdoc_9_25_8_2003.pdf [16 June 2014].
- Benabbou, A.K., Derriche, Z., Felix, C., Lejeune, P., and Guillard, C. 2007. Photocatalytic Inactivation of *Escherichia coli*: effect of concentration of TiO₂ and microorganism, nature, and intensity of UV Irradiation. *Applied Catalysis B: Environmental*, **76**, 257–263.

- Bennloch-Tinoco, M., Pina-Perez, M.C., Martinez-Navarrete, N., and Rodrigo, D. 2014. *Listeria monocytogenes* inactivation kinetics under microwave and conventional thermal processing in a kiwifruit puree. *Innovative Food Science and Emerging Technologies*, **22**, 131–136.
- Bermudez-Aguirre, D., Corradini, M.C., Mawson, R., and Barbosa-Canovas, G.V. 2009. Modeling the inactivation of *Listeria innocua* in raw whole milk treated under thermo-sonication. *Innovative Food Sciences and Emerging Technologies*, **10**, 172–178.
- Bialka, K.L., Demirci, A., and Puri, V.M. 2008. Modeling the inactivation of *Escherichia coli* O157:H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *Journal of Food Engineering*, **85**, 444–449.
- Bintsis, T., Tzanetaki, E.L., and Robinson, R.K. 2000. Existing and potential applications of ultraviolet light in the food industry – a critical review. *Journal of Food Science and Application*, **80**, 637–645.
- Bolton, J.R., and Linden, K.G. 2003. Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering – ASCE*, **129**, 209–215.
- Bradshaw, S.M., van Wyk, E.J., and de Swardt, J.B. 1998. Microwave heating principles and the application to the regeneration of granular activated carbon. *The Journal of the South African Institute of Mining and Metallurgy*, **July/August**, 201–212.
- Brody, A.L. 2012. The coming wave of microwave sterilization and pasteurization. *Food Technology Magazine*, **66**, 78–80.
- Buzrul, S. and Alpas, H. 2007. Modeling inactivation kinetics of food borne pathogens at a constant temperature. *LWT*, **40**, 632–637.
- Buzrul, S., Alpas, S., and Bozoglu, F. 2005. Use of Weibull frequency distribution model to describe the inactivation of *Alicyclobacillus acidoterrestris* by high pressure at different temperatures. *Food Research International*, **38**, 151–157.
- Byrne, B., Lyng, J.G., Dunne, G., and Bolon, D.J. 2010. Radio frequency heating of communitied meats – considerations in relation to microbial challenge studies. *Food Control*, **21**, 125–131.
- Cadet, J., Sage, E., and Douki, T. 2005. Ultraviolet radiation-mediated damage to cellular DNA. *Mutation Research*, **571**, 3–17.
- Char, C.D., Mitilinaki, E., Guerrero, S.R., and Alzamora S.M. 2010. Use of high intensity ultrasound and UV-C light to inactivate some microorganisms in fruit juices. *Food Bioprocess Technology*, **3**, 797–803.
- CFS. 2009. Safety of irradiated foods. Risk Assessment Studies, Report 37. Centre for Food Safety
- Food and Environmental Hygiene Department, The Government of the Hong Kong Special Administrative Region [Online]. Available at: http://www.cfs.gov.hk/english/programme/programme_rafs/files/RA_37_Safety_of_Irradiated_Food_final.pdf [11 June 2014].
- Chandrasekaran, S., Ramanathan, S., and Basak, T. 2013. Microwave food processing – a review. *Food Research International*, **52**, 243–261.
- Chick, H. 1908. An investigation into the laws of disinfection. *Journal of Hygiene (Cambridge)*, **8**, 92–158.
- Chmiel, H., Kaschek, M., Blöcher, C., Noronha, M., and Mavrov, V. 2002. Concepts for the treatment of spent process water in the food and beverage industries. *Desalination*, **152**, 307–314.
- Cho, M., Chung, H., and Yoon, J. 2003. Disinfection of water containing natural organic matter by using ozone-initiated radical reactions. *Applied and Environmental Microbiology*, **69**, 2284–2291.
- Choudhary, R., Bandla, S., Watson, D.G., Haddock, J., Abughazaleh, A., and Bhattacharya, B. 2011. Performance of coiled tube ultraviolet reactors to inactivate *Escherichia coli* W1485 and *Bacillus cereus* endospores in raw cow milk and commercially processed skimmed cow milk. *Journal of Food Engineering*, **107**, 14–20.
- Cieminis, K.G.K., Rančelien, V.M., Prijalgauskien, A.J., Tiunaitien, N V., Rudzianskait, A.M., and Jančys, Z.J. 1987. Chromosome and DNA damage and their repair in higher plants irradiated with short-wave ultraviolet light. *Mutation Research – Fundamental and Molecular Mechanisms of Mutagenesis*, **181**, 9–16.

- Daisuke, H., Toshitaka, U., Wenzhong, H., and Yaunaga, E. 2001. The short-time infrared ray sterilization of the cereal surface. In: *Proceedings of IFAC on Control Applications in Post-harvest and Processing Technology*, Tokyo, Japan, pp. 195–201.
- De La Vega-Miranda, B., Santiesteban-Lopez, N.A., Lopez-Malo, A., and Sosa-Morales, M.E. 2012. Inactivation of *Salmonella* Typhimurium in fresh vegetables using waterassisted microwave heating. *Food Control*, **26**, 19–22.
- Draper, N.R. 1988. Response surface designs. In: *Encyclopedia of Statistical Sciences*, pp. 107–119. John Wiley & Sons, Inc., New York, USA.
- Dreyfuss, M.S. and Chipley, J.R. 1980. Comparison of effects of sublethal microwave radiation and conventional heating on the metabolic activity of *Staphylococcus aureus*. *Applied Environmental Microbiology*, **39**, 13–16.
- Erdogdu, S.B. and Ekiz, H.I. 2011. Effect of ultraviolet and far infrared radiation on microbial decontamination and quality of cumin seeds. *Journal of Food Science*, **76**, 284–292.
- Erdogdu, S.B. and Ekiz H.I. 2013. Far infrared and ultraviolet radiaiton as a combined method for surface pasteurization of black pepper seeds. *Journal of Food Engineering*, **116**, 310–314.
- Fakruddin, M.D., Mazumder, R.M., and Mannan, K.H.B. 2011. *Ceylon Journal of Science (Bio. Sci.)*, **40**, 121–131.
- Farkas, J. 2006. Irradiation for better foods. *Trends in Food Science and Technology*, **17**, 148–152.
- Farkas, J. and Mohacsi-Farkas, C. 2011. History and future of food irradiation. *Trends in Food Science and Technology*, **22**, 121–126.
- Fernández, A., López, M., Bernardo, A., Condón, S., and Raso, J. 2010. Modelling thermal inactivation of *Listeria monocytogenes* in sucrose solutions of various water activities. *Food Microbiology*, **24**, 372–379.
- Franz, C.M.A.P., Specht, I., Cho, G., Graef, V., and Stahl, M.R. 2009. UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on Dean vortex technology. *Food Control*, **20**, 1103–1107.
- Geeraerd, A.H., Herremans, C.H., and Van Impe, J.F. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, **59**, 185–209.
- Gabriel, A.A. 2012. Inactivation of *Escherichia coli* O157:H7 and spoilage yeasts in germicidal UV-C-irradiated and heat-treated clear apple juice. *Food Control*, **25**, 425–432.
- Geveke, D.J. and Brunkhorst, C. 2004. Inactivation of *Escherichia coli* in apple juice by radio frequency electric fields. *Journal of Food Science*, **69**, FEP 134–FEP 138.
- Geveke, D.J. and Brunkhorst, C. 2008. Radio frequency electric fields inactivation of *Escherichia coli* in apple cider. *Journal of Food Engineering*, **85**, 215–221.
- Geveke, D.J. and Torres, D. 2012. Pasteurization of grapefruit juice using a centrifugal ultraviolet light irradiator. *Journal of Food Engineering*, **111**, 241–246.
- Geveke, D.J., Kozempel, M., Scullen, O.J., and Brunkhorst, C. 2002. Radio frequency energy effects on microorganisms in foods. *Innovative Food Science and Technologies*, **3**, 133–138.
- Geveke, D.J., Brunkhorst, C., and Fan, X. 2007. Radio frequency electric fields processing of orange juice. *Innovative Food Science and Emerging Technologies*, **8**, 549–554.
- Gibson, A.M., Bratchell, N., and Roberts, T.A. 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride, and storage temperature. *International Journal of Food Microbiology*, **6**, 155–178.
- Giuliani, R., Bevilacqua, A., Corbo, M.R., and Severini, C. 2010. Use of microwave processing to reduce the initial contamination by *Alicyclobacillus acidoterrestris* in a cream of asparagus and effect of the treatment on the lipid fraction. *Innovative Food Science and Emerging Technologies*, **11**, 328–334.
- Guerrero-Beltran, J.Á., Welte-Chanes, J., and Barbosa-Cánovas, G.V. 2009. Ultraviolet-C light processing of grape, cranberry and grapefruit juices to inactivate *Saccharomyces cerevisiae*. *Journal of Food Process Engineering*, **32**, 916–932.

- Haas, C.N., and Joffe, J. 1994. Disinfection under dynamic conditions: modification of Hom's model for decay. *Environmental Science Technology*, **28**, 1367–1369.
- Hamanaka, D., Dokan, S., Yasunaga, E., Kuroki, S., Uchino, T., and Akimoto, K. 2000. The sterilization effects on infrared ray of the agricultural products spoilage microorganisms (Part 1). An ASAE Meeting Presentation, Milwaukee, WI, July 9–12, 2000, No. 00 6090.
- Hassen, A., Mahrouk, M., Ouzari, H., Cherif, M., Boudabous, A., and Damelincourt, J.J. 2000. UV disinfection of treated wastewater in a large-scale pilot plant and inactivation of selected bacteria in a laboratory UV device. *Bioresource Technology*, **74**, 141–150.
- Huang, L. and Sites, J. 2012. Elimination of *Listeria monocytogenes* on cooked chicken breast meat surfaces by near infrared surface pasteurization prior to final packaging. *Journal of Food Process Engineering*, **35**, 1–15.
- Huang, H., Brooks, M.S.L., Huang, H.J., and Chen, X.D. 2009. Inactivation kinetics of yeast cells during infrared drying. *Drying Technology*, **27**, 1060–1068.
- ICGFI. 1999. Facts about Food Irradiation. A series of Fact Sheets from the International Consultative Group on Food Irradiation (ICGFI). Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. IAEA. Available at: <http://www.iaea.org/Publications/Booklets/foodirradiation.pdf> [11 June 2014]
- IFT. 2000. Kinetics of Microbial Inactivation for Alternative Food Processing Technologies – A Report of the Institute of Food Technologists for the Food and Drug Administration of the US Department of Health and Human Services, IFT/FDA Contract No. 223-98-2333.
- James, D.L., Jaczynski, J. and Matak, K.E. 2010. Electron beam irradiation on nalidixic acid resistant *Salmonella Montevideo* in cooked tomato puree of various pH values. *Journal of Food Safety*, **30**, 515–525.
- Jia, Q., Gao, M., Li, S., and Wang, Z. 2013. Effects of gamma and electron beam irradiation on the microbial quality of steamed tofu rolls. *Radiation Physics and Chemistry*, **82**, 119–121.
- Josephson, E.S. and Peterson, M.S. 2000. Preservation of Food by Ionizing Radiation (II), pp. 102–103. CRC Press, Boca Raton, FL, USA.
- Keener, K.M. 1998. Food Safety Irradiation. Available at: http://fbns.ncsu.edu/extension_program/documents/foodsafety_irradiation.pdf [11 June 2014].
- Keyser, M., Müller, I., Cilliers, F.P., Nel, W., and Gouws, P.A. 2008. UV radiation as a nonthermal treatment for the inactivation microorganisms in fruit juice. *Innovative Food Science on Emerging Technology*, **9**, 348–354.
- Koutchma, T. 2009. Advances in ultraviolet light technology for nonthermal processing of liquid foods. *Food and Bioprocess Technology*, **2**, 138–155.
- Koutchma, T., Keller, S., Chirtel, S., and Parisi, B. 2004. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. *Innovative Food Science and Engineering Technologies*, **5**, 179–189.
- Koutchma, T.N., Forney, L.J., and Moraru, C.I. 2009. *Ultraviolet Light in Food Technology: Principles and Applications*, pp. 2–3. CRC Press, New York, USA.
- Kozempel, M.F., Annous, B.A., Cook, R.D., Scullen, O.J., and Whiting, R.C. 1998. Inactivation of microorganisms with microwaves at reduced temperatures. *Journal of Food Protection*, **61**, 582–585.
- Krishnamurthy, K. 2006. Decontamination of milk and water by pulsed UV light and infrared heating. PhD Dissertation, Department of Agricultural and Biological Engineering, The Pennsylvania State University.
- Krishnamurthy, K., Khurana, H.K., Jun, S., Irudayaraj, J., and Demirci, A. 2008a. Infrared heating in food processing: an overview. *Comprehensive Reviews in Food Science and Food Safety*, **7**, 2–13.
- Krishnamurthy, K., Jun, S., Irudayaraj, J., and Demirci, A. 2008b. Efficiency of infrared heat treatment for inactivation of *Staphylococcus aureus* in milk. *Journal of Food Process Engineering*, **31**, 798–816.
- Krishnamurthy, K., Khurana, H.K., Jun, S., Irudayaraj, J.M., and Demirci, A. 2009. Infrared

- radiation for food processing. In: *Food Processing Operations Modeling: Design and Analysis* (eds Jun, S. and Irudayaraj, J.M.), pp. 115–142. CRC Press, Boca Raton, FL, USA.
- Kume, T., Furuta, M., Todoriki, S., Uenoyama, N., and Kobayashi, Y. 2009. Status of food irradiation in the world. *Radiation Physics and Chemistry*, **78**, 222–226.
- Kundu, D., Gill, A., Lui, C., Goswami, A.N., and Holley, R. 2014. Use of low dose e-beam irradiation to reduce *E. coli* O157:H7, non-O157 (VTEC) *E. coli* and *Salmonella* viability on meat surfaces. *Meat Science*, **96**, 413–418.
- Lee, Y. and Nam, S. 2002. Reflection of kinetic models to the chlorine disinfection for drinking water production. *The Journal of Microbiology*, **40**, 119–124.
- Lehtola, M.J., Miettinen, I.T., Lampola, T., Hirvonen, A., Vartiainen, T., and Martikainen, P.J. 2004. Pipeline materials modify the effectiveness of disinfectants in drinking water distribution systems. *Water Research*, **39**, 1962–1971.
- Liljed, H. and Landfald, B. 2000. Effects of high intensity light on ultraviolet-irradiated and non-irradiated fish pathogenic bacteria. *Water Research*, **34**, 481–486.
- Mahmoud, B.S.M. 2009. Effect of X-ray treatments on inoculated *Escherichia coli* O157:H7, *Salmonella enterica*, *Shigella flexneri* and *Vibrio parahaemolyticus* in ready to eat shrimp. *Food Microbiology*, **26**, 860–864.
- Mahmoud, B.S.M., Bachman, G., and Linton, R.H. 2010. Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri* on spinach leaves by X-ray. *Food Microbiology*, **27**, 24–28.
- Marra, F., Zhang, L., and Lyng, J.G. 2009. Radio frequency treatment of foods: review of recent advances. *Journal of Food Engineering*, **91**, 497–508.
- Marugán, J., Van Grieken, R., Sordo, C., and Cruz, C. 2008. Kinetics of the photocatalytic disinfection of *Escherichia coli* suspensions. *Applied Catalysis B: Environmental*, **82**, 27–36.
- Matak, K.E., Churey, J.J., Worobo, R.W., Sumner, S.S., Hovingh, E., Hackney, C.R., and Pierson, M.D. 2005. Efficacy of UV light for the reduction of *Listeria monocytogenes* in goat's milk. *Journal of Food Protection*, **68**, 2212–2216.
- Mattick, K.L., Legan, J.D., Humphrey, T.J., and Peleg, M. 2001. Calculating *Salmonella* survival during non-isothermal heat inactivation. *Journal of Food Protection*, **64**, 606–613.
- McMeekin, T.A., Olley, J., Ross, T., and Ratkowsky, D.A. 1993. *Predictive Microbiology: Theory and Application*. Research Studies Press, Taunton, UK.
- Miller, R.B. 2005. *Electronic Irradiation of Foods. An Introduction to the Technology*, pp. 5–6. Springer, New York, USA.
- Miller, R., Jeffrey, W., Mitchell, D., and Elasri, M. 1999. Bacterial responses to ultraviolet light. *ASM News*, **65**, 534–541.
- Monk, J.D., Bauchet, I.R., and Doyle, M.P. 1995. Irradiation inactivation of food borne microorganisms. *Journal of Food Protection*, **58**, 197–208.
- Moterjani, Y., Moy, G., and Todd, E. 2014. *Encyclopedia of Food Safety*, p. 182. Academic Press, San Diego, CA, USA.
- Ngadi, M., Smith, J.P., and Cayouette, B. 2003. Kinetics of ultraviolet light inactivation of *Escherichia coli* O157:H7 in liquid foods. *Journal of the Science of Food and Agriculture*, **83**, 1551–1555.
- Ochoa-Velasco, C.E. and Guerrero-Beltran, J.Á. 2012. Ultraviolet-C light effect on pitaya (*Stenocereus griseus*) juice. *Journal of Food Research*, **1**, 60–70.
- Oteiza, J.M., Giannuzzi, L., and Zaritzky, N. 2010. Ultraviolet treatment of orange juice to inactivate *E. coli* O157:H7 as affected by native microflora. *Food Bioprocess Technology*, **3**, 603–614.
- Peleg, M. and Normand, M.D. 2004. Calculating microbial survival parameters and predicting survival curves from non-isothermal inactivation data. *Critical Reviews in Food Science and Nutrition*, **40**, 159–172.
- Piyasena, P. and Dussault, C. 2003. Continuous radio-frequency heating of a model viscous solution: influence of active current, flow rate, and salt content on temperature rise. *Canadian Biosystem Engineering*, **45**, 327–334.
- Piyasena, P., Dussault, C., Koutchma, T., Ramaswamy, H.S., and Awuah, G.B. 2003. Radio

- frequency heating of foods: principles, applications and related properties – a review. *Critical Reviews in Food Science and Nutrition*, **43**, 587–606.
- Ramaswamy, H. and Tang, J. 2008. Microwave and radio frequency heating. *Food Science and Technology International*, **14**, O423–427.
- Rastogi, N.K. 2012. Recent trends and developments in infrared heating in food processing. *Critical Reviews of Food Science and Nutrition*, **52**, 737–760.
- Salazar-Gonzalez, C., San Martin-Gonzalez, M.F., Lopez-Malo, A., and Sosa-Morales, M.E. 2012. Recent studies related to microwave processing of fluid foods. *Food Bioprocess and Technology*, **5**, 31–46.
- Sawai, J., Sagara, K., Igarashi, H., Hashimoto, A., Kokugan, T., and Shimizu, M. 1995. Injury of *Escherichia coli* in physiological phosphate buffered saline induced by far-infrared irradiation. *Journal of Chemical Engineering of Japan*, **28**, 294–299.
- Sawai, J., Sagara, K., Hashimoto, A., Igarashi, H., and Shimizu, M. 2003. Inactivation characteristics shown by enzymes and bacteria treated with far-infrared radiative heating. *International Journal of Food Science and Technology*, **38**, 661–667.
- Sawai, J., Isomura, Y., Honma, T., and Kenmochi, H. 2006. Characteristics of the inactivation of *Escherichia coli* by infrared irradiative heating. *Biocontrol Science*, **11**, 85–90.
- Shazman, A., Mizrahi, S., Cogan, U., and Shimoni, E. 2007. Examining for possible nonthermal effects during heating in a microwave oven. *Food Chemistry*, **103**, 444–453.
- Shenga, E., Singh, R.P., and Yadav, A.S. 2010. Effect of pasteurization of shell egg on its quality characteristics under ambient storage. *Journal of Food Science and Technology – Mysore*, **47**, 420–425.
- Singh, S.P., Rai, S., Rai, A.K., Tiwari, S.P., Singh, S.S., and Samarketu, A.J. 1994. Athermal physiological effects of microwaves on a cyanobacterium *Nostoc muscorum*: evidence for EM-memory bits in water. *Medical and Biological Engineering and Computing*, **32**, 175–180.
- Sommers, C.H. 2004. D-10 values and elimination of foodborne pathogens. CIRMS 2004, November 3, 2004, Gaithersburg, Maryland, USA, p. 1. Available at: http://iapreview.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=171197 [16 June 2014].
- Sommers, C.H. and Fan, X. 2006. *Food Irradiation Research and Technology*, p. 127. Blackwell Publishers, Ames, Iowa, USA.
- Staack, N. 2008. Potential of infrared heating as a method for decontaminating food powder, Process development and impact on product quality. Doctoral Thesis, Faculty III – School of Process Sciences and Engineering, Institute of Food Technology and Food Chemistry, Department of Food Biotechnology and Food Process Engineering, Berlin.
- Taherigorabi, R., Matak, K.E., and Jaczynski, J. 2012. Application of e-beam to inactivate *Salmonella* in food: recent developments. *Food Research International*, **45**, 685–694.
- Tomac, A., Mascheroni, R.H., and Yeannes, M.I. 2013. Modelling the effect of gamma irradiation on the inactivation and growth kinetics of psychrotrophic bacteria in squid rings during refrigerated storage. *Shelf-life predictions. Journal of Food Engineering*, **117**, 211–216.
- Tornaletti, S. 2005. Transcription arrest at DNA damage sites. *Mutation Research*, **577**, 131–145.
- Ukuku, D.O. and Geveke, D.J. 2010. A combined treatment of UV-light and radio frequency electric field for the inactivation of *Escherichia coli* K-12 in apple juice. *International Journal of Food Microbiology*, **138**, 50–55.
- Unluturk, S. and Atilgan, M.R. 2014. UV-C irradiation of freshly squeezed grape juice and modeling inactivation kinetics. *Journal of Food Process Engineering*, **37**, 438–449.
- Unluturk, S.K., Arastoopur, H., and Koutchma, T. 2004. Modeling of UV dose distribution in a thin-film UV reactor for processing of apple cider. *Journal of Food Engineering*, **65**, 125–136.
- Unluturk, S., Atilgan, M.R., Baysal, A.H., and Tari, C. 2008. Use of UV-C radiation as a nonthermal process for liquid egg products (LEP). *Journal of Food Engineering*, **85**, 561–568.

- Unluturk, S., Atilgan, M.R., Baysal, A.H., and Unluturk, M.S. 2010. Modeling inactivation kinetics of liquid egg white exposed to UV-C irradiation. *International Journal of Food Microbiology*, **142**, 341–347.
- Valero, A., Cejudo, M., and Garcia-Gimeno, R.M. 2014. Inactivation kinetics for *Salmonella* Enteritidis in potato omelet using microwave heating treatments. *Food Control*, **43**, 175–182.
- Van Boekel, M.A.J.S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, **74**, 139–159.
- Van Boekel, M.A.J.S. 2009. *Kinetic Modeling of Reactions in Foods*, p. 767. CRC Press, Boca Raton, FL, USA.
- Wang, Y., Wig, T.D., Tang, J., and Hallberg, L.M. 2003. Sterilization of foodstuffs using radio frequency heating. *Journal of Food Science*, **68**, 539–544.
- Watson, H.E. 1908. A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *The Journal of Hygiene*, **8**, 536–542.
- Whiting, R.C. and Buchanan, R.L. 1993. A classification of models for predictive microbiology. *Food Microbiology*, **10**, 175–177.
- Wong, E., Linton, R.H., and Gerrard, D.E. 1998. Reduction of *E.coli* and *S. seftenberg* on pork skin and pork muscle using ultraviolet light. *Food Microbiology*, **15**, 415–423.

CHAPTER 9

Impact of high-pressure processing on the microbial ecology of foods[†]

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9.1 Introduction

In recent years, food chains have become more complicated in terms of handling, processing, and transporting; hence meeting consumer demands for more natural and healthier food and obtaining safe food are also becoming more difficult (Bilek and Turantaş, 2013; Caminiti *et al.*, 2011). An estimated 48 million cases of food-borne illness occur in the United States alone each year (Scallan *et al.*, 2011).

Traditional thermal treatments are the cornerstone of the food processing industry, providing required safety requirements and extension of the shelf life. However, this thermal processing may lead to loss of desired organoleptic properties and damage to heat-labile nutrients and vitamins (Cullen *et al.*, 2012). Also, certain microbes can survive under conditions of high temperature, for example, *E. coli* cells have shown resistance to heat treatment, and acidic media is generating more and more concern about pasteurized products with low pH, such as fruit juices (Gabriel and Nakano, 2011). Therefore, to ensure microbial safety and

preserve the quality of the food, it is essential to find new and alternative processing techniques.

At present, some novel and complementary approaches have been continuously sought and investigated, such as thermal electromagnetic technologies, ohmic and microwave heating, and some non-thermal processing methods that are effective at ambient or sublethal temperatures. Among these methods, particular attention has been paid to high hydrostatic pressure (HHP) processing, which is a non-thermal option and has been proposed as an alternative to the thermal processing technique for destroying food-borne organisms to enhance safety and shelf life of foods (Alpas *et al.*, 2000, 2003; Buzrul *et al.*, 2005a, 2005b).

In high hydrostatic pressure (HHP) processing, which is also referred to as ultra-high-pressure (UHP) processing or high-pressure processing (HPP), the packaged food is placed in the pressure vessel and subjected to water pressures ranging from 100 to 900 MPa. During pressure treatment, the process temperature may be varied from below 0 to above 100°C and exposure times may range from a few seconds

[†] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. USDA is an equal opportunity provider and employer.

to over 20 min (Butz and Tauscher, 2002) depending on the target pathogen. High hydrostatic pressure processing is a commercially available non-thermal technology, which is capable of inactivating certain microorganisms and enzymes in foods, while extending the shelf life of the products (Hugas *et al.*, 2002; Morales *et al.*, 2008). HHP processing can also efficiently enhance the microbial stability of food products while the changes in low molecular weight compounds such as vitamins, pigments, and flavoring components are minimal when evaluated immediately after pressurization (Ramirez *et al.*, 2009). In addition, some studies have demonstrated that HHP processing could enhance the antioxidant activity of juices compared to those that are untreated (De Ancos *et al.*, 2000; Garcia-Parra *et al.*, 2011). Based on the above advantages, HHP processing has been widely used to preserve liquid or solid, packaged or unpackaged foods, such as juices, jellies, fish, meats, salad dressing, rice cakes, and yogurts.

Therefore, the aim of this review is to provide an overview of the published data regarding HHP processing as well as basic knowledge and its current applications.

9.2 Processing operation

A high-pressure processing unit operation involves filling a sterile container with food that is then sealed and placed inside a pressure chamber filled with a pressure-transmitting hydraulic fluid for pressure transmission, with ethyl vinyl alcohol or polyvinyl alcohol suggested as the packing material (Masuda *et al.*, 1992). However, normal multilayer plastic and special aluminum packaging material can be used.

The product is packaged in a flexible container (usually a pouch or a plastic bottle) and is loaded into a high-pressure chamber filled with a pressure-transmitting (hydraulic) fluid. The hydraulic fluid (normally water) in the chamber is pressurized with a pump, and this pressure is transmitted through the package into the food

itself. Pressure is applied for a specific time, usually 3 to 5 minutes. The processed product is then removed and stored/distributed in the conventional manner. Because the pressure is transmitted uniformly (in all directions simultaneously), food retains its shape, even at extreme pressures. Also, the sensory characteristics of the food are retained without compromising microbial safety since heat is not employed.

9.3 Bacteria and enzyme inactivation

The use of high hydrostatic pressure at low pH has been shown to inactivate *E. coli* O157:H7 and *Salmonella* in fruit juices and other fruit products (Alex *et al.*, 2001; Bayndrl *et al.*, 2004; Jordan *et al.*, 2001; Linton *et al.*, 1999). Garcia-Graells *et al.* (1998) reported a 5.0 log cfu/ml decrease in the population of high-pressure-resistant mutants of *E. coli* bacteria in apple juice (pH 3.3) after 15 min of 300 MPa treatment at 20°C. When pressure was increased to 550 MPa and treatment conducted at ambient temperature (20°C) for 5 min, a 6.0 log cfu/ml inactivation of *E. coli* O157: H7 in orange juice (pH 3.9) was observed (Linton *et al.*, 1999). The mechanism of bacterial inactivation by HHP involves a combination of factors that affect the cell membrane, cell wall, proteins, enzymes, and genetic mechanisms. During high-pressure processing, low-energy covalent bonds of treated materials or food samples appeared to be unaffected, suggesting that primary structure and low-molecular-weight molecules, such as those responsible for the sensory and nutritional qualities of food, are left intact (Considine *et al.*, 2008; Patterson, 1999).

Most enzymes are made up of proteins and can dramatically affect metabolic activities. Several researchers have reported significant disruption of hydrophobic and electrostatic interactions that are responsible for maintaining the secondary and tertiary structure of proteins and other high-molecular-weight components (Heremans, 2001; Aymerich *et al.*,

2008; Ross *et al.*, 2003). The disruption of hydrophobic and electrostatic interactions leads to dysfunction of cellular enzymes and potential death of the microbes while producing foods of improved sensory and nutritional quality (Patterson, 1999).

Also, irreversible changes in the structure of membrane macromolecules such as proteins (Hoover *et al.*, 1989; Balny and Masson, 1993) result in membrane damage, with consequent changes in cell permeability affecting the cellular transport systems, loss of osmotic responsiveness, and cell incapacity to maintain internal pH (Aymerich *et al.*, 2008). Permeability of the membrane is also affected by compression of the membrane's bilayer and reduction in the cross-sectional area of the phospholipid molecules (Sale *et al.*, 1970; Hoover *et al.*, 1989; Datta and Deeth, 1999). In addition, the lengthening of the cell, the compression of gas vacuoles (Patterson, 2005), and the condensation of nuclear material (Manas and Mackey, 2004; Wouters *et al.*, 1998) were also reported. For example, hydrogen bonds and covalent bonds associated with DNA were broken after HHP treatment, and this affected the integrity and the chain of chromosome DNA (Yang, 2012).

Figure 9.1(a) and (b) shows a smooth intact cell wall, cell membrane, uniform cell cytoplasm, and electron-transparent regions of nucleoid in *E. coli* and *S. aureus* respectively as seen with scanning electron micrographs before pressure treatment. HPP treatment at 500 and 400 MPa caused cell disruptions. For example, 500 MPa treatment for 30 min resulted in the expansion of nucleoid regions and the compaction of interior regions (Figure 9.1 (a') and (b')) (Ritz *et al.*, 2001; Yang *et al.*, 2012).

The use of pressure with heat to achieve a better inactivation of spores has also been reported. Stewart *et al.* (2000) found that elevated temperature enhanced the destruction of *Bacillus subtilis* and *Clostridium sporogenes* spores and *Geobacillus stearothermophilus* spores (Ananta *et al.*, 2001).

Temperature is known to influence the effects of pressure on the inactivation of bacteria

(Carlez *et al.*, 1993). For *Listeria innocua*, pressurization at 4°C caused greater inactivation than pressure treatments at 35°C, and at 250 to 300 MPa, pressurization at 4 and 50°C were as efficient (about 3 log cycles). Patterson and Kilpatrick (1998) found that a 5 min treatment of 500 MPa pressure at 50°C resulted in a 6.0 log₁₀ reduction of *S. aureus* in UHT milk, while about 1.0 log₁₀ reduction was achieved with either treatment alone. Several studies have reported pressure and temperature treatment at -2, 15, 20, and 50°C lead inactivation of *Salmonella enterica* serovar Enteritidis and *E. coli* (Ponce *et al.*, 1998, 1999). Similarly, Carlez *et al.* (1993) reported that pressure and temperature (4, 20, 35, and 50°C) treatments enhanced the destruction of *Citrobacter freundii*, *Pseudomonas fluorescens*, and *Listeria innocua* in minced beef muscle. The survival curves of *Listeria monocytogenes* Scott A in UHT whole milk treated with high hydrostatic pressure were obtained at four temperatures (22, 40, 45, and 50°C) and two pressure levels (400 and 500 MPa) (Chen and Hoover, 2003b). In this study, the authors reported a more than 8 log₁₀ reduction of *L. monocytogenes* after a 5 min treatment of 500 MPa pressure at 50°C. When the pressure treatment (500 MPa) was carried out at a reduced temperature of 22°C for 35 min, the same level of inactivation for *L. monocytogenes* was achieved. Additionally, Erkmen (2009) indicated that inactivation rates of *S. typhimurium* were higher ($p < 0.05$) at 45°C than at 15, 25, and 35°C when pressure treatment ranged from 200 to 350 MPa.

High-pressure treatments do not break covalent bonds, but can alter hydrophobic and electrostatic bonds responsible for holding proteins in their biologically active form, thus resulting in the irreversible denaturation of one or more critical proteins in the microbe. In many cases, microbial inactivation under HHP treatment exhibits non-linear kinetics (e.g., Buzrul *et al.*, 2008a; Chen and Hoover, 2003a, 2003b, 2004; Tassou *et al.*, 2008). For example, inactivation of several bacteria and yeast followed first-order kinetics (Carlez *et al.*, 1993; Palou *et al.*, 1997; Zook *et al.*, 1999). Other

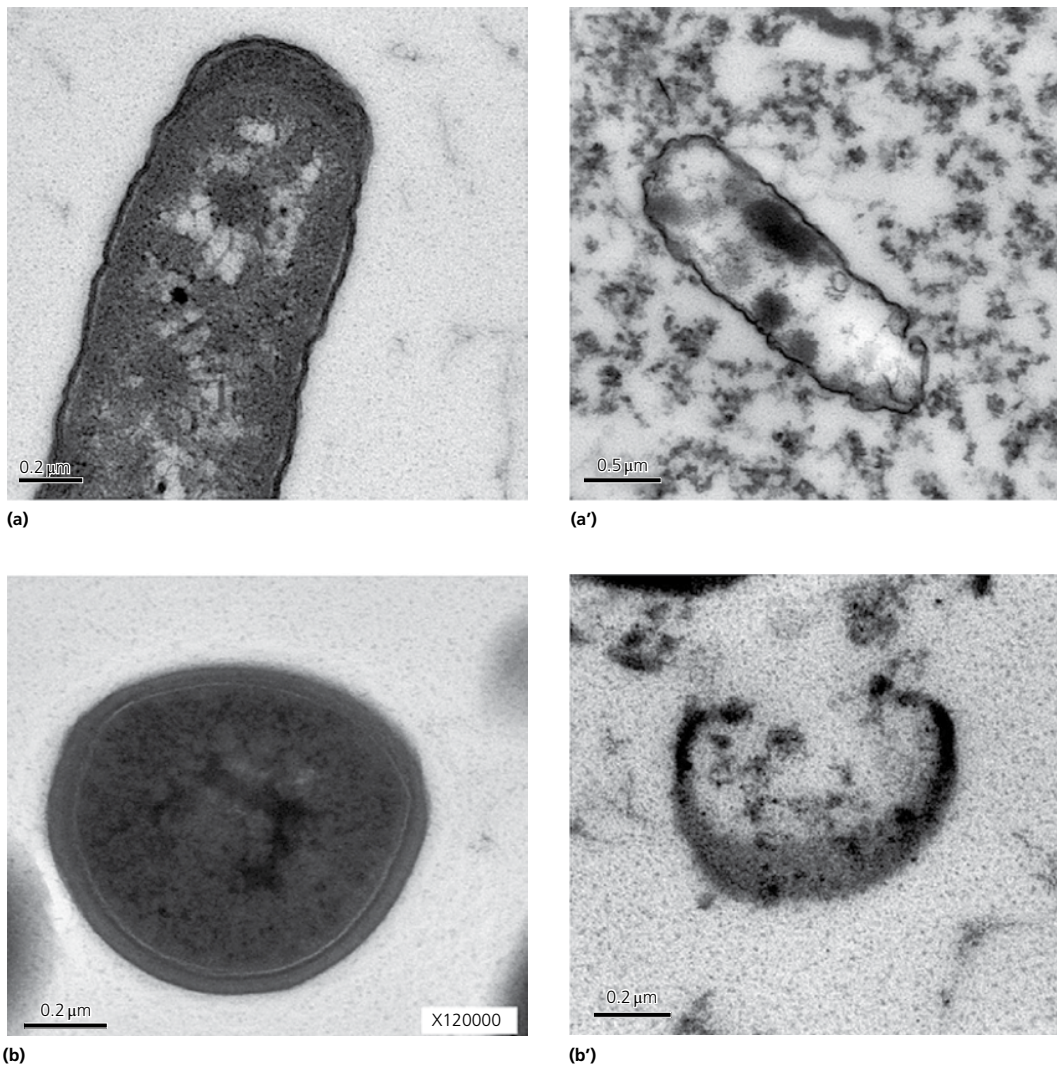


Figure 9.1 Cell structures of untreated and 500 MPa for 30 min treated *E. coli* and *S. aureus* under transmission electron microscopy (TEM) (Source: Yang *et al.*, 2012, *Food Control*, **28**, 273–278+).

authors reported changes in the slope and a two-phase inactivation phenomenon, the first fraction of the population being quickly inactivated whereas the second fraction of the population was to be much more resistant (Cheftel, 1995). The pattern of inactivation kinetics is influenced by pressure, temperature, and the composition of the medium (Palou *et al.*, 1997; Zook *et al.*, 1999). Smelt and Rijke (1992) reported first-order kinetics for high-pressure

inactivation of *E. coli* in a phosphate buffer saline, with *D* values of 25.9, 8.0, 2.5, and 0.8 min for treatments at 200, 250, 300, and 350 MPa, respectively. Furthermore, Patterson *et al.* (1995a) reported that the *D* value of *E. coli* at 700 MPa HHP treatments was about 13 min in the same media. In tryptic soy broth, a bacterial nutrient media, the calculated *D* value for *E. coli* was 143 min at 100 MPa (Furukawa *et al.*, 2002). Patterson *et al.* (1995b) reported that more than

6 log units of *C. freundii* were reduced with 280 MPa pressure at 20°C, 230 MPa at 4°C, and 150 MPa at 50°C. Ritz *et al.* (2000) indicated that HHP treatment (200–600 MPa) of *L. monocytogenes* at 20°C is least efficient as compared to treatments at 4 and 40°C.

9.4 Effect of high pressure on fruit and vegetable products

The effects of HHP processing on a wide variety of fruit and vegetable products, especially fresh produce and minimally processed food, has been investigated. Hsu *et al.* (2014) explored the potential application of HHP to eliminate Shiga toxin-producing *E. coli* (STEC) in fresh strawberry puree. The authors found that HPP treatments at 250 and 350 MPa for 5 min at 10°C resulted in greater than a 5 log reduction of *E. coli* O157:H7 and the “Big Six” non-O157 STEC cocktails, respectively. A steady decrease in the population of *Escherichia coli* O157:H7 and *Salmonella* spp. in frozen strawberry puree was observed at an increasing pressure treatment (Huang, Y., *et al.*, 2013). Treatment of strawberry puree with a low inoculum level of *Salmonella* at ~3 log cfu/g with 300 Mpa for 2 min led to an undetectable bacterial population. Higher pressure treatments of 450 and 350 Mpa for 2 min were able to eliminate *E. coli* O157:H7 and *Salmonella* with a high inoculation level (6 log cfu/g). Similar log reductions of *E. coli* O157 in apple and orange juice treated at 500 MPa at 20°C for 5 min have been reported. A 4 log inactivation of *E. coli* O157:H7 was reported in kiwi-fruit juice by pressure treatment of 300 MPa for 5 min at 20°C, while only a 1 log reduction was achieved in pineapple juice (Buzrul *et al.*, 2008b). A significant viability loss ($p < 0.05$) of more than 8 log cycles for selected relatively pressure-resistant pathogens (*Staphylococcus aureus* 485, *Escherichia coli* O157:H7 933, and *Salmonella enteritidis*) in apple, orange, apricot, and sour cherry juices was achieved after HPP treatment for 5 min at 350 MPa and 40°C (Bayindirli *et al.*, 2006). Neetoo *et al.* (2008)

applied HHP to eliminate *Escherichia coli* O157:H7 on alfalfa sprouted seeds.

Zhou *et al.* (2014) examined the effect of HHP on the microbiological quality of pumpkin. After HHP treatment, the total aerobic bacteria (TAB) count was significantly reduced. The surviving number of natural microorganisms in pumpkin after treatment at 450 MPa for 15 min and 550 MPa for 10 min averaged $1.44 \pm 0.01 \log_{10}$ cfu/g and $1.30 \pm 0.01 \log_{10}$ cfu/g, respectively. Similar results were reported for purple sweet potato nectar (Wang *et al.*, 2012), mango pulp (Liu *et al.*, 2013), kiwifruit and pineapple juices (Buzrul *et al.*, 2008b), apple juice (Valdramidis *et al.*, 2009), and lotus root (Dong *et al.*, 2013), indicating that HHP treatment is effective in reducing the TAB in different fruit and vegetable products. According to Li *et al.* (2010), the counts of TAB in sour Chinese cabbage were significantly reduced by 2.7–4.0 $\log_{10}$ cfu/g at 400 MPa and 4.2–4.5 $\log_{10}$ cfu/g at 600 MPa from 6.2 $\log_{10}$ cfu/g; the counts of lactic acid bacteria (LAB) were also reduced by 2.4–4.3 $\log_{10}$ cfu/g at 400 MPa from 7.0 $\log_{10}$ cfu/g and LAB was completely inactivated at 600 MPa.

9.5 Effect of HHP on meat and other food products

Efficacy of HHP treatment has been reported on meat and seafood products (Ginson *et al.*, 2013) including bay scallops (Yi *et al.*, 2013) and oyster (Cruz-Romero *et al.*, 2007, 2008). Yuste *et al.* (1999) reported a bactericidal effect of HPP on *L. innocua* in vacuum-packaged poultry meat pressurized at 350, 400, 450, and 500 MPa for a duration of 5, 10, 15, and 30 min at each pressure and a treatment temperature of 2, 10, and 20°C. The authors achieved ≥ 7.5 log reductions by HPP treatment. In another study, using similar bacterium, Al-Habsi *et al.* (2012) achieved 78.83% inhibition in honey treated at 800 MPa for 15 min. Efficacy of HPP for inactivation of *Salmonella*, *Escherichia coli*, *Shigella*, and *Staphylococcus aureus* in raw milk has been

investigated and its application for milk pasteurization has been proposed (Trujillo, 2002). In shrimp marinated with wine and subjected to high hydrostatic pressure, the total plate count (TPC) was reduced to below the detection limit in all treated samples. However, the bacterial populations in HHP-treated samples increased to 2.57 and 2.84 log₁₀ cfu/g after storage at 4 and 25°C for 90 days, respectively (Yi *et al.*, 2013).

9.6 Effect of added antimicrobial on pathogen inactivation by high-pressure processing (hurdle approach)

High-pressure processing has been recognized as a viable alternative to thermal processing among emerging food preservation technologies (Roberts and Hoover, 1996; Farkas and Hoover, 2000; Linton *et al.*, 2001). To further increase the shelf life and the safety of pressurized food products, HHP is being developed in combination with other treatments such as bacteriocins or other natural antimicrobials (Rastogi *et al.*, 2007). Nisin is the only bacteriocin recognized as GRAS and its use is approved in cooked meat sauces and soups in the United States (FSIS, 2002). Ponce *et al.* (1998) examined the potential for inactivation of *Listeria innocua* and *Escherichia coli* in liquid whole egg by HHP combined with the addition of nisin. They reported a reduction of almost 5 log₁₀ units for *E. coli* counts and more than 6 log₁₀ units for *L. innocua* at 450 MPa in the presence of 5 mg/l of nisin. Authors also observed no growth effect when samples were stored at 20°C after pressurization, except for samples with *L. innocua*. A similar synergistic effect of HHP and nisin combination was reported by Lee and Kaletunç (2010). In their study, the viability of two *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) strains were evaluated by differential scanning calorimetry (DSC) and plate counting. HHP (up to 200 MPa) or the nisin alone did not affect the

viability and cellular components of either strain. However, an 8 log cfu/ml reduction was observed after a pressure treatment at 500 MPa in the presence of nisin. Additionally, Jofre *et al.* (2008) reported that the combination of HHP, nisin, and refrigeration at 6°C was necessary to decrease the levels of *S. aureus* by 2.4 log cfu/g after 3 months of storage. Several studies have shown the synergism between HHP and antimicrobials such as enterocin LM-2 (Liu *et al.*, 2012), essential oils (Abriouel *et al.*, 2014; Espina *et al.*, 2013), and enterocin AS-48 (Ananou *et al.*, 2010), on inactivation of several food-borne pathogens and spoilage organisms (Garriga *et al.*, 2002; Jofre *et al.*, 2008; Zhang *et al.*, 2010). The combination of HHP and bacteriocins would increase the organism death rate because cells surviving pressurization also become sublethally injured and are then killed by antimicrobials.

Current HHP research is also exploring the combination of HHP and other non-thermal processing methods (Bermúdez-Aguirre and Barbosa-Cánovas, 2011). Chai *et al.* (2014) investigated a combination of TiO₂-UV photocatalysis and HHP for processing of freshly squeezed *Angelica keiskei* juice and reported inactivation of naturally occurring microbes, especially *B. cereus*. The authors presented research results that showed complete reduction of colonies of yeasts and molds, coliform bacteria, *Pseudomonas*, and *B. cereus* in *Angelica keiskei* juice after processing with a combination of TiO₂-UV photocatalysis and HHP. Compared to unprocessed *Angelica keiskei* juice, the *B. cereus* cell recovery and growth was much less in combined processed juice. Al-Nehlawi *et al.* (2014) investigated the synergistic effect of high hydrostatic pressure and carbon dioxide atmosphere packaging on inactivation of spoilage bacteria such as *Leuconostoc carnosum*, *Brochothrix thermosphacta*, *Salmonella enteritidis*, *Campylobacter jejuni*, and *Listeria innocua* on poultry sausages. The results demonstrated a strong synergistic effect of two combined processing and preservation technologies against all the

microorganisms studied, except in *S. enteritidis*, which showed a greater resistance under CO₂ atmospheres, and *C. jejuni*, which is especially sensitive at high pressures. Microbial cell damage caused by high pressure can facilitate the penetration of carbon dioxide into the microorganism cells, affecting their metabolism and consequently their growth. Several other studies have reported the synergistic effect between HHP and CO₂ against certain enzymes and microorganisms, such as *Lactobacillus plantarum* and *Escherichia coli* (Corwin and Shellhammer, 2002), *Bacillus subtilis*, *Staphylococcus aureus*, *Fusarium oxysporum*, and *Fusarium sporotrichioides* (Park *et al.*, 2003). In a combination treatment strategy of CO₂ with HHP, the pressure could be lowered without compromising the reduction of microbial counts.

9.7 High-pressure carbon dioxide (HPCD) disinfection

High-pressure CO₂ application uses pressures less than 50 MPa (Damar and Balaban, 2006), which is far less than the pressure commonly used in HPP applications (400–600 MPa). The main influencing parameter here is CO₂ and not high pressure, though pressure helps in improved penetration of CO₂ into the cell membrane. Though many other gases (N₂, N₂O, C₂H₄, Ar, and tetrafluoroethane) were tried for their microbial reduction capabilities, they were found to be not as effective as CO₂ in terms of gas sorption and desorption by the cells (Debs-Louka *et al.*, 1999; Dillow *et al.*, 1999; Enomoto *et al.*, 1997; Lin *et al.*, 1991; Nakamura *et al.*, 1994; Wei *et al.*, 1991). Pressurized CO₂ imparts lethality on bacteria by either directly affecting the molecular constituents or by dissociating itself into its reactive ionic species such as bicarbonates (HCO₃⁻), carbonates (CO₃²⁻) and hydrogen (H⁺) (Daniels *et al.*, 1985; Garcia-Gonzalez *et al.*, 2007; Haas *et al.*, 1989; Kamihira *et al.*, 1987). These in turn will affect the cell membrane permeability

and intracellular constituent properties (Debs-Louka *et al.*, 1999; Dixon and Kell, 1989; Haas *et al.*, 1989).

Dissolution of CO₂ in the water of food products generates carbonic acid (H₂CO₃) and the resulting low pH enhances the cell membrane penetration (Lin *et al.*, 1992; Wei *et al.*, 1991). The affected cell's metabolic function is altered by changes in its pH, enzyme activity, electrolytic balance, and by leaching of internal constituents. None of these changes are proved to be singularly responsible for the microbicidal effect of pressurized CO₂. It is hypothesized to be synergistic in many of these effects (Garcia-Gonzalez *et al.*, 2007). This technology is also termed cold pasteurization because of the mild processing conditions used (Damar and Balaban, 2006; Garcia-Gonzalez *et al.*, 2007). Mild processing conditions are easier to manage and control and that helps easy adoption of this technology (Spilimbergo *et al.*, 2002). The basic aspect of this technology is diffusion and dissolution of CO₂ into the food and through the embedded microbial cells. Density of CO₂ could be changed by changing its pressure and temperature. When these parameters are increased to above its critical values ($T_c = 31.1^\circ\text{C}$, $P = 7.38\text{ MPa}$), CO₂ reaches a single supercritical phase and possesses gas-like diffusivity and liquid-like solubility (Garcia-Gonzalez *et al.*, 2007; Ishikawa *et al.*, 1995; Mazzoni *et al.*, 2001). Increase in pressure accelerates solubility but is limited by the saturated solubility of CO₂ in the suspending medium (Spilimbergo *et al.*, 2002; Sims, 1999; Sims and Estigarribia, 2003; Spilimbergo and Bertucco, 2003). Spilimbergo *et al.* (2002) observed no significant increase in solubility of CO₂ and lowering of pH beyond 10 MPa pressure. In Erkmen (2000) experiments with HPCD on *Salmonella* Typhimurium showed accelerated inactivation with increasing CO₂ pressure from 15 to 60 atm (1.5–6 MPa). Similar to high-temperature short-time (HTST) heat processing, high pressure of CO₂ required only a short exposure time for achieving the same level of *Salmonella* inactivation (Erkmen and Karaman, 2001). Decimal reduction time

(DHPCD) values decreased with a rise of CO₂ pressure (Erkmen, 2000; Erkmen and Karaman, 2001; Hata *et al.*, 1996; Sirisee *et al.*, 1998).

Similarly, the use of high temperature required a shorter processing time. Erkmen (2000) equally inactivated *Salmonella* Typhimurium at 60 atm (0.6 MPa) CO₂ pressure under various temperature–time combinations (25°C for 30 min; 35°C for 15 min; 45°C for 12 min). An increase in temperature stimulates CO₂ diffusivity but decreases CO₂ solubility at much higher temperatures than its critical level, apart from deteriorating the food quality (Hong and Pyun, 1999; Lin *et al.*, 1994). Hence, it is recommended to hover around critical conditions on the supercritical side in order to maximize the benefit (Gunes *et al.*, 2006; Garcia-Gonzalez *et al.*, 2007).

The ratio of CO₂ to product masks the direct effect of temperature and pressure, especially in a flowthrough system (Gunes *et al.*, 2006; Tomasula, 2003). Agitation improves the contact of CO₂ with the product (Dillow *et al.*, 1999; Lin *et al.*, 1994; Hong *et al.*, 1997). The presence of water in the food enhances solubility and increased inactivation (Kumagai *et al.*, 1997).

Because of the complex physicochemical nature of food systems and wide variations in test conditions, contrasting results were reported in the literature. Wei *et al.* (1991) demonstrated complete inactivation of *Salmonella* in egg yolk but only limited inactivation in whole egg using the same CO₂ treatment (13.7 MPa, 35°C, 2 h). This was in contrast to the protective effect of lipid and fat components reported by Lin *et al.* (1994). A number of authors have hypothesized that the chief destructive effect on microbial cells is due to rapid and sudden release of CO₂ pressure and the resulting cell rupture (Fraser, 1951; Lin *et al.*, 1992; Nakamura *et al.*, 1994). This was contradicted by a few other studies that showed no significant effect during the depressurization step and the rate of depressurization (Enomoto *et al.*, 1997; Kumagai *et al.*, 1997; Debs-Louka *et al.*, 1999). Lin *et al.* (1992) used a rapid depressurization technique in both subcritical and supercritical temperatures to disrupt microbial cells and to recover various intracellular enzymes

and organelles for use in industry and medicine. Disruptive rates were influenced positively by temperature, pressure, and by the addition of cell lytic entrainer enzymes (Lin *et al.*, 1991, 1992). The same authors concluded that the disruption could be maximized by repetition of pressure release. Not much research has been reported on this as this may be of help only for microorganisms with robust and rigid cell walls, like those of yeast (Lin *et al.*, 1991, 1992; Hunter and Asenjo, 1988). Dillow *et al.* (1999) achieved a 3 log₁₀ cfu/ml reduction of *Salmonella* Salford in growth medium by cyclic pressurization and depressurization (20.5 MPa, 34°C, 36 min, 3 and 6 cycles). Park *et al.* (2002) studied the effects of combined (sequential application) HPCD and high hydrostatic pressure on inactivation of aerobic microorganisms in carrot juice. Compared with their individual applications, the combined effect on juice quality and safety was better. Not much recent literature was available on similar combined applications involving HPCD and other emerging technologies, especially on microbial inactivation. This may be because of the practical constraints in executing them.

As CO₂ and its highly reactive constituent species have a profound effect on enzymes and other sensory components of the food system, it is necessary to consider them alongside microbial inactivation (Del Pozo-Insfran *et al.*, 2006; Garcia-Gonzalez *et al.*, 2007). In addition to its potential for continuous application (Balaban, 2004; Connery *et al.*, 2005), this technology also satisfies the 5 log pathogen reduction rule for juice products to be included in the HACCP program. This may very well serve as an alternative to thermal pasteurization (Connery *et al.*, 2005; Werner and Hotchkiss, 2006).

9.8 Effect of HHP on bacteria, virus, insects, and other organisms

A large number of studies have shown that as a non-thermal process, HHP plays an important role in inactivation of pathogenic and spoilage

microorganisms. However, other applications, such as inactivation of viruses, insects, and removal of pesticide residue, have also been explored. Iizuka *et al.* (2013a) and Iizuka and Shimizu (2013) demonstrated that HHP has a function in reducing Chlorpyrifos, a commonly used broad-spectrum insecticide in pest control, in cherry tomatoes. The optimum HHP conditions for reduction of Chlorpyrifos were about 75 MPa at 5°C. Under this condition, the removal rate of Chlorpyrifos was around 75%, which is slightly higher than other treatments such as ultrasonication. Chlorpyrifos removed from samples was migrated to the surrounding water and no accumulation of toxic intermediates of Chlorpyrifos was detected in HHP-treated samples. The thick wall of cherry tomatoes was barely broken below 75 MPa, and almost all the pesticide remained in the cuticle layer without penetration (Iizuka *et al.*, 2013b).

HHP can kill most microorganisms without significantly affecting the sensory and nutritional properties, and thus increase the shelf life of these products. Sohn and Lee (1999) reported that the count of total aerobic bacteria (TAB) in kimchi, a fermented food made of vegetables and seasonings, was reduced to 4 log₁₀ cfu/ml by HPP at 400 MPa for 10 min without any change in the texture. Additionally, Lee *et al.* (2003) found that lactic acid bacteria (LAB) was significantly reduced from 7 to 3 log₁₀ cfu/ml at 600 MPa for 5 min, which prevented gas production in kimchi and excessive acidity during storage at 10°C. Furthermore, Peñas *et al.* (2010) demonstrated that 300 MPa for 10 min at 4°C led to a reduction of TAB and LAB (4–5 log₁₀ cfu/g) in sauerkraut immediately after the treatment. Li *et al.* (2010) applied HHP on sour Chinese cabbage to inactivate TAB, LAB, and yeasts. The results showed that the counts of TAB, LAB, and yeasts were significantly reduced by 2.7–4.0 log₁₀ cfu/g, 2.4–4.3 log₁₀ cfu/g and 1.5–2.0 log₁₀ cfu/g, respectively, at 400 MPa pressure. When the pressure was increased by 600 MPa, LAB was completely inactivated and the population of TAB was reduced by 4.2–4.5 log₁₀ cfu/g.

Cronobacter sakazakii is considered an emerging opportunistic pathogen of concern for public health (Drudy *et al.*, 2006; Iversen and Forsythe, 2003; Pagotto *et al.*, 2008) and, to date, there are only a few studies that have been reported on the application of HHP to control *C. sakazakii*. Koseki *et al.* (2009) developed a remarkable probabilistic model capable of identifying a minimum processing condition for a required log₁₀ reduction of *C. sakazakii* cells. Other types of bacteria, such as *Vibrio parahaemolyticus* and *Vibrio vulnificus*, have also been studied. Ye *et al.* (2012) proved that pressurization at 200–300 MPa for 2 min can cause various degrees of inactivation, from 1.2 to >7 log the most probable number (MPN)/g reductions, which means that HHP has a potential to be the processing choice for the oyster industry by virtue of its economic benefits and improved food safety.

López-Pedemonte *et al.* (2003) reported that the application of HHP alone to cheeses can result in only a small amount of inactivation, less than 1 log, for *B. cereus*. Additionally, Moerman (2005) reported that for food of neutral pH, HPP alone cannot provide sufficient inactivation to accomplish a state of pasteurization/sterilization. Moreover, bacterial spores have quite a strong resistance to high pressure (>1000 MPa). Hence, HHP should be combined with other sterilization processes in these scenarios. Gao *et al.* (2006a) obtained a 6 log-cycles reduction of *Geobacillus stearothermophilus* spores in milk buffer by HPP treatment at 625 MPa for 14 min at a high temperature of 86°C. About 6 log-cycles reduction of *Bacillus subtilis* spores were also achieved at 576 MPa pressure and at 87°C for a holding time of 13 min (Gao *et al.*, 2006b). A similar reduction for *Bacillus cereus* spores were reported by Ju *et al.* (2008) when milk buffer was treated by HPP at 540 MPa for 16.8 min at 71°C. Aouadhi *et al.* (2013) investigated the efficiency of HHP in combination with a moderate temperature to inactivate spores and achieved a 5 log reduction of *Bacillus sporothermodurans* spores at 477 MPa/48°C for 26 min and 495 MPa/49°C for

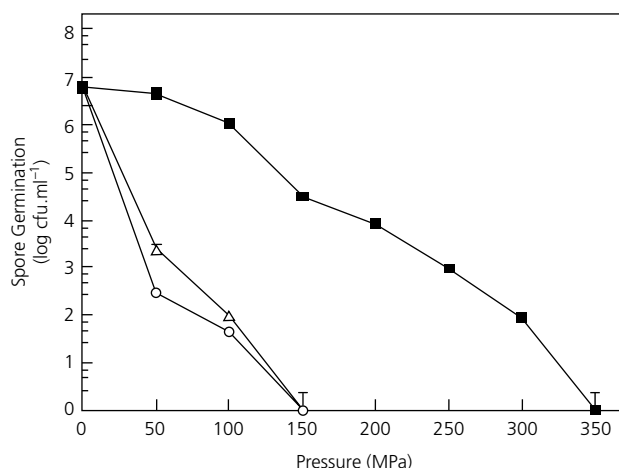


Figure 9.2 Efficacy of non-thermal treatment of HPP and plant essential oils for 30 min, alone and in combination, on viability of *C. gloeosporioides* spores: (■) without oil, (Δ) with 0.75 mg/ml citral oil, and (○) with 0.75 mg/ml lemongrass essential oil (Source: Palhano *et al.*, 2004, *International Journal of Food Microbiology*, **95**, 61).

30 min in water and in milk, respectively. Palhano *et al.* (2004) reported combined non-thermal treatment of HPP with citral oil or lemongrass essential oil for successful inactivation of viable mold (*Colletotrichum gloeosporioides*) spores compared with the reduction caused by HPP alone. The authors reported only a 2 log reduction of spores after a 150 MPa pressure treatment in the absence of oil (Figure 9.2).

In comparison to food-borne pathogenic bacteria, little information on pressure inactivation of viruses exists. The investigation of Overman and Lewis (1959) showed that the infectivity and antigenicity of some influenza virus strains were markedly affected by pressure at approximately 1600 atm. Similarly, Nakagami *et al.* (1992) found virus particles (virions) and their infection could be drastically affected by treatment at pressure greater than 300 MPa for 10 min at 25°C, and infective titers of HSV-1 (herpes simplex type 1) and HCMV (human cytomegalovirus) could be reduced by more than 7 and 4 log units, respectively, at pressures greater than 400 MPa. Calci *et al.* (2005) demonstrated that greater than 1, 2, or 3 log₁₀ PFU reductions of HAV (hepatitis A virus) per oyster can be obtained by 1 min HPP treatments at 350, 375, and 400 MPa pressures, respectively,

within a temperature range of 8.7 to 10.3°C. Chen *et al.* (2005) carried out HHP on *Feline calicivirus* (FCV) and indicated that a 4 min treatment at 200 MPa at temperatures of -10 and 50°C reduced the titer of FCV by 5.0 and 4.0 log units, respectively, whereas at 20°C the same treatment reduced the titer only by 0.3 log units. The results point to the potential for operating temperatures, particularly below room temperature, to lower the pressure required to achieve a desired level of virus inactivation. Other studies on virus inactivation by pressure were also reported (Basset, 1932; Basset *et al.*, 1938; Kovač *et al.*, 2012; Leonard *et al.*, 2007; Wilkinson *et al.*, 2001; Lou *et al.*, 2011; Kingsley *et al.*, 2002; Kingsley *et al.*, 2004).

9.9 Effect of HHP on quality: color, flavor, texture, sugar, totally soluble, and insolubles

High hydrostatic pressure is a non-thermal technology and the processing temperatures usually do not increase beyond 40°C (Welti-Chanes *et al.*, 2009). During processing, isostatic pressure transmits instantly to the product,

independent of size, shape, and composition of the foods (Patras *et al.*, 2009).

Numerous studies have demonstrated that HHP treatment is efficient enough to destroy microorganisms in fruit juices with little or no effect on sensory properties and nutritional value (Knorr, 1993; Norton and Sun, 2008). In addition, some studies have suggested that HHP processing may enhance the antioxidant activity of juices when compared to untreated product (De Ancos *et al.*, 2000; Garcia-Parra *et al.*, 2011). Laboissière *et al.* (2007) investigated the effects of HHP on the sensory properties of passion fruit juice by quantitative descriptive analysis (QDA). According to QDA and principal components analysis (PCA) results, they found that the innovative technology has an important role to play in minimizing loss of sensory and nutritional quality. Tao *et al.* (2012) also indicated that HHP processing at 650 MPa for 2 h significantly reduced the intensities of the sour and fruity odor of red wine. Additionally, Boluda-Aguilar *et al.* (2013) reported that Jasmine rice treated at 300 and 400 MPa for 2 and 4 min led to quick cooking rice with good texture and sensory characteristics.

High-pressure processing can produce structural changes in macromolecules such as proteins, causing alterations in color and texture. However, the covalent bonds are little affected, which are attributed to important quality parameters. Cheftel (1995) demonstrated that at ambient temperatures, application of pressures in the range of 300–500 MPa, reduces the activity of enzymes combined with retention of small molecules responsible for color, taste, vitamins, and nutrients, resulting in a pressurized product that can be stored for a considerable time at 4–6°C. Canto *et al.* (2012) evaluated the effect of HHP on the color and texture parameters of alligator tail meat, which were treated with 200, 300, and 400 MPa pressure for 10 min and stored at 4±1°C for 45 days. The results showed that at lower pressures there was a positive alteration in sensory characteristics of the alligator meat, especially with respect to color, flavor, and tenderness.

However, Kim *et al.* (2014) found that the hardness and the color parameters, L^* (lightness), a^* (redness), and b^* (yellowness), of garlic samples decreased after HHP treatment, while the cohesiveness value of garlic samples was increased significantly.

Zhou *et al.* (2014) proposed two different non-linear mathematical models to explain inactivation kinetics during high-pressure processing of pumpkins ranging from 350 to 550 MPa for 0.5 to 30 min. Results showed a greater retention of the original color, vitamin C, and antioxidant capacity and increased total phenols content for pumpkin samples after processing at 550 MPa for 10 min. The effects of high hydrostatic pressure (HHP) treatment at 300–500 MPa for 5–20 min on enzymes, phenolics, carotenoids, and color of apricot nectars were investigated by Huang, W., *et al.* (2013). The results of this study showed that HHP treatments also had no effect on total carotenoids and individual carotenes in apricot nectars and the color of HPP-treated apricot nectars was similar to the untreated apricot nectars. Varela-Santos *et al.* (2012) studied storage stability of pomegranate juice after high-pressure treatment and reported that pH, °Brix, and titratable acid of pomegranate juice were not significantly affected by pressure treatments during 15 days of storage. Microbiological quality and physico-chemical properties of HHP processed lotus root during 90 days of refrigerated storage was investigated by Dong *et al.* (2013). Results indicated that high-pressure processing at 600 MPa for 2.5 min was an effective non-thermal treatment approach for an extended shelf life of lotus root. No significant changes ($p < 0.05$) in color parameters (L , a , and b values) or product pH were observed during the 90 day storage.

In a comparative evaluation of high hydrostatic pressure (HHP, 600 MPa/1 min) and high-temperature short-time (HTST, 110°C/8.6 s) processing of mango nectars, it was observed (Liu *et al.*, 2014) that both yeast and mold populations in treated mango nectars were less than 1 log cfu/ml and the total aerobic bacteria counts were less than 1.70 log cfu/ml due to

both thermal and non-thermal processing. There was a significant decrease in fructose, glucose, and total sugar but a significant increase in 5-hydroxymethylfurfural and cloudiness of mango nectars, while the changes in sucrose, pH, and titratable acid were insignificant after both types of treatment.

Furthermore, during 16 weeks of storage, the fructose, glucose, titratable acid, and 5-hydroxymethylfurfural content increased, while sucrose, total sugar, pH, and cloudiness of nectar decreased significantly. Briones-Labarca *et al.* (2012) evaluated the effect of high hydrostatic pressure on quality changes such as color, texture, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), etc., of red abalone (*Haliotis rufecens*) during storage at 4°C. Results have shown that protein and fat contents of pressure-treated abalones were significantly ($p < 0.05$) lower compared to untreated samples. Treated abalones have a significantly higher pH, moisture, and ash content than untreated abalones. Pressure-treated samples showed a much less negative effect on tissue color of abalone. TVB-N and TMA levels for HHP-treated abalones increased during the storage period but did not exceed 28 mg TVB-N/100 g and 3 mg TMA/100 g, respectively, at the end of 60 days of storage. The untreated sample, however, exceeded the allowable limit for TVB-N and TMA within 30 days of storage.

9.10 Advantages and disadvantages of using HHP

High hydrostatic pressure is a non-thermal technology that eliminates microorganisms with a minimal effect on the quality of the foods. Compared to thermal processing, pressure has less detrimental effects on food and therefore the products preserve most of their natural colors and flavors and health-promoting substances (Kingsley *et al.*, 2005; Wilkinson *et al.*, 2001). For fruit and vegetable products, HPP offers the option of making a product of high quality, greater safety, and increased shelflife

(Butz *et al.*, 2003). For fruit juice and beverages, HPP can also produce innovative products with fresh-like quality attributes, natural colors, etc., which are valued by consumers (Deliza *et al.*, 2005). Other advantages are reduced process times, minimal physical and chemical changes, retention of freshness, flavor, texture, appearance, and color, reduction of loss of vitamin C, and other fine functional properties compared to traditional thermal processing (Flick, 2003). However, HHP can sometimes affect the yield, sensory qualities such as color and texture, and can induce biochemical changes affecting natural food properties, but these effects are less severe than those experienced during thermal processing techniques (Buckow and Heinz, 2008; Hogan *et al.*, 2005). In addition, these side effects on food properties can be attenuated by a suitable selection of processing parameters such as temperature, time, and pressure (Buckow and Heinz, 2008).

HHP can affect smaller molecules such as vitamin C and β -carotene or inactivate some types of enzymes (Butz *et al.*, 2002; Bull *et al.*, 2004; Cheftel, 1995). It can also alter the food rheological properties (Patterson *et al.*, 2007). Furthermore, HHP may also affect food quality attributes such as color, flavor, and nutritional value to a lesser extent (Oey *et al.*, 2008b; Rastogi *et al.*, 2007; Rendueles *et al.*, 2011).

9.11 Applications and conclusions

High-pressure processing is preferred by the food industry because of its capacity to diminish the microbial load while preserving most of the sensory, nutritional, and functional properties of the treated products (Rivalain *et al.*, 2010). The first HHP-treated product that appeared on the market was a high acid jam in Japan in the early 1990s. Since then, a wide spectrum of food products have been commercialized, for example, fish and seafood products, meat products such as cooked or cured ham, fruit products such as guacamole,

fruit jellies, and juices, and ready-to-eat (RTE) products (Considine *et al.*, 2008; Goh *et al.*, 2007; Murchie *et al.*, 2005; Smelt, 1998; Torres and Velazquez, 2005).

In conclusion, the efficacy of HPP for inactivating bacteria for heat-sensitive foods is very promising. More study associated with the cost of operations is needed for an effective utilization of this technology. Similarly, the effect of treatments on injured populations of bacteria should be investigated further as certain bacterial microflora injured during processing were found to repair and survive in certain media. Due to the lack of sufficient published scientific literature concerning growth kinetics of injured HPP-treated cells, the food industry and risk assessors would find it difficult estimating the likelihood that any of the pathogen, if present in treated samples, would grow in response to the treatments and at storage temperatures. It is important to state that any model used for predicting the behavior of injured and surviving bacterial cells in HPP-treated food samples should consider the ability of injured bacteria to repair and survive in treated samples stored at room or refrigerated temperatures. These variables as suggested above should not be the sole means of ensuring the microbiological safety of the treated foods but as prediction guidelines that would ensure better microbial food safety.

References

- Abriouel, H., Benomar, N., Gálvez, A., and Pulido, R.P. 2014. Preservation of Manzanilla Aloreña cracked green table olives by high hydrostatic pressure treatments singly or in combination with natural antimicrobials. *LWT – Food Science and Technology*, **56**, 427–431.
- Alex, Y.-L.T., Ravishankar, S., and Sizer, C.E. 2001. Effect of low-temperature, high-pressure treatment on the survival of *Escherichia coli* O157:H7 and *Salmonella* in unpasteurized fruit juices. *Journal of Food Protection*, **64**, 1122–1127.
- Al-Habsi, N.A. and Niranjana, K. 2012. Effect of high hydrostatic pressure on antimicrobial activity and quality of Manuka honey. *Food Chemistry*, **135**, 1448–1454.
- Al-Nehlawi, A., Guri, S., Guamis, B., and Saldo, J. 2014. Synergistic effect of carbon dioxide atmospheres and high hydrostatic pressure to reduce spoilage bacteria on poultry sausages. *LWT – Food Science and Technology*, **58**, 404–411.
- Alpas, H., Kalchayanand, N., Bozoglu, F., and Ray, B. 2000. Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *International Journal of Food Microbiology*, **60**, 33–42.
- Alpas, H., Lee, J., Bozoglu, F., and Kaletunc, G. 2003. Evaluation of high hydrostatic pressure sensitivity of *Staphylococcus aureus* and *Escherichia coli* O157:H7 by differential scanning calorimetry. *International Journal of Food Microbiology*, **87**, 229–237.
- Ananou, S., Garriga, M., Jofré, A., Aymerich, T., Gálvez, A., Maqueda, M., Martínez-Bueno, M., and Valdivia, E. 2010. Combined effect of enterocin AS-48 and high hydrostatic pressure to control food-borne pathogens inoculated in low acid fermented sausages. *Meat Science*, **84**, 594–600.
- Ananta, E., Heinz, V., Schlüter, C., and Knorr, D. 2001. Kinetic studies on high-pressure inactivation of *Bacillus stearothermophilus* spores suspended in food matrices. *Innovative Food Science and Emerging Technologies*, **2**, 261–272.
- Aouadhi, C., Simonin, H., Prévost, H., de Lamballerie, M., Maaroufi, A., and Mejri, S. 2013. Inactivation of *Bacillus sporothermodurans* LTIS27 spores by high hydrostatic pressure and moderate heat studied by response surface methodology. *LWT – Food Science and Technology*, **50**, 50–56.
- Aymerich, T., Picouet, P.A., and Monfort, J.M. 2008. Decontamination technologies for meat products. *Meat Science*, **78**, 114–129.
- Balaban, M.O. 2004. Method and apparatus for continuous flow reduction of microbial and/or

- enzymatic activity in a liquid product using carbon dioxide. US Patent 6,723,365 B2 (April 20, 2004) and US Patent Application 2004/0131739 A1 (July 8, 2004).
- Balny, C. and Masson, P. 1993. Effects of high pressure on proteins. *Food Reviews International*, **9**, 611–628.
- Basset, F. 1932. Etude sur les effets biologiques des ultrapressions: resistance des bactéries, des diastases et des toxines aux pressions trts & levees. *C.R. Acad. Sci.*, **195**, 1431.
- Basset, F., Gratis, A., Macheborref, P., and Monil, P. 1938. Action of high pressures on plant viruses. *Proceedings of the Society of Experimental Biology and Medicine*, **38**, 248.
- Bayindirli, A., Alpas, H., Bozoglu, F., and Hizal, M. 2006. Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. *Food Control*, **17**, 52–58.
- Bermúdez-Aguirre, D. and Barbosa-Cánovas, G. 2011. An update on high hydrostatic pressure, from the laboratory to industrial applications. *Food Engineering Reviews*, **3**, 44–61.
- Bilek, S.E. and Turantaş, F. 2013. Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. *International Journal of Food Microbiology*, **166**, 155–162.
- Boluda-Aguilar, M., Taboada-Rodríguez, A., López-Gómez, A., Marín-Iniesta, F., and Barbosa-Cánovas, G.V. 2013. Quick cooking rice by high hydrostatic pressure processing. *LWT – Food Science Technology*, **51**, 196–204.
- Briones-Labarca, V., Perez-Won, M., Zamarca, M., Aguilera-Radic, J.M., and Tabilo-Munizaga, G. 2012. Effects of high hydrostatic pressure on microstructure, texture, color and biochemical changes of red abalone (*Haliotis rufecens*) during cold storage time. *Innovative Food Science and Emerging Technologies*, **13**, 42–50.
- Buckow, R. and Heinz, V. 2008. High pressure processing – a database of kinetic information. *Chemie Ingenieur Technik*, **80**, 1081–1095.
- Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., et al. 2004. The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technologies*, **5**, 135–149.
- Butz, P. and Tauscher, B. 2002. Emerging technologies: chemical aspects. *Food Research International*, **35**, 279–284.
- Butz, P., Edenharter, R., Fernandez Garcia, A., Fister, H., Merkel, C., and Tauscher, B. (2002). Changes in functional properties of vegetables induced by high pressure treatment. *Food Research International*, **35**, 295–300.
- Butz, P., Fernandez-Garcia, A., Lindauer, R., Dieterich, S., Bognar, A., and Tauscher, B. 2003. Influence of ultra high pressure processing on fruit and vegetable products. *Journal of Food Engineering*, **56**, 233–236.
- Buzrul, S., Alpas, H., and Bozoglu, F. 2005a. Effects of high hydrostatic pressure on shelf life of lager beer. *European Food Research and Technology*, **220**, 615–618.
- Buzrul, S., Alpas, H., and Bozoglu, F. 2005b. Effect of high hydrostatic pressure on quality parameters of lager beer. *Journal of the Science of Food and Agriculture*, **85**, 1672–1676.
- Buzrul, S., Alpas, H., Largeteau, A., and Demazeau, G. 2008a. Inactivation of *Escherichia coli* and *Listeria innocua* in kiwifruit and pineapple juices by high hydrostatic pressure. *International Journal of Food Microbiology*, **124**, 275–278.
- Buzrul, S., Alpas, H., Largeteau, A., and Demazeau, G. 2008b. Modeling high pressure inactivation of *Escherichia coli* and *Listeria innocua* in whole milk. *European Food Research and Technology*, **227**, 443–448.
- Calci, K.R., Meade, G.K., Tezloff, R.C., and Kingsley, D.H. 2005. High-pressure inactivation of hepatitis A virus within oysters. *Applied and Environmental Microbiology*, 339–343.
- Caminiti, I.M., Noci, F., Muñoz, A., Whyte, P., Morgan, D.J., Cronin, D.A., and Lyng, J.G. 2011. Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chemistry*, **124**, 1387–1392.
- Canto, A.C.V.C.S., Lima, B.R.C.C., Cruz, A.G., Lázaro, C.A., Freitas, D.G.C., Faria, J.A.F., Torrezan, R., Freitas, M.Q., and Silva, T.P.J. 2012.

- Effect of high hydrostatic pressure on the color and texture parameters of refrigerated Caiman (*Caiman crocodilus yacare*) tail meat. *Meat Science*, **91**, 255–260.
- Carlez, A., Rosec, J.P., Richard, N., and Cheftel, J.C. 1993. High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle. *Lebensm.-Wiss.-Technol.*, **26**, 357–363.
- Chai, C.-H., Lee, J.-Y., Lee, Y.-J., Na, S.-Y., Park, J.-J. 2014. A combination of TiO₂/UV photocatalysis and high hydrostatic pressure to inactivate *Bacillus cereus* in freshly squeezed Angelica keiskei juice. *LWT – Food Science and Technology*, **55**, 104–109.
- Cheftel, J.C. (1995). Review: high-pressure, microbial inactivation and food preservation. *Food Science and Technology International*, **1**, 75–90.
- Chen, H.Q. and Hoover, D.G. 2003a. Modeling the combined effect of high hydrostatic pressure and mild heat on the inactivation kinetics of *Listeria monocytogenes* Scott A in whole milk. *Innovative Food Science and Emerging Technologies*, **4**, 25–34.
- Chen, H.Q. and Hoover, D.G. 2003b. Pressure inactivation kinetics of *Yersinia enterocolitica* ATCC 35669. *International Journal of Food Microbiology*, **87**, 161–171.
- Chen, H., Hoover, D.G., and Kingsley, D.H. 2004. Use of Weibull model to describe and predict pressure inactivation of *Listeria monocytogenes* Scott A in whole milk. *Innovative Food Science and Emerging Technologies*, **5**, 269–276.
- Chen, H., Hoover, D.G., and Kingsley, D.H. 2005. Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection*, **68**, 2389–2394.
- Connery, K.A., Shah, P., Coleman, L., and Hunek, B. 2005. Commercialization of Better Than Fresh™ dense phase carbon dioxide processing for liquid food. *International Symposium on Supercritical Fluids (ISSF)*, Orlando (US), 2005.
- Considine, K.M., Nelly, A.L., Fitzgerald, G.F., Hill, C., and Sleator, R.D. 2008. High-pressure processing effects on microbial food safety and food quality. *FEMS Microbiology Letters*, **281**, 1–9.
- Corwin, H. and Shellhammer, T.H. 2002. Combined carbon dioxide and high pressure 449 inactivation of pectin methylesterase, polyphenol oxidase, *Lactobacillus plantarum* and *Escherichia coli*. *Journal of Food Science*, **67**, 697–701.
- Cruz-Romero, M.C., Kelly, A.L., and Kerry, J.P. 2007. Effect of high-pressure and heat treatments on physical and biochemical characteristics of oysters (*Crassostrea gigas*). *Innovative Food Science and Emerging Technologies*, **8**, 30–38.
- Cruz-Romero, M., Kerry, J.P., and Kelly, A.L. 2008. Changes in the microbiological and physicochemical quality of high-pressure-treated oysters (*Crassostrea gigas*) during chilled storage. *Food Control*, **19**, 1139–1147.
- Cullen, P.J., Tiwari, B.K., and Valdramidis, V.P. 2012. Status and trends of novel thermal and non-thermal technologies for fluid foods, Chapter 1. In: *Novel Thermal and Non-Thermal Technologies for Fluid Foods*, pp. 1–6. Academic Press, Cambridge, MA, USA.
- Damar, S. and Balaban, M.O. 2006. Review of dense phase CO₂ technology: microbial and enzyme inactivation, and effects on food quality. *Journal of Food Science*, **71**, R1–R11.
- Daniels, J.A., Krishnamurthi, R., and Rizvi, S.S.H. 1985. A review of effects of carbon dioxide on microbial growth and food quality. *Journal of Food Protection*, **48**, 532–537.
- Datta, N., Deeth, H.C. 1999. High pressure processing of milk and dairy products. *Australian Journal of Dairy Technology*, **54**, 41–48.
- De Ancos, B., Gonzalez, E., and Cano, M.P. 2000. Effect of high-pressure treatment on the carotenoid composition and the radical scavenging activity of persimmon fruit purees. *Journal of Agricultural and Food Chemistry*, **48**, 3542–3548.
- Debs-Louka, E., Louka, N., Abraham, G., Chabot, V., and Allaf, K. 1999. Effect of compressed carbon dioxide on microbial cell viability. *Applied and Environmental Microbiology*, **65**, 626–631.

- Deliza, R., Rosenthal, A., Abadio, F.B.D., Silva, C.H.O., and Castillo, C. 2005. Application of high pressure technology in the fruit juice processing: benefits perceived by consumers. *Journal of Food Engineering*, **67**, 241–246.
- Del Pozo-Insfran, D., Balaban, M.O., and Talcote, S.T. 2006. Microbial stability, phytochemical retention, and organoleptic attributes of dense phase CO₂ processed muscadine grape juice. *Journal of Agricultural and Food Chemistry*, **54**, 5468–5473.
- Dillow, A.K., Dehghani, F., Hrkach, J.S., Foster, N.R., and Langer, R. 1999. Bacterial inactivation by using near- and supercritical carbon dioxide. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 10344.
- Dixon, N.M. and Kell, D.B. 1989. The inhibition of CO₂ of the growth and metabolism of microorganisms. *Journal of Applied Bacteriology*, **67**, 109–136.
- Dong, P., Kong, M., Yao, J., Zhang, Y., Liao, X., Hu, X., et al. 2013. The effect of high hydrostatic pressure on the microbiological quality and physicochemical properties of lotus root during refrigerated storage. *Innovative Food Science and Emerging Technologies*, **19**, 79–84.
- Drudy, D., Mullane, N.R., Quinn, T., Wall, P.G., and Fanning, S. 2006. *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. *Clinical Infectious Diseases*, **42**, 996–1002.
- Enomoto, A., Nakamura, K., Nagai, K., Hashimoto, T., and Hakoda, M. 1997. Inactivation of food microorganisms by high-pressure carbon dioxide treatment with or without explosive decompression. *Bioscience, Biotechnology and Biochemistry*, **61**, 1133–1137.
- Erkmen, O. 2000. Inactivation of *Salmonella typhimurium* by high pressure carbon dioxide. *Food Microbiology*, **17**, 225–232.
- Erkmen, O. 2009. Mathematical modeling of *Salmonella typhimurium* inactivation under high hydrostatic pressure at different temperatures. *Food and Bioprocesses Processing*, **87**, 68–73.
- Erkmen, O. and Karaman, H. 2001. Kinetic studies on the high pressure carbon dioxide inactivation of *Salmonella Typhimurium*. *Journal of Food Engineering*, **50**, 25–28.
- Espina, L., García-Gonzalo, D., Laglaoui, A., Mackey, B.M., Pagán, R. 2013. Synergistic combinations of high hydrostatic pressure and essential oils or their constituents and their use in preservation of fruit juices. *International Journal of Food Microbiology*, **161**, 23–30.
- Farkas, D.F. and Hoover, D.G. (2000). High pressure processing. *Journal of Food Science*, **65**, 47–64.
- Flick, G. Jr. 2003. High hydrostatic pressure processing has potential. *Global Aquaculture Advocate*, **6**.
- Food Safety and Inspection Service (FSIS). 2002. Safe and suitable ingredients used in the production of meat and poultry products. Directive 7120.1, Washington, DC.
- Fraser, D. 1951. Bursting bacteria by release of gas pressure. *Nature*, **167**, 33–34.
- Furukawa, S., Noma, S., Shimoda, M., and Hayakawa, I. 2002. Effect of initial concentration of bacterial suspensions on their inactivation by high hydrostatic pressure. *International Journal of Food Science and Technology*, **37**, 573–577.
- Gabriel, A.A. and Nakano, H. 2011. Effects of culture conditions on the subsequent heat inactivation of *E. coli* O157: H7 in apple juice. *Food Control*, **22**, 1456–1460.
- Gaoa, Y.-L., Ju, X.-R., and Jiang, H.-H. 2006a. Analysis of reduction of *Geobacillus stearothermophilus* spores treated with high hydrostatic pressure and mild heat in milk buffer. *Journal of Biotechnology*, **125**, 351–360.
- Gaoa, Y.-L., Ju, X.-R., and Jiang, H.-H. 2006b. Studies on inactivation of *Bacillus subtilis* spores by high hydrostatic pressure and heat using design of experiments. *Journal of Food Engineering*, **77**, 672–679.
- Garcia-Gonzalez, L., Geeraerd, A.H., Spilimbergo, S., Elst, K., Van Ginneken, L., Debevere, J., Van Impe, J.F., and Devlieghere, F. 2007. High pressure carbon dioxide inactivation of microorganisms in foods: past, present and future. *International Journal of Food Microbiology*, **117**, 1–28.
- Garcia-Graells, C., Hauben, K. J. A., and Michiels, J. A. 1998. High-pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices. *Applied and Environment Microbiology*, **64**, 1566–1568.

- Garcia-Parra, J., Gonzalez-Cebrino, F., Delgado, J., Lozano, M., Hernandez, T., and Ramirez, R. 2011. Effect of thermal and high-pressure processing on the nutritional value and quality attributes of a nectarine puree with industrial origin during the refrigerated storage. *Journal of Food Science*, **76**, C618–C625.
- Garriga, M., Aymerich, M.T., Costa, S., Monfort, J.M., and Hugas, M. 2002. Bactericidal synergism through bacteriocins and high pressure in a meat model system during storage. *Food Microbiology*, **19**, 509–518.
- Ginson, J., Kamalakanth, C., Bindu, J., Venkateswarlu, R., Das, S., Chauhan, O., et al. 2013. Changes in *K* value, microbiological and sensory acceptability of high pressure processed Indian white prawn (*Fenneropenaeus indicus*). *Food and Bioprocess Technology*, **6**, 1175–1180.
- Goh, E.L.C., Hocking, A.D., Stewart, C.M., Buckle, K.A., and Fleet, G.H. 2007. Baroprotective effect of increased solute concentrations on yeast and moulds during high pressure processing. *Innovative Food Science and Emerging Technologies*, **8**, 535–542.
- Gunes, G., Blum, L.K., and Hotchkiss, J.H. 2006. Inactivation of *Escherichia coli* (ATCC 4157) in diluted apple cider by dense-phase carbon dioxide. *Journal of Food Protection*, **69**, 12–16.
- Haas, G.J., Prescott, H.E., Dudley, E., Dik, R., Hintlian, C., and Keane, L. 1989. Inactivation of microorganisms by carbon dioxide under pressure. *Journal of Food Safety*, **9**, 253–265.
- Hata, C., Kumagai, H., and Nakamura, K. 1996. Rate analysis of the sterilization of microbial cells in high pressure carbon dioxide. *Food Science and Technology International*, **2**, 229–233.
- Heremans, K. 2001. The effects of pressure on biomaterials. In: *Ultra High Pressure Treatments of Foods* (eds Hendrickx, M.E.G. and Knorr, D.), pp. 23–51. Academic/Plenum Publishers, New York.
- Hogan, E., Kelly, A.L., and Sun, D.-W. 2005. High pressure processing of foods: an overview. In: *Emerging Technologies for Food Processing* (ed. Da Wen, S.), pp. 3–31. Academic Press.
- Hong, S.-I. and Pyun, Y.-R. 1999. Inactivation kinetics of *Lactobacillus plantarum* by high pressure carbon dioxide. *Journal of Food Science*, **64**, 728–733.
- Hong, S.-I., Park, W.-S., and Pyun, Y.-R. 1997. Non-thermal inactivation of *Lactobacillus plantarum* as influenced by pressure and temperature of pressurized carbon dioxide. *International Journal of Food Science and Technology*, **34**, 125–130.
- Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.E., and Knorr, D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology*, **43**, 99–107.
- Hsu, H.-Y., Sheen, S., Sites, J., Huang, L., and Wu, J.S.-W. 2014. Effect of high pressure treatment on the survival of Shiga toxin-producing *Escherichia coli* in strawberry puree. *Food Microbiology*, **40**, 25–30.
- Huang, W., Bi, X., Zhang, X., Liao, X., Hu, X., and Wu, J. 2013. Comparative study of enzymes, phenolics, carotenoids and color of apricot nectars treated by high hydrostatic pressure and high temperature short time. *Innovative Food Science and Emerging Technologies*, **18**, 74–82.
- Huang, Y., Mu, Y., and Chen, H. 2013. Inactivation of *Escherichia coli* O157:H7 and *Salmonella* spp. in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. *International Journal of Food Microbiology*, **160**, 337–343.
- Hugas, M., Garriga, M., and Monfort, J.M. 2002. New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, **62**(3), 359–371.
- Hunter, J.B. and Asenjo, J.A. 1988. A structured mechanistic model of the kinetics of enzymatic lysis and disruption of yeast cells. *Biotechnology and Bioengineering*, **31**, 929–943.
- Iizuka, T. and Shimizu, A. 2013. Removal of pesticide residue from cherry tomatoes by hydrostatic pressure. *Innovative Food Science and Emerging Technologies*, **26**, 34–39.
- Iizuka, T., Maeda, S., and Shimizu, A. 2013a. Removal of pesticide residue in cherry tomato by hydrostatic pressure. *Journal of Food Engineering*, **116**, 796–800.

- Iizuka, T., Yahata, M., and Shimizu, A. 2013b. Potential mechanism involved in removal of hydrophobic pesticides from vegetables by hydrostatic pressure. *Journal of Food Engineering*, **119**, 1–6.
- Ishikawa, H., Shimoda, M., Shiratsuchi, H., and Osajima, Y. 1995. Sterilization of microorganisms by the supercritical carbon dioxide micro-bubble method. *Bioscience, Biotechnology and Biochemistry*, **59**, 1949–1950.
- Iversen, C. and Forsythe, S.J. 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. *Trends in Food Science and Technology*, **14**, 443–454.
- Jofre, A., Aymerich, T., and Garriga, M. 2008. Assessment of the effectiveness of antimicrobial packaging combined with high pressure to control *Salmonella* sp. in cooked ham. *Food Control*, **19**, 634–638.
- Jordan, S.L., Pascual, C., Bracey, E., and Mackey, B.M. 2001. Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in fruit juices. *Journal of Applied Microbiology*, **91**, 463–469.
- Ju, X.-R., Gao, Y.-L., Yao, M.-L., and Qian, Y. 2008. Response of *Bacillus cereus* spores to high hydrostatic pressure and moderate heat. *LWT – Food Science and Technology*, **41**, 2104–2112.
- Kamihira, M., Taniguchi, M., and Kobayashi, T. 1987. Sterilization of microorganisms with supercritical carbon dioxide. *Agricultural and Biological Chemistry*, **51**, 407–412.
- Kim, K.-W., Kim, Y.-T., Kim, M., Noh, B.-S., and Choi, W.-S. 2014. Effect of high hydrostatic pressure (HHP) treatment on flavor, physico-chemical properties and biological functionalities of garlic. *LWT – Food Science and Technology*, **55**, 347–354.
- Kingsley, D.H., Hoover, D.G., Papafragkou, E., and Richards, G.P. 2002. Inactivation of hepatitis A virus and calicivirus by high hydrostatic pressure. *Journal of Food Protection*, **65**, 1605–1609.
- Kingsley, D.H., Chen, H., and Hoover, D.G. 2004. Inactivation of selected picornaviruses by high hydrostatic pressure. *Virus Research*, **102**, 221–224.
- Kingsley, D.H., Guan, D., and Hoover, D.G. 2005. Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. *Journal of Food Protection*, **68**, 1748–1751.
- Knorr, D. 1993. Effects of high-hydrostatic-pressure processes on food safety and quality. *Food Technology*, **47**, 156–163.
- Koseki, S., Matsubara, M., Yamamoto, K. 2009. Prediction of a required log reduction with probability for *Enterobacter sakazakii* during high-pressure processing, using a survival/death interface model. *Applied and Environmental Microbiology*, **75**, 1885–1891.
- Kovač, K., Bouwknecht, M., Diez-Valcarce, M., Raspor, P., Hernández, M., and Rodríguez-Lázaro, D. 2012. Evaluation of high hydrostatic pressure effect on human adenovirus using molecular methods and cell culture. *International Journal of Food Microbiology*, **157**, 368–374.
- Kumagai, H., Hata, C., and Nakamura, K. 1997. CO₂ sorption by microbial cells and sterilization by high-pressure CO₂. *Bioscience, Biotechnology and Biochemistry*, **61**, 931–935.
- Laboissière, L.H.E.S., Deliza, R., Barros-Marcellini, A.M., Rosenthal, A., Camargo, L.M.A.Q., and Junqueira, R.G. 2007. Effects of high hydrostatic pressure (HHP) on sensory characteristics of yellow passion fruit juice. *Innovative Food Science and Emerging Technologies*, **8**, 469–477.
- Lee, J. and Kaletunç, G. 2010. Inactivation of *Salmonella Enteritidis* strains by combination of high hydrostatic pressure and nisin. *International Journal of Food Microbiology*, **140**, 49–56.
- Lee, J.W., Cha, D.S., Hwang, K.T., and Park, H.J. 2003. Effects of CO₂ absorbent and high pressure treatment on the shelf-life of packaged Kimchi products, Korea. *International Journal of Food Science and Technology*, **38**, 519–524.
- Leonard, J.N., Ferstl, P., Delgado, A., and Schaffer, D.V. 2007. Enhanced preparation of adeno-associated viral vectors by using high hydrostatic pressure to selectively inactivate helper adenovirus. *Biotechnology and Bioengineering*, **97**, 1170–1179.
- Li, L., Feng, L., Yi, J., Hua, C., Chen, F., Liao, X., Wang, Z., and Hu, X. 2010. High hydrostatic pressure inactivation of total aerobic bacteria,

- lactic acid bacteria, yeasts in sour Chinese cabbage. *International Journal of Food Microbiology*, **142**, 180–184.
- Lin, H.-M., Chan, E.C., Chen, C., and Chen, L.F. 1991. Disintegration of yeast cells by pressurized carbon dioxide. *Biotechnology Progress*, **7**, 201–204.
- Lin, H.-M., Yang, Z.Y., and Chen, L.F. 1992. An improved method for disruption of microbial cells with pressurized carbon dioxide. *Biotechnology Progress*, **8**, 165–166.
- Lin, H.-M., Cao, N.J., and Chen, L.F. 1994. Antimicrobial effect of pressurized carbon dioxide on *Listeria monocytogenes*. *Journal of Food Science*, **59**, 657–659.
- Linton, M., McClements, J. M., and Patterson, M.F. 1999. Inactivation of *Escherichia coli* O157:H7 in orange juice using a combination of high pressure and mild heat. *Journal of Food Protection*, **62**, 277–279.
- Linton, M., McClements, J.M.J., and Patterson, M.F. 2001. Inactivation of pathogenic *Escherichia coli* in skimmed milk using high hydrostatic pressure. *Innovative Food Science and Emerging Technologies*, **2**, 99–104.
- Liu, G.-R., Wang, Y., Gui, M., Zheng, H., Dai, R., Li, P. 2012. Combined effect of high hydrostatic pressure and enterocin LM-2 on the refrigerated shelf life of ready-to-eat sliced vacuum-packed cooked ham. *Food Control*, **24**, 64–71.
- Liu, F., Wang, Y., Bi, X., Guo, X., Fu, S., and Liao, X. 2013. Comparison of microbial inactivation and rheological characteristics of mango pulp after high hydrostatic pressure treatment and high temperature short time treatment. *Food Bioprocess Technology*, **6**, 2675–2684.
- Liu, F.-X., Wang, Y.-T., Li, R., Bi, X.-F., and Liao, X.O. 2014. Effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and color of mango nectars. *Innovative Food Science and Emerging Technologies*, **21**, 35–43.
- López-Pedemonte, T.J., Roig-Sagués, A.X., Trujillo, A.J., Capellas, M., and Guamis, B. 2003. Inactivation of Spores of *Bacillus cereus* in Cheese by High Hydrostatic Pressure with the Addition of Nisin or Lysozyme. *Journal of Dairy Science*, **86**, 3075–3081.
- Lou, F., Neetoo, H., Chen, H., and Li, J. 2011. Inactivation of a human norovirus surrogate by high-pressure processing: effectiveness, mechanism, and potential application in the fresh produce industry. *Applied and Environmental Microbiology*, **77**, 1862–1871.
- Manas, P. and Mackey, B.M. 2004. Morphological and physiological changes induced by high hydrostatic pressure in exponential- and stationary-phase cells of *Escherichia coli*: relationship with cell death. *Applied and Environmental Microbiology*, **70**, 1545–1554.
- Masuda, M., Saiti, Y., Iwanami, T., and Hirai, Y. 1992. Effects of hydrostatic pressure on packaging materials for food. In: *High Pressure and Biotechnology* (eds Balny, C., Hayashi, R., Heremans, K., and Massons, P.), pp. 545–547. John Libbey Eurotext, London, UK.
- Mazzoni, A.M., Sharma, R.R., Demirci, A., and Ziegler, G.R. 2001. Supercritical carbon dioxide treatment to inactivate aerobic microorganisms on alfalfa seeds. *Journal of Food Safety*, **21**, 215–233.
- Moerman, F. 2005. High hydrostatic pressure inactivation of vegetative microorganisms, aerobic and anaerobic spores in pork Marengo, a low acidic particulate food product. *Meat Science*, **69**, 225–232.
- Morales, P., Calzada, J., Avila, M., and Nunez, M. 2008. Inactivation of *Escherichia coli* O157: H7 in ground beef by single-cycle and multiple-cycle high-pressure treatments. *Journal of Food Protection*, **71**, 811–815.
- Murchie, L.W., Cruz-Romero, M., Ferry, J.P., Linton, M., Patterson, M.F., Smiddy, M., and Kelly, A.L. 2005. High pressure processing of shellfish: a review of microbiological and other quality aspects. *Innovative Food Science and Emerging Technologies*, **6**, 257–270.
- Nakagami, T., Shigehisaa, T., Ohmori, T., Tajia, S., Haseb, A., Kimurab, T., and Yamanishi, K. 1992. Inactivation of herpes viruses by high hydrostatic pressure. *Journal of Virological Methods*, **38**, 255–262.
- Nakamura, K., Enomoto, A., Fukushima, H., Nagai, K., and Hakoda, M. 1994. Disruption of microbial cells by the flash discharge of high pressure

- carbon dioxide. *Bioscience, Biotechnology and Biochemistry*, **58**, 1297–1301.
- Neetoo, H., Ye, M., and Chen, H. 2008. Potential application of high hydrostatic pressure to eliminate *Escherichia coli* O157:H7 on alfalfa sprouted seeds. *International Journal of Food Microbiology*, **128**, 348–353.
- Norton, T. and Sun, D.-W. 2008. Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, **1**, 2–34.
- Oey, I., Lille, M., Van Loey, A., and Hendrickx, M. 2008a. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in Food Science and Technology*, **19**, 320–328.
- Oey, I., Van der Plancken, I., Van Loey, A., and Hendrickx, M. 2008b. Does high pressure processing influence nutritional aspects of plant based food systems? *Trends in Food Science and Technology*, **19**, 300–308.
- Overman, J.R. and Lewis, A.M., Jr. 1959. Effects of high pressures on influenza viruses. *Proceedings of Society of Experimental Biology and Medicine*, **102**, 329.
- Pagotto, F., Farber, J.M., Lenati, R. 2008. Pathogenicity of *Enterobacter sakazakii*. In: *Enterobacter sakazakii. Emerging Issues in Food Safety* (eds Farber, J.M. and Forsythe, S.J.), pp. 127–144. ASM Press, Washington, DC.
- Palhano, F.L., Vilcheb, T.T.B., Santos, R.B., Orlando, M.T.D., Ventura, J.A., and Fernandes, P.M.B. 2004. Inactivation of *Colletotrichum gloeosporioides* spores by high hydrostatic pressure combined with citral or lemongrass essential oil. *International Journal of Food Microbiology*, **95**, 61–66.
- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J., and Swanson, B.G. 1997. High hydrostatic pressure as a hurdle for *Zygosaccharomyces bailii* inactivation. *Journal of Food Science*, **62**, 855–857.
- Park, S.-J., Lee, J.-I., and Park, J. 2002. Effects of a combined process of high-pressure carbon dioxide and high hydrostatic pressure on the quality of carrot juice. *Journal of Food Science*, **67**, 1827–1834.
- Park, S.-J., Park, H.-W., and Park, J. 2003. Inactivation kinetics of food poisoning microorganisms by carbon dioxide and high hydrostatic pressure. *Journal of Food Science*, **68**, 976–981.
- Patras, A., Brunton, N.P., Da Pieve, S., and Butler, F. 2009. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purees. *Innovative Food Science and Emerging Technology*, **10**, 308–313.
- Patterson, M.F. 1999. High-pressure treatment of foods. In: *The Encyclopedia of Food Microbiology* (eds Robinson, R.K., Batt, C.A., and Patel, P.D.), pp. 1059–1065. Academic Press, New York.
- Patterson, M.F. and Kilpatrick, D.J. 1998. The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. *Journal of Food Protection*, **61**, 432–436.
- Patterson, M.F., Quinn, M., Simpson, R., and Gilmour, A. 1995a. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *Journal of Food Protection*, **61**, 432–436.
- Patterson, M.F., Quinn, M., Simpson, R., and Gilmour, A. 1995b. Effects of high pressure on vegetative pathogens. In: *High Pressure Processing of Foods* (eds Ledward, D.A., Johnston, D.E., and Earnshaw, R.G.), pp. 47–63. Nottingham University Press, Nottingham.
- Patterson, M.F., Linton, M., and Doona, C.J. 2007. Introduction to high pressure processing of foods. In *High Pressure Processing of Foods* (eds Donna, C.J. and Feeherry, F.E.), pp. 1–14. Blackwell Publishing Professional, Iowa, USA.
- Peñas, E., Frias, J., Gomez, R., Vidal-Valverde, C. 2010. High hydrostatic pressure can improve the microbial quality of sauerkraut during storage, Spain. *Food Control*, **21**, 524–528.
- Ponce, E., Pla, R., Capellas, M., Guamis, B., and Mor-Mur, M. 1998. Inactivation of *Escherichia coli* inoculated in liquid whole egg by high hydrostatic pressure. *Food Microbiology*, **15**, 265–272.

- Ponce, E., Pla, R., Sendra, E., Guamis, B., and Mor-Mur, M. 1999. Destruction of *Salmonella enteritidis* inoculated in liquid whole egg by high hydrostatic pressure: comparative study in selective and non-selective media. *Food Microbiology*, **16**, 357–365.
- Ramirez, R., Saraiva, J.A., Perez Lamela, C., and Torres, J.A. 2009. Reaction kinetics analysis of chemical changes in pressure-assisted thermal processing. *Food Engineering Reviews*, **1**, 16–30.
- Rastogi, N.K., Raghavarao, K.S.M.S., Balasubramaniam, V.M., Niranjan, K., and Knorr, D. 2007. Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition*, **47**, 69–112.
- Rendueles, E., Omer, M.K., Alyseike, O., Alonso-Calleja, C., Capita, R., and Prieto, M. 2011. Microbiological food safety assessment of high hydrostatic pressure processing: a review. *LWT – Food Science and Technology*, **44**, 1251–1260.
- Ritz, M., Freulet, M., Orange, N., and Federighi, M. 2000. Effects of high hydrostatic pressure on membrane proteins of *Salmonella typhimurium*. *International Journal of Food Microbiology*, **55**, 115–119.
- Ritz, M., Tholozan, J.L., Federighi, M., and Pilet, M.F. 2001. Morphological and physiological characterization of *Listeria monocytogenes* subjected to high hydrostatic pressure. *Applied and Environmental Microbiology*, **67**, 2240–2247.
- Rivalain, N., Roquain, J., and Demazeau, G. 2010. Development of high hydrostatic pressure in biosciences: Pressure effect on biological structures and potential applications in Biotechnologies. *Biotechnology Advances*, **28**, 659–672.
- Roberts, C.M. and Hoover, D.G. 1996. Sensitivity of *Bacillus coagulans* spores to combinations of high hydrostatic pressure, heat, acidity and nisin. *Journal of Applied Bacteriology*, **81**, 363–368.
- Ross, A.I.V., Griffiths, M.W., Mittal, G.S., and Deeth, H.C. 2003. Combining nonthermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology*, **89**, 125–138.
- Sale, A.J.H., Gould, G.W., and Hamilton, W.A. 1970. Inactivation of bacterial spores by hydrostatic pressure. *Journal of General Microbiology*, **60**, 323–334.
- Scallan, E., Griffin, P.M., Angulo, F.J., Tauxe, R.V., and Hoekstra, R.M. 2011. Foodborne illness acquired in the United States from unspecified agents. *Emerging Infectious Disease*, **17**, 16–22.
- Sims, M. 1999. Method and membrane system for sterilizing and preserving liquids using carbon dioxide. US Patent 6,331,272 B1 (December 18, 2001) and WO Patent 04/41805 (July 20, 2000).
- Sims, M. and Estigarribia, E. 2003. Membrane carbon dioxide sterilization of liquid foods: scale up of a commercial continuous process. *Proceedings of the 6th International Symposium on Supercritical Fluids*, 28–30 April, Versailles, France, pp. 1457–1460.
- Sirisee, U., Heish, F., and Huff, H.E. 1998. Microbial safety of supercritical carbon dioxide processes. *Journal of Food Processing and Preservation*, **22**, 387–403.
- Smelt, J.P.P.M. 1998. Recent advances in the microbiology of high pressure processing. *Trends in Food Science and Technology*, **9**, 152–158.
- Smelt, J. and Rijke, G. 1992. High pressure treatment as a tool for pasteurization of foods. In: *High Pressure and Biotechnology* (eds Balny, C., Hayashi, R., Heremans, K., and Masson, P.), pp. 361–363. Colloque Inserm/John Libbey Eurotex Ltd.
- Sohn, K.-H. and Lee, H.-J. 1999. Effects of high pressure treatment on the quality and storage of kimchi, Japan. *International Journal of Food Science and Technology*, **33**, 359–365.
- Spilimbergo, S. and Bertucco, A. 2003. Non-thermal bacteria inactivation with dense CO₂. *Biotechnology and Bioengineering*, **84**, 627–638.
- Spilimbergo, S., Elvassore, N., and Bertucco, A. 2002. Microbial inactivation by high pressure. *Journal of Supercritical Fluids*, **22**, 55–63.
- Stewart, C.M., Dunne, C.P., Sikes, A., and Hoover, D.G. 2000. Sensitivity of spores of *Bacillus subtilis*

- and *Clostridium sporogenes* PA 3679 to combinations of high hydrostatic pressure and other processing parameters. *Innovative Food Science and Emerging Technologies*, **1**, 49–56.
- Tao, Y., Sun, D.-W., Górecki, A., Błaszczak, W., Lamparski, G., Amarowicz, R., Fornal, J., and Jeliński, T. 2012. Effects of high hydrostatic pressure processing on the physicochemical and sensorial properties of a red wine. *Innovative Food Science and Emerging Technologies*, **16**, 409–416.
- Tassou, C.C., Panagou, E.Z., Samaras, F.J., Galiatsatou, P., and Mallidis, C.G. 2008. Temperature-assisted high hydrostatic pressure inactivation of *Staphylococcus aureus* in a ham model system: evaluation in selective and nonselective medium. *Journal of Applied Microbiology*, **104**, 1764–1773.
- Tomasula, P.M. 2003. Supercritical fluid extraction of foods. In: *Encyclopedia of Agricultural, Food and Biological Engineering* (ed. Heldman, D.). Marcel Dekker, Inc., New York, USA.
- Torres, J.A., and Velazquez, G. 2005. Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering*, **67**, 95–112.
- Trujillo, A.J. 2002. Applications of high-hydrostatic pressure on milk and dairy products. *High Pressure Research*, **22**, 619–626.
- Valdramidis, V.P., Graham, W.D., Beattie, A., Linton, M., McKay, A., Fearon, A.M., *et al.* 2009. Defining the stability interfaces of apple juice: implications on the optimization and design of high hydrostatic pressure treatment. *Innovative Food Science and Emerging Technologies*, **10**, 396–404.
- Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V., and Morales-Castro, J. 2012. Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science and Emerging Technologies*, **13**, 13–22.
- Wang, Y., Liu, F., Cao, X., Chen, F., Hu, X., and Liao, X. 2012. Comparison of high hydrostatic pressure and high temperature short time processing on quality of purple sweet potato nectar. *Innovative Food Science and Emerging Technologies*, **16**, 326–334.
- Wei, C.I., Balaban, M.O., Fernando, S.Y., and Peplow, A.J. 1991. Bacterial effect of high pressure CO₂ treatment on foods spiked with *Listeria* and *Salmonella*. *Journal of Food Protection*, **54**, 189–193.
- Welti-Chanes, J., Ochoa-Velasco, C.E., and Guerrero-Beltrán, J.A. 2009. High-pressure homogenization of orange juice to inactivate pectinmethylesterase. *Innovative Food Science and Emerging Technologies*, **10**, 457–462.
- Werner, B.G. and Hotchkiss, J.H. 2006. Continuous flow nonthermal CO₂ processing: the lethal effects of subcritical and supercritical CO₂ on total microbial populations and bacterial spores in raw milk. *Journal of Dairy Science*, **89**, 872–881.
- Wilkinson, N., Kurdziel, A.S., Langton, S., Needs, E., and Cook, N. 2001. Resistance of poliovirus to inactivation by high hydrostatic pressures. *Innovative Food Science and Emerging Technologies*, **2**, 95–98.
- Wouters, P.C., Glaasker, E., and Smelt, J.P. 1998. Effects of high pressure on inactivation kinetics and events related to proton efflux in *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, **64**, 509–514.
- Yang, B.-W., Shi, Y., Xia, X.-D., Xi, M., Wang, X., Ji, B., and Meng, J.-H. 2012. Inactivation of food-borne pathogens in raw milk using high hydrostatic pressure. *Food Control*, **28**, 273–278.
- Ye, M., Huang, Y., and Chen, H. 2012. Inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters by high-hydrostatic pressure and mild heat. *Food Microbiology*, **32**, 179–184.
- Yi, J., Xu, Q., Hu, X., Dong, P., Liao, X., and Zhang, Y. (2013). Shucking of bay scallop (*Argopecten irradians*) using high hydrostatic pressure and its effect on microbiological and physical quality of adductor muscle. *Innovative Food Science and Emerging Technologies*, **18**, 57–64.
- Yuste, J., Mor-Mur, M., Capellas, M., and Pla, R. 1999. *Listeria innocua* and aerobic mesophiles during chill storage of inoculated mechanically recovered poultry meat treated with high hydrostatic pressure. *Meat Science*, **53**, 251–257.

- Zhang, J., Liu, G., Li, P., and Qu, Y. 2010. Pentocin 31-1, a novel meat-borne bacteriocin and its application as biopreservative in chill-stored tray-packaged pork meat. *Food Control*, **21**, 198–202.
- Zhou, C.-L., Liu, W., Zhao, J., Yuan, C., Song, Y., Chen, D., Ni, Y.-Y., and Li, Q.-H. 2014. The effect of high hydrostatic pressure on the microbiological quality and physical–chemical characteristics of pumpkin (*Cucurbita maxima* Duch.) during refrigerated storage. *Innovative Food Science and Emerging Technologies*, **21**, 24–34.
- Zook, C.D., Parish, M.E., Braddock, R.J., Balaban, M.O. 1999. High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juices. *Journal of Food Science*, **64**, 533–535.

CHAPTER 10

Impact of Vacuum packaging, modified and controlled atmosphere on the microbial ecology of foods

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10.1 Introduction

Food products have different deterioration rates depending on several aspects, such as composition, formulation, packaging, and storage conditions, and for this reason the most effective preservation techniques are often a mixture of different strategies (Del Nobile and Conte, 2013). Food problems can vary from the most harmless, such as the loss of color or flavor, to much more dangerous issues, such as microbial deterioration. It is for this reason that most preservation techniques focus on the reduction of microbial growth or prevention of contamination. So far, the use of vacuum packaging (VP) or the modification of the atmosphere around the product (MAP or CA) represent the most common methods used to control food quality (Devlieghere *et al.*, 2004). The VP process creates a vacuum and seals hermetically and the material used does not allow the access of gases and moisture into the package (Brody, 1989). MAP may be defined as the enclosure of food products in gas-barrier materials, in which the gaseous environment has been changed (Church and Parsons, 1995; Phillips, 1996). There are mainly two ways to modify the atmosphere, passively through the food product or deliberately (Kader *et al.*, 1989). The passive MAP may

develop within an hermetically sealed package due to respiration of the product, and the change in the atmosphere can be perceived, for example, through oxygen consumption or carbon dioxide creation. A good matching between film permeability and product respiration can create a passive beneficial modified atmosphere within the package. The active MAP consists in pulling a small vacuum in the package, thus replacing the atmosphere with a proper gas mixture. This process is faster compared to the passive modified atmosphere. One of the most important aspects to be considered in an MAP application is the optimal definition of the gas atmosphere and the specific packaging design for each food product, because potential health risks due to the vulnerability of MAP products can occur (Charles *et al.*, 2003; Kader and Watkins, 2000). Under controlled atmospheric conditions (CA), the atmosphere is modified from that of the ambient atmosphere, and these conditions are maintained throughout storage. MAP uses the same principles as CA, but it is used on smaller quantities of produce and the atmosphere is only initially modified (Kader, 1986; Saltveit, 2003). Advantages and disadvantages, as well as the main capacity of each technique to preserve food products are all detailed in the following, with a particular emphasis on MAP.

10.2 Vacuum packaging

A vacuum is simply the absence of air. It can be created in a plastic bag or in a container by sucking out all of the air and then sealing it to prevent air from leaking back in. The single most important reason for creating a vacuum is to remove oxygen from around the thing that it has to be stored in. Sealing foods under vacuum gives the following benefits: elimination of oxygen and oxidation prevention, flavor preservation, freezer burn prevention, natural moisture control, and mold inhibition. Once a food is in contact with the air it begins to lose its flavor and its appearance changes, for example, a banana and apple quickly show signs of oxidation by browning. This reaction affects all fresh foods to some extent, even if the effects are not immediately obvious. VP eliminates contact with the air and therefore eliminates oxidation. With increasing emphasis on the use of natural oils in modern diets more consumers are trying to eat fish, which is rich in eicosapentaenoic oil and helps prevent cardiovascular disease. Fish oils deteriorate and become rancid faster than the fat found in meat such as beef, chicken, and pork. Fish well-wrapped in packages can be stored in a fridge for 2 days and 2 months if frozen. By vacuum packing such foods all their natural goodness can be preserved for 4–6 days in a fridge or frozen for 2 years. Furthermore, whilst freezing foods offers greater flexibility it is often negative in terms of texture and final taste. This is due to oxidation, dehydration, and slow bacterial growth, which causes freezer burn – all need oxygen to occur. Another reason to package or wrap food is to keep it from drying out because air causes moisture to evaporate. Therefore, by removing air with VP the food's natural moisture is maintained along with all its flavor and texture attributes. It will thus not be necessary to throw away the top slices of a loaf to find the soft one or have sliced meat curling up at the edges – everything will retain its own natural moisture. Keeping cereals, muesli, and crackers crisp and fresh is another problem. This is because dehydrated

foods, unless they are kept in an airtight container, quickly spoil as moisture in the air softens them. The use of VP on these types of foods is far superior in the control of their natural texture and flavor attributes as it not only removes the air but also the moisture is taken out. In the seafood industry, VP is often combined with refrigeration and other non-thermal processing techniques to commercialize minimally processed products that provide consumers with products possessing better nutritional and sensory properties. Moreover, VP is also often combined with pre-cooking treatments to increase the number of ready-to-eat seafood products. Atrea *et al.* (2009) evaluated the effect of VP individually or in combination with the addition of oregano oil on the shelf life extension of fresh Mediterranean octopus (*Octopus vulgaris*) stored under refrigeration by evaluating microbiological, chemical, and sensory parameters. They found that the shelflife was 6 days (under aerobic storage), 9 days (under vacuum), 17 and 23 days for products treated with oregano oil (0.2 and 0.4%, v/w) under VP. Ntzimani *et al.* (2010) evaluated the effect of VP in combination with EDTA and lysozyme on the shelf life of semi-cooked coated chicken fillets stored at 4°C. Based on both microbiological and sensory analyses, treatments with VP produced a shelf life extension of 7–8 days, as compared to the control samples. More recently, Pérez-Gregorio *et al.* (2011) studied the impact of minimal processing preservation technologies on the flavonol and anthocyanin content in red onions. The technologies studied were cutting, washing with sodium hypochlorite solution, VP, and refrigerated storage for 16 days, in the absence and presence of light. It was found that vacuum packaging of sliced onions maintained the initial level of total flavonols in the samples, improving also the sensorial and microbiological characteristics. Moderate vacuum packaging is designed by Burg (1975) and consists in the storage, under an atmospheric pressure in the range of 10 to 100 mbar, of products at refrigerated temperatures. At this low pressure, a constant circulation of fresh air, substantially

saturated with water (RH of 80 to 100%) is maintained and additional facilities to constantly scrub carbon dioxide and ethylene may be included. In the moderate VP system, storage pressure is set at 400 mbar and is reduced by simply evacuating the air from the storage facility. This reduces the amount of oxygen in the storage atmosphere, slowing down the respiration rate of the stored product and inhibiting the growth of microorganisms. Compared to MAP systems that use sophisticated equipment, a moderate VP system is technically simple and when used in a rigid-container version, it may be recycled.

10.3 Controlled atmosphere

There are a number of commercially available systems for creating CA conditions, for example, the use of an external gas generator that operates on the open-flame or catalytic burner principles, removing oxygen from the incoming air. This system operation procedure results in a very flexible and fast removal of oxygen. Another system used for CA consists in the use of liquid nitrogen atmospheric generators, which ensures the maintainance of a controlled atmosphere by flushing with sprayed liquid nitrogen placed in front of the evaporator blowers. Excess carbon dioxide is absorbed by lime bags and a sensor detects rising oxygen levels and corrects them by spraying more liquid nitrogen. Furthermore, gas separator systems are commonly used and are of two different types. The first is a pressure-swing adsorption system that absorbs oxygen by means of a membrane that contains a filtering system. Nitrogen-rich gas is exported and the bound oxygen is flushed by depressurization of the vessel. Indeed, the hollow fiber membrane system operates by heating the compressed air that is forced through hollow fibers made of semi-permeable membranes. In this way carbon dioxide and oxygen are selectively removed by the membrane and the nitrogen continues into the storage space. Another CA system is that of

hypobaric storage, which is a form of low-pressure storage mediated by a vacuum pump that evacuates the container until the desired pressure is reached. All gas levels are reduced and ethylene diffusion from the product is enhanced. Moisture loss is also reduced. This system is recommended for the curing of onions.

Other CA systems use different scrubbing reagents, such as caustic soda, water, hydrated lime, activated charcoal, and molecular sieves in order to remove carbon dioxide and for this reason they are called carbon dioxide control systems. A further CA system is the ethylene controller, which removes ethylene by means of a scrubber-heated catalyst system where ethylene is oxidized to yield carbon dioxide and water vapor. These two gases are then removed from the room, or by means of an absorbent bead scrubber where ethylene is bound to aluminum silicate spheres mixed with potassium permanganate. In the latter, as bead saturation occurs, they turn from purple to brown. Commercial use of CA storage is greatest for apples and pears worldwide; it is used less for cabbages, sweet onions, kiwifruits, avocados, persimmons, pomegranates, and nuts and dried fruits and vegetables. CA can have a direct or indirect effect on post-harvest pathogens (bacteria and fungi) and consequently decay incidence and severity. For example, carbon dioxide at 10 to 15% significantly inhibits development of botrytis rot on strawberries, cherries, and other perishables. Low oxygen (<1%) and elevated carbon dioxide (40 to 60%) can be a useful tool for insect control in some fresh and dried fruits, flowers, vegetables, and dried nuts and grains. The disadvantages are, for example, the irregular ripening of fruits, such as banana, mango, pear, and tomato, which can result from exposure to oxygen levels below 2% and/or carbon dioxide levels above 5% for more than 1 month; the development of off-flavors and off-odors at very low oxygen concentrations (as a result of anaerobic respiration) and very high carbon dioxide levels (as a result of fermentative metabolism); and the increased

susceptibility to decay when the fruit is physiologically injured by too-low oxygen or too-high carbon dioxide concentrations (Wang, 1990). Generally CA is more costly than MAP due to the need for constant maintenance of gas levels for large quantities of product and for this reason is less used than MAP application.

10.4 Modified atmosphere packaging

MAP has been used since 1927 to extend the shelf life of apples stored in a reduced oxygen atmosphere with a higher carbon dioxide concentration. A few years later, it was used during transportation of fruit in cargo ships and at the same time it was found that the increased concentration of carbon dioxide improved the shelf life of beef carcasses, which has almost doubled during long-distance transports (Davies, 1995). Despite this, MAP has only been applied in Europe for commercial use in retail packs starting from the 1970s, with Marks and Spencer only introducing MAP meat in the UK in 1979. The great success of this product and the increasing consumer demand for food with a longer shelf life helped to develop further MAP applications. It is important to underline that MAP is not designed to improve food quality but just to keep it unchanged or close to its initial quality level by slowing down or inhibiting the product's deterioration (Farber, 1991). To be improved by the use of MAP, a product should be of prime quality, it should be handled in an appropriate manner in order to minimize contamination and deterioration risks, and it should be kept at an appropriate storage temperature (Sivertsvik *et al.*, 2002). The advantages of MAP are: the increment of shelf life; the reduction in retail waste; the clear presentation of product and all round visibility; the easy separation of sliced products; the use of little or no chemical preservatives; the reduction of transport costs due to less frequent deliveries; and the reduced costs of production due to better utilization of space and equipment. Conversely,

the disadvantages are mainly related to the increase in the cost of the packaging (machinery, materials, analytical equipment), and also to the major pack volume that limits the transport of high product quantities. There are also some aspects that make the use of MAP inconvenient, such as, for example, for products in which yeasts are the major cause of spoilage, it is known that yeast produces carbon dioxide and high levels of carbon dioxide mainly stimulates their growth. Other food-associated pathogens such as *Clostridium perfringens* and *Clostridium botulinum* are influenced by the presence of carbon dioxide and grow easily in anaerobic conditions.

In order to have an optimized gas packaging balance, the choice of the appropriate equipment is a very important aspect. Two techniques can be used to replace air: the first is gas flushing while the second is a compensated vacuum. The first technique is realized through a form-fill seal machine that replaces, with a continuous gas stream, the air inside the package. Once the air surrounding the product is diluted the package is well sealed. An important consideration must be done due to the fact that there is a limit efficiency of this technique that reduces the oxygen level in a gas flushed pack to 2–5% oxygen; moreover, if the food product is particularly oxygen-sensitive this is not suitable. For this reason it is always very important to consider the oxygen sensitivity of a product before applying such a packaging system. An advantage of this method is the machine's speed, thanks to its continuous action and a packaging production rate that is extremely high.

The compensated vacuum technique consists in the removal of air pulling a vacuum in the atmosphere of the package and breaking it with a gas mixture. This technique is carried out through a two-step process and for this reason is slower than the flush gas method. However, there is a higher efficiency with respect to the residual oxygen level, which is much lower. Therefore, compensated vacuum machines should be used with food products that are highly sensitive to oxygen.

Carbon dioxide, nitrogen, and oxygen are some of the most used gases for MAP, even though research is being done to examine other gases such as argon (E938), helium (E939), nitrous oxide (E942), and sulfur dioxide (E220) (Enfors *et al.*, 1979; Jamie and Saltveit, 2002; Nasar-Abbas *et al.*, 2008; Rocculi *et al.*, 2005). Meng *et al.* (2012) investigated the effect of pressurized argon treatments on fresh-cut green peppers placed in polystyrene packages with a gas combination of 5 and 8% oxygen and carbon dioxide, respectively, and stored at 4°C and 90% humidity for 12 days. Various studies have also been carried out on carbon monoxide, hydrogen, and chlorine, even though at the moment their commercial application is restricted (Krause *et al.*, 2003; Gee and Brown, 1981). Several companies have also requested the FDA to prohibit the use of CO in the packaging of fresh meats because the use of carbon monoxide maintains the fresh looking color even after the product has reached its microbial contamination limit. In fact, by using carbon monoxide in MAP systems it is possible to maintain a stable red color of meat thanks to its ability to reduce myoglobin and the concomitant formation of carboxymyoglobin, which is an oxidant-stable pigment (Narasimha and Sachindra, 2002).

Nitrogen is inert and tasteless and on its own displays little or no antimicrobial activity, but thanks to its low solubility in water and fat, it can prevent package collapse. Sometimes its utilization in MAP is strictly necessary; for example, in the dairy sector, for creams, butter, and yogurts, an atmosphere consisting only of nitrogen is recommended because the absorption of carbon dioxide could affect the flavor properties (Gill, 1988).

An oxygen presence in the package is known to help aerobic microbial spoilage, even at low storage temperatures. Oxygen is a colorless, odorless gas that has a relatively low solubility in water, supports combustion (is explosive), and is very reactive with a wide variety of biological compounds (Mullan and McDowell, 2003). It is involved in many deteriorative food reactions

such as browning, rancidity development, fat oxidation, and pigment oxidation. For this reason, oxygen is generally excluded in MAP or is maintained at extremely low levels. However, with some types of food such as fruit, vegetables, red meat and fish, and some dairy products, oxygen is necessary either for respiration, color retention, avoidance of anaerobic conditions, or viability of mold in blue cheese. Specifically, oxygen in seafood products is necessary to inhibit the growth of *C. botulinum*, which is known to be an extremely toxic anaerobic pathogen. In meat it allows myoglobin to be maintained in its oxygenated form (oxymyoglobin), giving meat its bright red color, commonly linked to meat freshness (Kropft, 2004).

Carbon dioxide is water and lipid soluble and even if it is not specifically a bactericide or fungicide it has both of these properties; indeed its overall effect on microorganisms is to prolong the lag phase, followed by a reduction in the growth rate (Jakobsen and Nertelsen, 2002). Because of carbon dioxide absorption, its antimicrobial function is related to its concentration in food and not in the package headspace (Dixon and Kell, 1989; Gill and Tan, 1980). This means that only if the quantity of carbon dioxide is larger than the saturation limit of the product will the preservative effect be guaranteed. The other two factors that affect the absorption of carbon dioxide into the food product are the pH of the food matrix and the carbon dioxide partial pressure. Gill (1988) stated that carbon dioxide solubility into a muscle tissue with a pH of 5.5 and a storage temperature of 0°C was approximately 960 ml/kg of tissue, with the solubility increasing by 360 ml/kg for each pH unit increment. The solubility decreased by 19 ml/kg for each 1°C temperature increment. The same author also concluded that the solubility of carbon dioxide in fat tissues initially increased as the temperature was raised above 0°C, but then declined at higher temperatures. To sum up, a maximum storage life could be reached storing meat products at the lowest temperature without freezing the product. An increase in storage temperature of 1°C affects storage life,

reducing it by about 10%. For fresh cheeses it is important to choose the optimal carbon dioxide concentration to inhibit Gram-negative bacteria, with concentration not exceeding 20–30% in order to prevent sour flavors, consistency alteration, and package explosion. Many theories have been developed to describe its influence on bacterial cells. The main ones are related to: (i) the alteration of cell membrane function including effects on nutrient uptake and absorption; (ii) direct inhibition of enzymes or a decrease in the rate of enzyme reactions; (iii) penetration of bacterial membranes, leading to intracellular pH changes; and (iv) direct changing of the physiochemical properties of proteins. Many factors influence the effect of carbon dioxide, such as its concentration, the pressure, the type of microorganisms, the load of bacterial population and their growth phase, the temperature at which products are stored, the acidity, and the water activity of the product. Carbon dioxide is particularly effective when the spoilage organisms are aerobic, Gram-negative, and psychotropic bacteria.

Among the various food categories, the application of MAP to fresh-cut produce is of particular interest for the food industry, due to the fact that MAP is able to retard the respiration rate of the produce and delay enzymatic degradation of complex substrates (Conte *et al.*, 2012; Del Nobile *et al.*, 2012). When a fresh vegetable product is packaged it is important to adapt the gas film permeability to the product respiration rate. In this way an immediate equilibrium modified atmosphere will be established in the package (passive MAP) and the shelf life of the product can be increased. A product's respiration rate and film permeability depend on extrinsic factors such as temperature, so for this reason MAP is applied to maintain a desirable atmosphere within a specific temperature range. A slight change in temperature, even just a few degrees, will also change the package atmosphere, which may become inappropriate or even injurious to the product. Respiration is a metabolic activity that provides the energy needed for other plant biochemical

reactions; in fact aerobic respiration determines the oxidative breakdown of carbohydrates, lipids, and organic acids into simpler molecules, including carbon dioxide and water, with the release of energy (Fonseca *et al.*, 2002). In order to reduce the respiration rate it is necessary to decrease oxygen concentration surrounding the product, in order to decrease the activity of oxidizing enzymes such as polyphenoloxidase, glycolic acid oxidase, and ascorbic acid oxidase (Kader, 1986). Decreasing the respiration rate by means of MAP and lowering the storage temperature helps to prevent enzymatic degradation of complex substrates and reduces sensitivity to ethylene synthesis, significantly extending the shelf life. An oxygen level below 1% may lead to tissue deterioration as well as the production of off-flavors (Ares *et al.*, 2007). Concerning the use of carbon dioxide in packaging systems, different theories have been proposed. As carbon dioxide is a product of the respiration process it may cause feedback inhibition or it might affect the Krebs cycle's enzymes and intermediates at high concentrations, and again carbon dioxide might inhibit ethylene production instead of having a direct influence on the respiration process (Fonseca *et al.*, 2002). There is abundant literature dealing with the use of MAP on fresh-cut products that confirms the view that the individualization of optimal headspace conditions requires specific

Table 10.1 Recommended gas mixtures for retail MAP of main food commodities.

Food product	CO ₂ (%)	O ₂ (%)	N ₂ (%)
Raw red meat	30	70	
Raw fish and seafood products	40	30	30
Cooked and processed meat	30	–	70
Cooked and processed fish	30	–	70
Ready meals	30	–	70
Fresh pasta	50	–	50
Hard cheese	100	–	–
Soft cheese	30	–	70
Other dairy products	–	–	100
Cooked and stuffed vegetable products	30	–	70

knowledge of fresh-cut product characteristics, thus not making it possible to generalize on the nature of the gas mixture (Allende *et al.*, 2004; Caleb *et al.*, 2012; Conte *et al.*, 2011; Costa *et al.*, 2013; Liu and Li 2006; Lucera *et al.*, 2012; Mastromatteo *et al.*, 2011; Parentelli *et al.*, 2007; Plestenjak *et al.*, 2008; Soliva-Fortuny *et al.*, 2005). A summary of generally recommended gas mixtures that maintain the sensory quality and prolong the shelf life of major food products is reported in Table 10.1.

References

- Allende, A., Luo, Y., McEvoy, J.L., Artes, F., and Wang, C.Y. 2004. Microbial and quality changes in minimally processed baby spinach leaves stored under super atmospheric oxygen and modified atmosphere conditions. *Postharvest Biology and Technology*, **33**, 51–59.
- Ares, G., Lareo, C., and Lema, P. 2007. Modified atmosphere packaging for postharvest storage of mushrooms. A review. *Fresh Produce*, **1**, 32–40.
- Atrea, I., Papavergou, A., Amvrosiadis, I., and Savvaidis, I.N. 2009. Combined effect of vacuum packaging and oregano essential oil on the shelf-life of Mediterranean octopus (*Octopus vulgaris*) from the Aegean Sea stored at 4°C. *Food Microbiology*, **26**, 166–172.
- Brody, A.L. 1989. Controlled/modified atmosphere/vacuum packaging of meat. In: *Controlled/Modified Atmosphere/Vacuum Packaging of Foods* (ed. Brody, A.L.), pp. 17–38. Nutrition Press, Trumbull, CT, USA.
- Burg, S.P. 1975. Hypobaric storage and transportation of fresh fruits and vegetables. In: *Postharvest Biology and Handling of Fruits and Vegetables* (eds Haard, N.F. and Salunkhe, D.K.), pp. 172–188. AVI, Westport, CT, USA.
- Caleb, O.J., Opara, U.L., and Witthuhn, C.R. 2012. Modified atmosphere packaging of pomegranate fruit and arils: a review. *Food and Bioprocess Technology*, **5**, 15–30.
- Charles, F., Sanchez, J., and Gontard, N. 2003. Active modified atmosphere packaging of fresh fruits and vegetables: modeling with tomatoes and oxygen absorber. *Journal of Food Science*, **68**, 1736–1742.
- Church, I.J. and Parsons, A.L. 1995. Modified atmosphere packaging technology: a review. *Journal of the Science of Food and Agriculture*, **67**, 143–152.
- Conte, A., Lucera, A., Costa, C., Mastromatteo, M., and Del Nobile, M.A. 2011. Combined strategies for ready-to-cook lampascioni (*Muscari comosum* L.). *Food Processing and Preservation*, **35**, 861–868.
- Conte, A., Mastromatteo, M., & Del Nobile, M.A. 2012. Packaging optimization for minimally processed fruit and vegetables. In: *Food Packaging: Procedures, Management and Trends*, Edited by Nova Science Publishers, Inc., pp. 37–62.
- Costa, C., Lucera, A., Conte, A., and Del Nobile, M.A. 2013. Antimicrobial treatments to preserve packaged ready-to-eat table grapes. *Food Technology and Biotechnology*, **51**, 301–307.
- Davies, A.R. 1995. *Advances in Modified-Atmosphere Packaging, New Methods of Food Preservation* (ed. Gould, G.W.), pp. 304–320. Blackie, Glasgow, UK.
- Del Nobile, M.A. and Conte, A. 2013. *Packaging for Food Preservation*, Food Engineering Series. Springer Science + Business Media, New York.
- Del Nobile, M.A., Conte, A., Mastromatteo, M., and Mastromatteo, M. 2012. Modified atmosphere packaging. In: *Decontamination of Fresh and Minimally Processed Produce* (ed. Gomez-Lopez, V.M.), pp. 453–467. Wiley-Blackwell. ISBN 978-0-8138-2384-3.
- Devlieghere, F., Vermeiren, L., and Debevere, J. 2004. New preservation technologies: possibilities and limitation. *International Dairy Journal*, **14**, 273–285.
- Dixon, N. and Kell, D. 1989. The inhibition by CO₂ of the growth and metabolism of microorganisms. *Journal of Applied Bacteriology*, **67**, 109–136.
- Enfors, S., Molin, G., and Ternstrom, A. 1979. Effect of packaging under carbon dioxide, nitrogen, or air on the microflora of pork stored at 4°C. *Journal of Applied Bacteriology*, **47**, 197–208.

- Farber, J.M. 1991. Microbiological aspects of modified-atmosphere packaging technology – a review. *Journal of Food Protection*, **54**, 58–70.
- Fonseca, S.C., Oliveira, F.A.R., and Brecht, J.K. 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, **52**, 99–119.
- Gee, D. and Brown, D. 1981. The effect of carbon monoxide on bacterial growth. *Meat Science*, **5**, 215–222.
- Gill, C. 1988. The solubility of carbon dioxide in meat. *Meat Science*, **22**, 65–71.
- Gill, C. and Tan, K. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. *Applied and Environmental Microbiology*, **39**, 317–319.
- Jakobsen, M. and Nertelsen, G. 2002. The use of CO₂ in packaging of fresh red meats and its effect on chemical quality changes in the meat: a review. *Journal of Muscle Foods*, **13**, 143–168.
- Jamie, P. and Saltveit, M.E. 2002. Postharvest changes in broccoli and lettuce during storage in argon, helium, and nitrogen atmospheres containing 2% oxygen. *Postharvest Biology and Technology*, **26**, 113–118.
- Kader, A.A. 1986 Biochemical and physiological basis for effects of controlled and modified atmospheres on fruit and vegetables. *Food Technology*, **40**, 99–104.
- Kader, A.A. and Watkins, C.B. 2000. Modified atmosphere packaging – toward 2000 and beyond. *Hort Technology*, **10**, 483–486.
- Kader, A.A., Zagory, D., and Kerbel, E.L. 1989. Modified atmosphere packaging of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, **28**, 1–30.
- Krause, T., Sebranek, J., Rust, R., and Honeyman, M. 2003. Use of carbon monoxide packaging for improving the shelf life of pork. *Journal of Food Science*, **68**, 2596–2603.
- Kropf, D. 2004. Packaging/modified- and controlled-atmosphere. In: *Encyclopedia of Meat Sciences* (eds Jensen, W., Devine, C., and Dikeman, M.), pp. 962–969. Elsevier, Oxford, UK.
- Liu, F. and Li, Y. 2006. Storage characteristics and relationships between microbial growth parameters and shelf life of MAP sliced onions. *Postharvest Biology and Technology*, **40**, 262–268.
- Lucera, A., Conte, A., and Del Nobile, M.A. 2012. Shelf life of ready-to-cook cauliflower mixtures as affected by packaging film mass transport properties. *International Journal of Food Science and Technology*, **47**, 1598–1604.
- Mastromatteo, M., Mastromatteo, M., Conte, A., and Del Nobile, M.A. 2011. Packaging strategies to prolong the shelf life of minimally processed kiwifruit (*Actinidia deliciosa* cv. Hayward). *Food Research International*, **44**, 1224–1230.
- Meng, X., Zhang, M., and Adhikari, B. 2012. Extending shelf-life of fresh-cut green peppers using pressurized argon treatment. *Postharvest Biology and Technology*, **71**, 13–20.
- Mullan, M. and McDowell, D. 2003. Modified atmosphere packaging. In: *Food Packaging Technology* (eds Coles, R., McDowell, D., and Kirwan, M.), pp. 303–339. CRC Press, Boca Raton, FL, USA.
- Narasimha, R. and Sachindra, N. 2002. Modified atmosphere and vacuum packaging of meat and poultry products. *Food Reviews International*, **18**, 263–293.
- Nasar-Abbas, S.M., Plummer, J.A., Siddique, K.H.M., and White, P.F. 2008. Nitrogen retards and oxygen accelerates colour darkening in faba bean (*Vicia faba* L.) during storage. *Postharvest Biology and Technology*, **47**, 113–118.
- Ntzimani, A.G., Giatrakou, V.I., and Savvaidis, I.N. 2010. Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) on semi cooked coated chicken meat stored in vacuum packages at 4°C: microbiological and sensory evaluation. *Innovative Food Science and Emerging Technologies*, **11**, 187–196.
- Parentelli, C., Ares, G., Corona, M., Lareo, C., Gambaro, A., and Soubes, M. 2007. Sensory and microbiological quality of shiitake mushrooms in modified atmosphere packages. *Journal of the Science of Food and Agriculture*, **87**, 1645–1652.
- Pérez-Gregorio, M.R., García-Falcón, M.S., and Simal-Gándara, J. 2011. Flavonoids changes in fresh-cut onions during storage in different packaging systems. *Food Chemistry*, **124**, 652–658.

- Phillips, C.A. 1996. Review: modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology*, **31**, 463–479.
- Plestenjak, A., Pozrl, T., Hribar, J., Unuk, T., and Vidrih, R. 2008. Regulation of metabolic changes in shredded cabbage by modified atmosphere packaging. *Food Technology and Biotechnology*, **46**, 427–433.
- Rocculi, P., Romani, S., and Rosa, M.D. 2005. Effect of MAP with argon and nitrous oxide on quality maintenance of minimally processed kiwifruit. *Postharvest Biology and Technology*, **35**, 319–328.
- Saltveit, M.E. 2003. Is it possible to find an optimal controlled atmosphere? *Postharvest Biology and Technology*, **27**, 3–13.
- Sivertsvik, M., Jeksrud, W.K., and Rosnes, J.T. 2002. A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology*, **37**, 107–127.
- Soliva-Fortuny, R.C., Ricart-Coll, M., and Martin-Belloso, O. 2005. Sensory quality and internal atmosphere of fresh-cut Golden Delicious apples. *International Journal of Food Science and Technology*, **40**, 369–375.
- Wang, C.Y. 1990. Physiological and biochemical effects of controlled atmosphere on fruits and vegetables. In: *Food Preservation by Modified Atmospheres* (eds Calderon, M. and Barkai-Golan, R.), pp. 197–223. CRC Press, Boca Raton, FL, USA.

CHAPTER 11

Impact of fermentation on the microbial ecology of foods

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11.1 Introduction

The role of the fermentation process of fermented foods is twofold, contributing not only to the organoleptic characteristics of the products but also to its stability (safety). In case of insufficient fermentation of the product, this may lead to an unsafe final product because pathogenic bacteria can survive in the process. Fermented foods are generally recognized as safe, but some notable outbreaks of food-borne illness associated with fermented foods have occurred (Adams and Mitchell, 2002; Comi *et al.*, 1992; Cowden *et al.*, 1989; Norrung *et al.*, 1999; Pontello *et al.*, 1998; Taplin, 1982). Several outbreaks of illness have been attributed to the consumption of fermented sausages contaminated with *Staphylococcus aureus* and *Salmonella* spp. In addition, pathogens like *Listeria monocytogenes* and *Escherichia coli* O157:H7, have been identified as causative organisms in outbreaks involving fermented products such as sausages, cheeses, and yogurt (Beumer, 1997; Farber and Peterkin, 1991; Nissen and Holck, 1998; Warburton *et al.*, 1987).

Hence, the extent to which fermented foods are considered safe is primarily dependent on the way the fermentation process is conducted. Based on the manufacturing conditions, e.g., pH, water activity (a_w), and the time–temperature profile applied for fermentation, food-

borne pathogens may survive at the end of the process (Drosinos *et al.*, 2006; Mataragas *et al.*, 2015a, 2015b). All raw materials used in fermentation processes, e.g., meat, milk, vegetables, etc., can carry pathogenic bacteria (Roberts, 1990; Gareis *et al.*, 2011a). Depending on the type of fermentation, these pathogens may be totally or partly eliminated or their growth is inhibited by the antibacterial substances that are produced during fermentation. Usually, to ensure a satisfactory fermentation and to control the growth of food-borne pathogens, the use of specific starters is recommended (Beumer, 2001).

In most fermented foods, the inhibition of growth is more likely and can often ensure safety if the contamination levels are relatively low. With infectious pathogens, however, particularly those with a small minimum infectious dose, some degree of inactivation is still necessary to provide an acceptable level of safety (Beumer, 2001). Thus, studies using artificially contaminated raw materials to identify critical control points, e.g., fermentation pH, and critical limits, e.g., pH 4.6, that can be integrated into an HACCP plan (Lucke, 2000) to ensure safe products have been conducted (Arocha *et al.*, 1992; Bachmann and Spahr, 1995; Barazi and Erkmen, 2008; Degenhardt and Sant' Anna, 2007; Drosinos *et al.*, 2006; Farber *et al.*, 1993;

Foegeding, *et al.*, 1992; Gareis *et al.*, 2011b, 2011c, 2012; Hwang *et al.*, 2009; Kanellou *et al.*, 2013; Lahti *et al.*, 2001; Lindqvist and Lindblad, 2009; Maher *et al.*, 2001; Mataragas *et al.*, 2015a, 2015b; Nightingale *et al.*, 2006; Nissen and Holck, 1998; Porto-Fett *et al.*, 2008; Ramsaran *et al.*, 1998; Reitsma and Henning, 1996; Saad *et al.*, 2001; Thevenot *et al.*, 2005).

11.2 Fermentations: microbial ecology and activity

Food fermentations are among the most ancient technologies through which man could make safe and stable perishable raw materials, such as milk, meat, and vegetables, maintaining, or even increasing, their nutritional value (Bourdichon *et al.*, 2012). Fermentations are biological processes in which microorganisms, mainly bacteria and yeasts, but also filamentous fungi, with both their direct metabolic activity (e.g. production of ethanol or lactic acid) and production of enzymes (like proteases, lipases, amylases, etc.), transform raw materials into final products that possess different physicochemical and organoleptic characteristics, have a longer shelf life, and are safe (Caplice and Fitzgerald, 1999).

The main food fermentations nowadays exploited at industrial level are:

- lactic acid fermentation, for the production of dairy products, fermented meats and fish, sourdoughs and several fermented vegetables (e.g., olives, pickles and sauerkrauts), in which lactic acid bacteria (LAB) are primarily involved;
- alcoholic fermentation, for the production of alcoholic beverages, such as wine and beer, but is also involved in the fermentation of bakery products, with yeasts, and more specifically *Saccharomyces cerevisiae*, as the fermentation agent;
- acetic acid fermentation, for the production of vinegar, in which a suroxidation of ethanol takes place with the production of acetic acid mediated by the acetic acid bacteria (AAB).

Several other fermentations are exploited in the food industry (e.g., propionic fermentation in

Swiss-type cheese), but the ones mentioned above are the most important from an economic point of view.

11.3 Factors affecting food-borne pathogen inactivation during fermentation

Food-borne pathogens associated with fermented foods are mainly introduced into this type of product through the raw materials used for fermentation: meat, milk, fish, vegetables, and fruits. These may be contaminated with food-borne pathogens entering in the production process. Important pathogenic bacteria associated with the raw materials used in fermentation processes are *Aeromonas hydrophila*, *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Mycobacterium avium* subsp. *paratuberculosis*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Vibrio* spp., and *Yersinia enterocolitica* (Beumer, 2001; Spahr and Schaefroth, 2001). Each food-borne pathogen does not have the same frequency of occurrence in each category of the raw materials. Depending on the raw material, the presence of each food-borne pathogen is more or less likely.

Before the start of the fermentation process, other non-thermal processes such as peeling, washing, soaking, mixing, and/or grinding may (not) have an effect on the concentration of pathogens present in food. Inactivation or survival during fermentation depends on product characteristics such as pH and a_w , the presence of preservative agents (e.g., salt), factors associated with the microorganisms themselves such as the physiological state of the pathogens, antagonism and production of antibacterial substances, and process parameters, e.g., the fermentation temperature and time of processing. The raw material of some fermented products may receive thermal treatment, e.g., raw milk during cheese production, which is

expected to lower or eliminate the presence of vegetative bacterial cells.

11.3.1 Factors associated with the product

The main factors controlling the food-borne pathogens in fermented foods are the raw material quality, pH, a_w , the presence of preservative agents like salt, and the production of antimicrobial substances by the starter cultures. A proper fermented food will effectively control, in most of the cases, the growth of pathogens (Table 11.1). However, food-borne pathogens can survive the process or even grow if the final pH and/or a_w values are relatively high (Drosinos *et al.*, 2006; Mataragas *et al.*, 2015a, 2015b; Withers and Couper, 2012) (Table 11.2). The critical stage for the survival of the food-borne pathogens is the beginning of the fermentation process, when the pH and a_w are still high and the competitive effect of the starter culture has not yet fully been deployed (Shay, 1993). During fermentation, various antimicrobial compounds are produced by the starter cultures added, particularly by the LAB. Such substances are the weak organic acids like

Table 11.1 Limits of growth for the food-borne pathogens associated with the raw materials used in the fermentation processes in relation to product characteristics (pH and a_w).

Microorganisms	min pH	min a_w
<i>Aeromonas hydrophila</i>	4.50	0.960–0.970
<i>Bacillus cereus</i>	4.35–4.90	0.912–0.950
<i>Campylobacter</i> spp.	4.90	0.980
<i>Clostridium botulinum</i>		
Proteolytic types A, B, F	4.60–4.80	0.940
Non-proteolytic types B, E, F	5.00	0.970
<i>Escherichia coli</i> O157:H7	4.20	0.950
<i>Listeria monocytogenes</i>	4.50	0.890–0.900
<i>Salmonella</i> spp.	4.20	0.940
<i>Shigella</i> spp.	4.80–5.00	0.948
<i>Staphylococcus aureus</i>		
Growth	4.00	0.840–0.860
Toxin production	4.50	0.870
<i>Vibrio</i> spp.	4.80–5.00	0.940–0.970
<i>Yersinia enterocolitica</i>	4.20–4.80	0.945

Table 11.2 Final product characteristics in terms of pH and a_w after the fermentation of the raw materials.

Raw materials	Final pH	Final a_w
Meat	4.50–6.00	0.850–0.990
Milk	<4.00–5.50	>0.960
Fish	<5.00–6.50	>0.950
Vegetables	<4.00–5.50	>0.960
Fruits	<4.00	>0.960

lactic acid and acetic acid contributing primarily to the antimicrobial activity. Other substances are hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocins.

11.3.2 Factors associated with the microorganisms

The physiological state of the bacterial cells appears to be a significant factor in fermentation treatment. During processing, microorganisms in the food undergo various kinds of stresses, and these stresses have a fundamental impact on the behavior of the stress-exposed cells. Bacteria have evolved adaptive networks to face the challenges of changing environments (Abee and Wouters, 1999). Fermented foods are a paramount example for such environments and challenges. Upon triggering of the adaptive networks, the so-called “adaptive stress response”, the bacteria gain increased resistance towards the processing conditions over time, leading to the reduction of the inactivation rate, a phenomenon known as tailing of the inactivation curve. Earlier work has shown that *S. enterica* and *L. monocytogenes* may adapt to the dynamically changing environment of the fermented sausages, allowing their better survival (Mataragas *et al.*, 2015a, 2015b).

11.3.3 Process parameters

Fermentation treatment is defined by temperature, time of processing, relative humidity, and air circulation in the fermentation and aging chamber, as well as by pH and a_w of the product, which are changing over time. From this point of view, fermentation is more complex than heat treatment because the process parameters that

have a critical role in the latter case are only temperature and time. All these factors are related during the non-thermal inactivation of the pathogens. In general, inactivation of vegetative bacteria increases with increasing temperature while keeping the other process parameters constant, e.g., during storage (Mataragas *et al.*, 2015c). During processing, however, the effect of the food matrix, pH, and a_w is significant, making the inactivation of the bacterial cells more complicated.

The environmental conditions prevailing in the early fermentation are critical with regard to growth and subsequent survival rate of food-borne pathogens. For instance, in fermented meats, the rapid decrease of pH is an important contributing factor to the inactivation of the pathogens for fermentation temperatures above 20 °C, but a_w becomes more important than a decrease in pH at temperatures ≤ 20 °C (Gounadaki *et al.*, 2005). It has been shown that *E. coli* O157:H7 multiply during the early stages of production of several cheeses (Arocha *et al.*, 1992; Maher *et al.*, 2001; Ramsaran *et al.*, 1998; Reitsma and Henning, 1996; Saad *et al.*, 2001). In the fermented meats, however, this has been seen to a lesser degree due to the presence of curing salts in the mix inhibiting the growth. Later on during process and maturation of cheeses, *E. coli* O157:H7 concentration declines, but often the microorganism has been detected after extended periods of storage (Maher *et al.*, 2001; Reitsma and Henning, 1996).

11.4 Challenge tests

Challenge tests are useful because they provide robust data by determining the fate of the food-borne pathogens inoculated into raw materials through the actual course of fermentation and storage (Adams and Mitchell, 2002). They can be used for various purposes. One such purpose is the validation of a process that should meet a criterion related to the performance of a lethality step. In the case of fermentation, an example is

the 5 log reduction of *E. coli* O157:H7 for fermented meats (Ellajosyula *et al.*, 1998). The challenge test validates whether the specific process is performing as it is expected in relation to the predetermined performance standard (process challenge test). Thus, the objective of the process challenge test is to investigate whether a selected pathogen survives a process like fermentation and ripening as opposed to a product challenge test in which the growth or not of the selected pathogen is examined (Beumer, 2001). Data from challenge tests can be analyzed quantitatively to predict the fate of the selected pathogen using predictive modeling tools, as described in the Section 11.5, "Predictive modeling".

When performing a challenge test, some factors should be taken into consideration, such as (FDA, 2013; Vestergaard, 2001):

- a The selection of the food-borne pathogens to be inoculated in the product or their surrogates. It is preferable to use a cocktail of strains isolated from identical or similar products.
- b The level of the inoculum. The inoculum level that will be used in the challenge test is dependent on the objective of performing the challenge test, i.e., to determine the shelf life and stability of the product or to validate the performance standard of a step in the process aiming to reduce the pathogen concentration.
- c The preparation of the inoculum and the inoculation method. When the preparation of the inoculum is taking place, any stress that could reduce cell viability should be avoided. Food-borne pathogens contaminating a product could be adapted to factors associated with the product, e.g., low pH and a_w . Therefore, it is often necessary to allow microorganisms to experience a kind of stress-like growth at low pH or a_w for reliable results.
- d The study duration. It is also dependent on the objective of the study. It is desired, however, to challenge the product during its manufacturing process or for its entire shelf life and beyond.

- e The storage conditions and the formulation factors. Test samples should be manufactured, packaged, and stored at conditions that are very close, as they possibly can be, to the commercial conditions of the product.
- f The analysis of samples. The level of the selected food-borne pathogen is enumerated by direct plating or the most probable number at each predetermined time interval (sampling point). It is desired to have two or three samples per sampling point (replicates). For a higher degree of certainty, the experiment should be performed again using different batch(es) of the product (independent experiment).

11.5 Predictive modeling

Once the challenge testing is accomplished, the data are analyzed to investigate the fate of the food-borne pathogens, i.e., how the pathogens behave over time. Combining the quantitative data for each sampling point with the data on the background microflora and physicochemical properties of the product gives an overview of the microbiological stability of the formulation (FDA, 2013). As regards fermentation, analysis of the data will reveal if the process is able to perform as was expected, i.e., at the required level of lethality. The data from the challenge tests can be used in developing predictive models. Predictive models are computer-based programs simulating the way in which food-borne pathogens will behave in a formulation under specific conditions, e.g., fermentation.

11.5.1 Behavior of the food-borne pathogens during inactivation

The inactivation curve of the food-borne pathogens due to fermentation can take many forms. Therefore, the inactivation pattern should be identified that best describes this inhibition. The most frequent inhibition pattern is the linear inactivation of the bacterial population, which uses a constant rate to describe the log-linear

microbial inactivation over time (Black and Davidson, 2008). Data in the literature, however, regarding inactivation of the food-borne pathogens have shown that there are substantial deviations from the log-linear inactivation. Inactivation curves may appear as sigmoidal-like curves with both a shoulder and tail, log-linear curves with a preceding shoulder or with a tail only, biphasic consisting of two linear parts, biphasic with a shoulder, concave and convex curves, convex/concave curves followed by tailing, and curves with a double concave/convex shape (Albert and Mafart, 2005; Bigelow and Esty, 1920; Cerf, 1977; Corroler *et al.*, 2006; Geeraerd *et al.*, 2000, 2005; Mafart *et al.*, 2002).

Another observation is that although the fermentation and ripening temperatures alone may not cause the destruction of the bacterial cells, the temperature applied is an important explanatory variable of the inactivation rate, and hence of the total inactivation, i.e., the higher the temperature the faster is the inactivation. This has been observed both for *E. coli* O157:H7 (McQuestin *et al.*, 2009) and *L. monocytogenes* (Mataragas *et al.*, 2015d) during their inactivation in fermented meats where higher inactivation is achieved if these processes are being performed at elevated temperatures for increased times. Other factors such as pH and a_w are also important in pathogens inactivation, but increased holding times and temperatures will enhance their inactivation.

In general, the underlying cause(s) for such deviations from the log-linear inactivation of food-borne pathogens is/are not well understood. There may be various reasons for this. For instance, there are different theories for explaining tailing phenomena in the inactivation curves (Cerf, 1977; Geeraerd *et al.*, 2000). The resistant population (tail) is considered to be a subpopulation that is very resistant to the inactivation treatment (vitalistic theory), is inaccessible or adapted to the inactivation treatment (mechanistic theory I where the tail is a normal feature), and is genetically more resistant or not receiving the same lethal dose (mechanistic theory II where the tail is an

artifact). Shoulders in the inactivation curves could be an indication that destruction of the bacterial cells occurs after the achievement of a certain threshold of cell damage. Moreover, cell clumping cannot be excluded, which might cause the appearance of a shoulder in the inactivation curves.

Various models have been proposed in the literature to describe all the aforementioned inactivation patterns (Geeraerd *et al.*, 2005). A model describing the inactivation of the food-borne pathogen as a function of time is fitted to the experimental data obtained during fermentation to calculate the kinetic parameters such as inactivation rate, time needed for the first decimal reduction of the pathogen, time needed for 1 or 4 log reductions of the pathogen, or total inactivation. Challenge tests from which the inactivation data are coming have the advantage that all the factors, described previously, affecting the inactivation of the microbial cells are “included” in the model and the calculated kinetic parameters.

11.5.2 Kinetic behavior (inactivation) models

As already mentioned, the classical inactivation curve is the log-linear regression (Bigelow and Esty, 1920). The assumption of this model is that all cells of a population have an equal sensitivity to the inactivation treatment. This kind of model has widespread use, but deviations from this linear approach have been identified, indicating that inactivation may not always follow a linear trend. For this reason, some non-linear inactivation models have been developed to fill in this gap (Table 11.3).

The log-linear model with a shoulder and/or tail is the most used non-linear model, in which the inactivation includes a preceding lag phase (shoulder) where the microbial population remains unchanged and/or saturation (tail) of the decay where a resistant population survives the inactivation treatment (Geeraerd *et al.*, 2000). The model was developed as a couple of two differential equations, including a parameter related to the physiological state of the cells

(C_c). This parameter of the model describes the shoulder effect and is relied on the hypothesis of the presence of a pool of a critical or protective component (C_c) around the cell. This pool is gradually destroyed and towards the end of the shoulder phase becomes equal to one. Finally, the model includes a factor (N_{res}), which implies the existence of a more resistant subpopulation (tailing effect). This model covers four different shapes of inactivation curve: log-linear regression, log-linear with a preceding shoulder, log-linear with a tail, and sigmoidal-like with a shoulder and tail (Bevilacqua and Sinigaglia, 2010).

The next category of functions includes the Weibullian-type equations. These are based on the assumption of the existence of several subpopulations, each with its own inactivation kinetics (van Boekel, 2002). The most used equation is that of Mafart *et al.* (2002). A modified version of the Weibull equation introduces the tailing effect (Albert and Mafart, 2005) and the simultaneous presence of a concave and convex shape in the inactivation curve (Corroler *et al.*, 2006). The Weibullian-type models cover the following shapes of the inactivation curve: log-linear regression, concave or convex inactivation curve with or without a tail, and concave/convex inactivation curve.

The last category is the biphasic nature of the microbial inactivation. These models are based on the assumption of the existence of two different subpopulations (a sensitive and a more resistant to the inactivation treatment subpopulation) with different inactivation rates. Two models exist, the model of two populations proposed as a two-fraction model by Cerf (1977) and the model describing the biphasic microbial inactivation with an initial shoulder (Geeraerd *et al.*, 2005). This category of models covers the inactivation curves of log-linear regression, log-linear with tailing, biphasic, and biphasic with a shoulder.

In order to select the best model fitting the experimental data and calculating the kinetic parameters of microbial inactivation there are several criteria. The most frequently used are

Table 11.3 Various inactivation models covering different shapes of the inactivation curve.

Inactivation model	Equation applied to experimental data	Reference
Log-linear regression	$\log_{10}(N_t) = \log_{10}(N_0) - \frac{k_{max} \times t}{\ln(10)} = \log_{10}(N_0) - \frac{t}{D}$	Bigelow and Esty, 1920
Log-linear+shoulder + tail	$\log_{10}(N_t) = \log_{10} \left[\left(10^{\log_{10}(N_0)} - 10^{\log_{10}(N_{res})} \right) \times e^{-k_{max} \times t} \times \left(\frac{e^{k_{max} \times S_t}}{1 + (e^{k_{max} \times S_t} \times e^{-k_{max} \times t})} \right) + 10^{\log_{10}(N_{res})} \right]$	Geeraerd et al., 2000
Log-linear + shoulder	The log-linear + shoulder + tail equation by setting $N_{res} = 0$	Geeraerd et al., 2000
Log-linear + tail	The log-linear + shoulder + tail equation by setting $S_t = 0$	Geeraerd et al., 2000
Weibull	$\log_{10}(N_t) = \log_{10}(N_0) - \left(\frac{t}{\delta} \right)^p$	Mafart et al., 2002
Weibull + tail	$\log_{10}(N_t) = \left[\left(10^{\log_{10}(N_0)} - 10^{\log_{10}(N_{res})} \times 10^{-\left(\frac{t}{\delta} \right)^p} + 10^{\log_{10}(N_{res})} \right) \right]$	Albert and Mafart, 2005
Double Weibull	$\log_{10}(N_t) = \log_{10} \left[\frac{10^{N_0}}{1 + 10^a} \times 10^{\left(-\frac{t}{\delta_1} \right)^p + a} + 10^{\left(-\frac{t}{\delta_2} \right)^p} \right]$	Corroler et al., 2006
Biphasic model	$\log_{10}(N_t) = \log_{10}(N_0) + \log_{10} \left[f \times e^{-k_{max} t} + (1-f) \times e^{-k_{max} t} \right]$	Cerf, 1977
Biphasic+shoulder	$\log_{10}(N_t) = \log_{10}(N_0) + \log_{10} \left[f \times e^{-k_{max} t} \times \frac{e^{k_{max} t S_t}}{1 + (e^{k_{max} t S_t} - 1) \times e^{-k_{max} t}} + (1-f) \times e^{-k_{max} t} \times \left(\frac{e^{k_{max} t S_t}}{1 + (e^{k_{max} t S_t} - 1) \times e^{-k_{max} t}} \right)^{\frac{k_{max,2}}{k_{max,1}}} \right]$	Geeraerd et al., 2005

N_t , the microbial cell density at time t (cfu/g or ml); N_0 , the initial microbial cell density (cfu/g or ml); N_{res} , the final (resistant) microbial cell density (tail) (cfu/g or ml); S_t , the shoulder phase (time unit); t , the time (time unit); k_{max} , the inactivation rate (1/time unit); D , the decimal reduction time (time unit), i.e., the time needed for 1 log reduction of the food-borne pathogen of concern; δ , the time needed for the first decimal reduction of the food-borne pathogen of concern (time unit); δ_1 and δ_2 , the time needed for the first decimal reduction of the sensitive and resistant subpopulations, respectively (time unit); p , the shape parameter (if $p > 1$ then a convex curve is obtained, if $p < 1$ then a concave curve is obtained, and if $p = 1$ then a linear curve is obtained); f , the fraction of the major subpopulation (sensitive) (%); $1 - f$, the fraction of the minor subpopulation (resistant) (%); and $k_{max,1}$ and $k_{max,2}$, the inactivation rates in the sensitive and resistant subpopulations, respectively (1/time unit); a , a transformation of the f parameter equal to $a = \log_{10} \left(\frac{f}{1-f} \right)$ or $f = \frac{10^a}{1+10^a}$

the following: (a) the sum of squared errors (SSE) (Geeraerd *et al.*, 2005), (b) the mean square error (MSE) (Geeraerd *et al.*, 2005; Neter *et al.*, 1996), (c) the root mean square error (RMSE), (d) R^2 and $R^2_{adjusted}$ values (Neter *et al.*, 1996), (e) bias B_f and accuracy A_f factors (Ross, 1996), and (f) model simplicity (Diels *et al.*, 2007). In general, the fitting performance of a model increases with decreasing SSE, MSE, or RMSE, and with R^2 , $R^2_{adjusted}$, B_f and A_f values as near to 1 as possible. In addition, a good model should be as simple as possible to avoid overfitting. Thus, a correction for the number of parameters should be taken into account since models with more independent parameters tend to fit better. Criteria such as MSE and $R^2_{adjusted}$ take into account such a correction (Diels *et al.*, 2007).

11.5.3 Case study: process challenge test for fermented products artificially contaminated with *L. monocytogenes*

The survival data of *L. monocytogenes* inoculated in the batter of a fermented sausage (Sudjuk, Bosnia-Herzegovina) manufactured with the addition of starter culture (*Lactobacillus sakei* I151) (Drosinos *et al.*, 2006) were used for illustrative purposes. The *L. monocytogenes* population was measured by plate counting on PALCAM. The experimental inactivation data consisted of time (t) (days) and population ($\log_{10}$ (N) in cfu/g) were introduced in an MS Excel spreadsheet. The Excel add-in GInaFiT

(Geeraerd *et al.*, 2005) was used to model the non-thermal inactivation of the pathogen. Based on the shape of the survival data obtained, the data were fitted with three out of the ten models of the tool. As an example, the fit for the best model chosen according to the statistical indices (Table 11.4) is presented in Figure 11.1.

These three models lead to similar statistical measures and seem to describe the data well. However, the statistical measures (RMSE and $R^2_{adjusted}$ for models comparison) of the log-linear with shoulder model are the best compared to the other two models. In addition, Mossel *et al.* (1995) reported that a standard deviation of

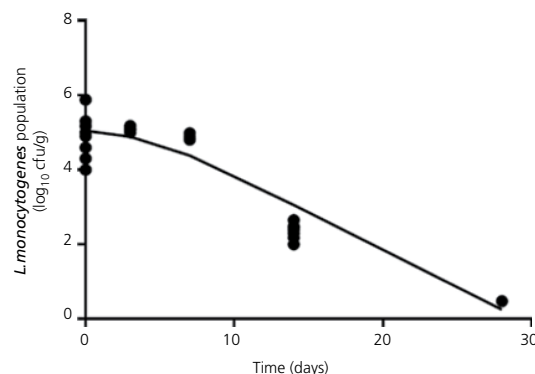


Figure 11.1 Non-thermal inactivation of the food-borne pathogen *L. monocytogenes* inoculated in the batter of a fermented sausage (Sudjuk) manufactured with the addition of starter culture (*Lactobacillus sakei* I151). Closed circles, observed data; solid line, predicted data according to the log-linear with a shoulder model.

Table 11.4 Statistical indices and kinetic parameters estimated when applying three models of the tool GInaFiT on the experimental inactivation data of *L. monocytogenes* in Sudjuk fermented sausage.

Inactivation model	MSE	RMSE	R^2	$R^2_{adjusted}$	k_{max} (1/days)	t_{4D} (days)	$\log_{10}(N_0)$ (cfu/g)	S_f (days)	δ (days)	p (–)
Log-linear regression	0.31	0.56	0.91	0.91	0.40	22.96	5.35			
Log-linear + shoulder	0.25	0.50	0.93	0.93	0.46	24.08	5.05	4.08		
Weibull	0.30	0.54	0.92	0.91		24.08	5.17		7.43	1.19

MSE, the mean square error; RMSE, the root mean square error; R^2 , the correlation coefficient; $R^2_{adjusted}$, the correlation coefficient accounting for the model parameters; k_{max} , the inactivation rate; t_{4D} , the time needed for 4 log reductions of *L. monocytogenes*; $\log_{10}(N_0)$, the initial population of *L. monocytogenes*; S_f , the duration of the shoulder phase; δ , the time needed for the first decimal reduction of *L. monocytogenes*; and p , the shape parameter.

Table 11.5 Quantitative data on the non-thermal inactivation of various food-borne pathogens in various fermented foods.

Food-borne pathogen	Food product	N_{tot} (log ₁₀ cfu/g)	Reference
<i>L. monocytogenes</i>	Sremska fermented sausage	3.82	Drosinos <i>et al.</i> , 2006
<i>L. monocytogenes</i>	Sudjok fermented sausage	4.49	Drosinos <i>et al.</i> , 2006
<i>L. monocytogenes</i>	Croatian-style fermented sausage	4.32	Drosinos <i>et al.</i> , 2006
<i>L. monocytogenes</i>	Hungarian-style fermented sausage	1.41	Drosinos <i>et al.</i> , 2006
<i>L. monocytogenes</i>	Italian-style fermented sausage	3.49	Degenhardt and Sant' Anna, 2007
<i>L. monocytogenes</i>	Sucuk	1.70	Barazi and Erkmen, 2008
<i>L. monocytogenes</i>	American-style fermented sausage	1.91–5.11	Foegeding <i>et al.</i> , 1992
<i>L. monocytogenes</i>	German-style minisalami	0.36–2.65	Gareis <i>et al.</i> , 2012
<i>St. aureus</i>	German-style minisalami	0.20–2.00	Gareis <i>et al.</i> , 2012
<i>E. coli</i> O157:H7	German-style minisalami	1.50–3.10	Gareis <i>et al.</i> , 2012
<i>Salmonella</i>	German-style minisalami	1.60–2.30	Gareis <i>et al.</i> , 2012
<i>L. monocytogenes</i>	Norwegian-style fermented sausage	0.20–0.74	Nissen and Holck, 1998
<i>E. coli</i> O157:H7	Norwegian-style fermented sausage	1.80–3.00	Nissen and Holck, 1998
<i>S. kentucky</i>	Norwegian-style fermented sausage	0.80–1.80	Nissen and Holck, 1998
<i>L. monocytogenes</i>	German-style fermented sausage	5.01	Farber <i>et al.</i> , 1993
<i>L. monocytogenes</i>	American-style fermented sausage	5.12	Farber <i>et al.</i> , 1993
<i>L. monocytogenes</i>	Soudjouk-style semi-dry fermented sausage	0.36–0.64	Porto-Fett <i>et al.</i> , 2008
<i>L. monocytogenes</i>	Soudjouk-style semi-dry fermented sausage	0.52–0.59	Porto-Fett <i>et al.</i> , 2008
<i>E. coli</i> O157:H7	Soudjouk-style semi-dry fermented sausage	2.31–2.97	Porto-Fett <i>et al.</i> , 2008
<i>St. aureus</i>	Soudjouk-style semi-dry fermented sausage	0.300.90	Nightingale <i>et al.</i> , 2006
<i>L. monocytogenes</i>	Italian-style salami	2.4–4.5	Nightingale <i>et al.</i> , 2006
<i>Salmonella</i>	Italian-style salami	2.4–4.5	Nightingale <i>et al.</i> , 2006
<i>L. monocytogenes</i>	Soudjouk-style semi-dry fermented sausage	0.10–0.90	Hwang <i>et al.</i> , 2009
<i>E. coli</i> O157:H7	Soudjouk-style semi-dry fermented sausage	0.00–4.40	Hwang <i>et al.</i> , 2009
<i>S. Typhimurium</i>	Soudjouk-style semi-dry fermented sausage	0.30–4.70	Hwang <i>et al.</i> , 2009
<i>L. monocytogenes</i>	Swedish-style fermented sausage	0.30–4.00	Lindqvist and Lindblad, 2009
<i>E. coli</i> O157:H7	Swedish-style fermented sausage	1.50–4.50	Lindqvist and Lindblad, 2009
<i>Y. enterocolitica</i>	Swedish-style fermented sausage	1.50–5.50	Lindqvist and Lindblad, 2009
<i>L. monocytogenes</i>	French-style fermented sausage	1.73–3.22	Thevenot <i>et al.</i> , 2005
<i>L. monocytogenes</i>	Finnish-style fermented sausage	0.71–2.07	Lahti <i>et al.</i> , 2001
<i>E. coli</i> O157:H7	Finnish-style fermented sausage	2.50–4.50	Lahti <i>et al.</i> , 2001
<i>L. monocytogenes</i>	Cacciatore fermented sausage	0.53	Mataragas <i>et al.</i> , 2015a
<i>L. monocytogenes</i>	Cacciatore fermented sausage	0.87	Mataragas <i>et al.</i> , 2015b
<i>S. enterica</i>	Cacciatore fermented sausage	1.08	Mataragas <i>et al.</i> , 2015a

<i>S. enterica</i>	Cacciatore fermented sausage	0.97	Mataragas <i>et al.</i> , 2015b
<i>L. monocytogenes</i>	Felino fermented sausage	0.24	Mataragas <i>et al.</i> , 2015a
<i>L. monocytogenes</i>	Felino fermented sausage	0.51	Mataragas <i>et al.</i> , 2015b
<i>S. enterica</i>	Felino fermented sausage	1.30	Mataragas <i>et al.</i> , 2015a
<i>S. enterica</i>	Felino fermented sausage	2.36	Mataragas <i>et al.</i> , 2015b
<i>L. monocytogenes</i>	Milano fermented sausage	0.66	Mataragas <i>et al.</i> , 2015b
<i>S. enterica</i>	Milano fermented sausage	1.51	Mataragas <i>et al.</i> , 2015b
<i>E. coli</i> O157:H7	Pepperoni	0.84–2.75	Riordan <i>et al.</i> , 1998
<i>E. coli</i> O157:H7	Lebanon Bologna	0.05–0.59	Ellajosyula <i>et al.</i> , 1998
<i>St. aureus</i>	Lebanon Bologna	0.03–1.18	Ellajosyula <i>et al.</i> , 1998
<i>E. coli</i> O157:H7	Pepperoni	1.9	Faith <i>et al.</i> , 1997
<i>E. coli</i> O157:H7	Pepperoni	1.2	Hinkens <i>et al.</i> , 1996
<i>St. aureus</i>	Pepperoni	2.14	Smith <i>et al.</i> , 1975
<i>E. coli</i> O157:H7	Soudjouk	0.88	Calicioglu <i>et al.</i> , 2002
<i>E. coli</i> O157:H7	Soudjouk	0.28–1.96	Calicioglu <i>et al.</i> , 2001
<i>E. coli</i> O157:H7	Cheddar cheese	≤3.00	Reitsma and Henning, 1996
<i>L. monocytogenes</i>	Cheddar cheese	≤2.70	Ryser and Marth, 1987a
<i>E. coli</i> O157:H7	Cheddar cheese	1.00	Teo and Schlesser, 2000
<i>Aeromonas</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>Campylobacter</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>E. coli</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>L. monocytogenes</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>P. aeruginosa</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>Salmonella</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>Staphylococcus</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>Yersinia</i>	Swiss hard/semi-hard cheeses	4.00–6.00	Bachman and Spahr, 1995
<i>L. monocytogenes</i>	Parmesan cheese	4.00–5.00	Yousef and Mart, 1990
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	Swiss hard cheese	4.30	Spahr and Schaefroth, 2001
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	Swiss semi-hard cheese	2.00	Spahr and Schaefroth, 2001
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>	Hispanic-style soft white cheese	1.50–2.00	Sung and Collins, 2000
<i>L. monocytogenes</i>	Pickles in brine (storage)	2.80–4.00	Kanellou <i>et al.</i> , 2013
<i>S. Typhimurium</i>	Pickles in brine (storage)	2.80–4.00	Kanellou <i>et al.</i> , 2013

N_{tot} total inactivation of the food-borne pathogen achieved during the manufacture of the product.

0.25–0.50 is accepted for regularly standardized bacterial colony counts. Although these values are referred to the measurement variation and not to the total experimental variation, by accepting this limitation the RMSE of the log-linear with the shoulder model appear to be acceptable (Geeraerd *et al.*, 2005).

The in situ non-thermal inactivation of various food-borne pathogens in different fermented foods is presented in Table 11.5. In this table only the inactivation of the food-borne pathogens is considered and not any potential growth that may occur during manufacture of the product (Ryser and Marth, 1987b). Because in many cases the inactivation of the food-borne pathogen did not follow first-order kinetics displaying shoulder and/or tailing, the total inactivation (N_{tot} , $\log_{10}$ cfu/g) during the manufacture of the product is only given. When inactivation of the microorganism is biphasic or displays a shoulder and/or tailing, the inactivation rate k_{max} and/or the time needed for 1 log reduction of the pathogen (D) are not suitable measures to display since these are referred only to the linear part of the inactivation curve.

11.6 Conclusions

Although many studies have been performed relative to the quantification of the inactivation of the food-borne pathogens in fermented foods, the reliable quantitative description of the process effect on inactivation of the microorganisms and on the quality characteristics of the products will remain an important issue. The various quantitative models that have been developed to fill in this gap will underpin process design and optimization and support food business operators to achieve the established food safety objectives and produce safe products (McQuestin *et al.*, 2009). The development of quantitative models for the non-thermal inactivation of the food-borne pathogens in the fermented foods is more complicated than for heat inactivation since fermentation is dependent on many factors such as pH, a_w , and

fermentation temperature. Therefore efforts should be made for improvement of the quality of the raw survival data. Finally, an important contribution to the improvement of the non-thermal (fermentation) inactivation models will be an understanding of the behavior of stress-adapted food-borne pathogens during the fermentation process. This task is highly challenging but, with such knowledge, control measures and hygiene practices can be developed for all types of fermentations in order to ensure safe products. Developments towards insight into the molecular background of the microbial inactivation during fermentation will substantially facilitate this task (Mataragas *et al.*, 2015e).

References

- Abee, T. and Wouters, J.A. 1999. Microbial stress response in minimal processing. *International Journal of Food Microbiology*, **50**, 65–91.
- Adams, M. and Mitchell, R. 2002. Fermentation and pathogen control: a risk assessment approach. *International Journal of Food Microbiology*, **79**, 75–83.
- Albert, I. and Mafart, P. 2005. A modified Weibull model for bacterial inactivation. *International Journal of Food Microbiology*, **100**, 197–211.
- Arocha, M.M., McVey, M., Loder, S.D., Rupnow, J.H., and Bullerman, L. 1992. Behaviour of enterohaemorrhagic *E. coli* O157:H7 during manufacture of cottage cheese. *Journal of Food Protection*, **55**, 379–381.
- Bachmann, H.P. and Spahr, U. 1995. The fate of potentially pathogenic bacteria in Swiss hard and semi-hard cheeses made from raw milk. *Journal of Dairy Science*, **78**, 476–483.
- Barazi, A. and Erkmen, O. 2008. Survival of *Listeria monocytogenes* in Sucuk during manufacturing and storage periods at different modified atmospheres. *Roumanian Biotechnological Letters*, **13**, S49–S58.
- Beumer, R.R. 1997. *Listeria monocytogenes*: detection and behavior in food and in the environment. PhD Dissertation, Wageningen University Research Center, The Netherlands.

- Beumer, R.R. 2001. Microbiological hazards and their control: bacteria. In: *Fermentation and Food Safety* (eds Adams, R.R. and Nout, M.J.R.), pp. 141–157. Aspen Publishers, Inc., Gaithersburg, MD, USA.
- Bevilacqua, A. and Sinigaglia, M. 2010. Food shelf life and safety: challenge tests, prediction and mathematical tools. In: *Application of Alternative Food-Preservation Technologies to Enhance Food Safety and Stability* (eds Bevilacqua, A., Corbo, M.R., and Sinigaglia, M.), pp. 161–187. Bentham Books, Foggia, Italy.
- Bigelow, W.D. and Esty, J.R. 1920. The thermal death point in relation to typical thermophilic organisms. *Journal of Infectious Diseases*, **27**, 602.
- Black, D.G. and Davidson, P.M. 2008. Use of modeling to enhance the microbiological safety of the food system. *Comprehensive Reviews in Food Science and Food Safety*, **7**, 159–167.
- Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., *et al.* 2012. Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology*, **154**, 87–97.
- Calicioglu, M., Faith, N.G., Buege, D.R., and Luchansky, J.B. 2001. Validation of a manufacturing process for fermented, semidry Turkish soudjouk to control *Escherichia coli* O157:H7. *Journal of Food Protection*, **64**, 1156–1161.
- Calicioglu, M., Faith, N.G., Buege, D.R., and Luchansky, J.B. 2002. Viability of *Escherichia coli* O157:H7 during manufacturing and storage of a fermented, semidry soudjouk-style sausage. *Journal of Food Protection*, **65**, 1541–1544.
- Caplice, E. and Fitzgerald, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*, **50**, 131–149.
- Cerf, O. 1977. A review. Tailing of survival curves of bacterial spores. *Journal of Applied Microbiology*, **42**, 1–19.
- Comi, G., Frigerio, R., and Cantoni, C. 1992. *Listeria monocytogenes* serotypes in Italian meat products. *Letters in Applied Microbiology*, **15**, 168–171.
- Coroller, L., Leguerinel, I., Mettler, E., Savy, N., and Mafart, P. 2006. General model, based on two mixed Weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. *Applied and Environmental Microbiology*, **72**, 6493–6502.
- Cowden, J.M., O'Mahony, M., Bartlett, C.L.R., Rana, B., *et al.* 1989. A national outbreak of *Salmonella typhimurium* DT124 caused by contaminated salami sticks. *Epidemiology and Infection*, **103**, 219–225.
- Degenhardt, R. and Sant' Anna, E.S. 2007. Survival of *Listeria monocytogenes* in low acid Italian sausage produced under Brazilian conditions. *Brazilian Journal of Microbiology*, **38**, 309–314.
- Diels, A., Van Opstal, I., Masschalck, B., and Michiels, C.W. 2007. Modelling of high-pressure inactivation of microorganisms in foods. In: *Modelling Microorganisms in Food* (eds Brul, S., van Gerwen, S., and Zwietering, M.), pp. 161–197. Woodhead Publishing Limited, Cambridge, UK.
- Drosinos, E.H., Mataragas, M., Veskovic-Moracanin, S., Gasparik-Reichardt, J., Hadziosmanovic, M., and Alagic, D. 2006. Quantifying non-thermal inactivation of *Listeria monocytogenes* in European fermented sausages using bacteriocinogenic lactic acid bacteria or their bacteriocins: a case study for risk assessment. *Journal of Food Protection*, **69**, 2648–2663.
- Ellajosyula, K.R., Doores, S., Mills, E.W., Wilson, R.A., Anantheswaran, R.C., and Knabel, S.J. 1998. Destruction of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in Lebanon bologna by interaction of fermentation pH, heating temperature, and time. *Journal of Food Protection*, **61**, 152–157.
- Faith, N.G., Parniere, N., Larson, T., Lorang, T.D., and Luchansky, J.B. 1997. Viability of *Escherichia coli* O157:H7 in pepperoni during the manufacture of sticks and the subsequent storage of slices at 21, 4 and –20 °C under air, vacuum and CO₂. *International Journal of Food Microbiology*, **37**, 47–54.
- Farber, J.M. and Peterkin, P.I. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Microbiological Reviews*, **55**, 476–511.
- Farber, J.M., Daley, E., Holley, R., and Osborne, W.R. 1993. Survival of *Listeria monocytogenes* during the production of uncooked German,

- American and Italian style fermented sausages. *Food Microbiology*, **10**, 123–132.
- Foegeding, P.M., Thomas, A.B., Pilkington, D.H., and Klaenhammer, T.R. 1992. Enhanced control of *Listeria monocytogenes* by *in situ*-produced pediocin during dry fermented sausage production. *Applied and Environmental Microbiology*, **58**, 884–890.
- Food and Drug Administration (FDA). 2013. Evaluation and definition of potentially hazardous foods – Chapter 6. Microbiological challenge testing. Accessed at 19 June 2014, <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm094154.htm>.
- Gareis, M., Kabisch, J., Pichner, R., and Hechelmann, H. 2011a. Occurrence of food-borne pathogens in minisalami. *Fleischwirtschaft International*, **26**, 69–73.
- Gareis, M., Kabisch, J., Pichner, R., and Hechelmann, H. 2011b. Behavior and survival of *Salmonella* spp. in minisalami. *Fleischwirtschaft International*, **26**, 70–77.
- Gareis, M., Kabisch, J., Pichner, R., and Hechelmann, H. 2011c. Behavior of Shigatoxigenic/enterohemorrhagic *E. coli* in minisalami. *Fleischwirtschaft International*, **26**, 67–70.
- Gareis, M., Kabisch, J., Pichner, R., and Hechelmann, H. 2012. Behavior of *L. monocytogenes* and *S. aureus* in minisalami. *Fleischwirtschaft International*, **27**, 73–78.
- Geeraerd, A.H., Herremans, C.H., and Van Impe, J.F. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology*, **59**, 185–209.
- Geeraerd, A.H., Valdramidis, V.P., and Van Impe, J.F. 2005. GInaFit, a freeware tool to assess non log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102**, 95–105.
- Gounadaki, A., Skandamis, P., Drosinos, E.H., and Nychas, G.J.E. 2005. Survival of *Listeria monocytogenes* during the fermentation and ripening process of sausages. In: *Proceedings of the 1st Hellenic Symposium in Food Biotechnology and Technology*, pp. 436–441. Association of Greek Chemists and Hellenic Association of Chemical Engineers, Athens, Greece.
- Hinkens, J.A., Faith, N.G., Lorang, T.D., Bailey, P., Buege, D.R., Kaspar, C.W., and Luchansky, J.B. 1996. Validation of pepperoni processes for control of *Escherichia coli* O157:H7. *Journal of Food Protection*, **59**, 1260–1266.
- Hwang, C.-A., Porto-Fett, A.C.S., Juneja, V.K., Ingham, S.C., Ingham, B.H., and Luchansky, J.B. 2009. Modeling the survival of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella Typhimurium* during fermentation, drying, and storage of soudjouk-style fermented sausage. *International Journal of Food Microbiology*, **129**, 244–252.
- Kanellou, G., Paramithiotis, S., Mataragas, M., and Drosinos, E.H. 2013. Field study on the microbiological quality of pickles in brine and survival of *Salmonella Typhimurium* and *Listeria monocytogenes* during storage at 4 °C. *European Food Research and Technology*, **236**, 391–397.
- Lahti, E., Johansson, T., Honkanen-Buzalski, T., Hill, P., and Nurmi, E. 2001. Survival and detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during the manufacture of dry sausage using two different starter cultures. *Food Microbiology*, **18**, 75–85.
- Lindqvist, R. and Lindblad, M. 2009. Inactivation of *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. *International Journal of Food Microbiology*, **129**, 59–67.
- Lucke, F.-K. 2000. Quality and safety issues in fermented meat products. Lecture presented at the Joint Meeting of the Society of Applied Microbiology (UK) and the Estonian Society for Microbiology on *Microbiological Safety of Food*, Tartu, Estonia.
- Mafart, P., Couvert, O., Gaillard, S., and Leguerinel, I. 2002. On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology*, **72**, 107–113.
- Maher, M.M., Jordan, K.N., Upton, M.E., and Coffey, A. 2001. Growth and survival of *E. coli* O157:H7 during the manufacture and ripening of a smear-ripened cheese produced from raw milk. *Journal of Applied Microbiology*, **90**, 201–207.

- Mataragas, M., Bellio, A., Rovetto, F., Astegiano, S., Decastelli, L., and Cocolin, L. 2015a. Risk-based control of food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* in the Italian fermented sausages Cacciatore and Felino. *Meat Science*, **103**, 39–45.
- Mataragas, M., Bellio, A., Rovetto, F., Astegiano, S., Greci, C., Hertel, C., Decastelli, L., and Cocolin, L. 2015b. Quantification of persistence of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* during manufacture of Italian fermented sausages. *Food Control*, **47**, 552–559.
- Mataragas, M., Alessandria, V., Rantsiou, K., and Cocolin, L. 2015c. Evaluation of the *Listeria monocytogenes* inactivation during the post-process storage of fermented sausages: a basis for the development of a decision support tool. *Food Control*, **50**, 568–573.
- Mataragas, M., Rantsiou, K., Alessandria, V., and Cocolin, L. 2015d. Estimating the nonthermal inactivation of *Listeria monocytogenes* in fermented sausages relative to temperature, pH and water activity. *Meat Science*, **100**, 171–178.
- Mataragas, M., Rovetto, F., Bellio, A., Alessandria, V., Rantsiou, K., Decastelli, L., and Cocolin, L. 2015e. Differential gene expression profiling of *Listeria monocytogenes* in Cacciatore and Felino salami to reveal potential stress resistance biomarkers. *Food Microbiology*, **46**, 408–417.
- McQuestin, O.J., Shadbolt, C.T., and Ross, T. 2009. Quantification of the relative effects of temperature, pH, and water activity on inactivation of *Escherichia coli* in fermented meat by meta-analysis. *Applied and Environmental Microbiology*, **75**, 6963–6972.
- Mossel, D.A.A., Corry, J.E.L., Struijk, C.B., and Baird, R.M. 1995. *Essentials of the Microbiology of Foods*. John Wiley & Sons Ltd, Chichester, West Sussex, UK.
- Neter, J., Kutner, M., Nachtsheim, C., and Wasserman, W. 1996. *Applied Linear Statistical Models*. McGraw-Hill, New York, USA.
- Nightingale, K.K., Thippareddi, H., Phebus, R.K., Marsden, J.L., and Nutsch, A.L. 2006. Validation of a traditional Italian-style salami manufacturing process for control of *Salmonella* and *Listeria monocytogenes*. *Journal of Food Protection*, **69**, 794–800.
- Nissen, H. and Hoick, A. 1998. Survival of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella kentucky* in Norwegian fermented, dry sausage. *Food Microbiology*, **15**, 273–279.
- Norrung, B., Andersen, J.K., and Schlundt, J. 1999. Incidence and control of *Listeria monocytogenes* in foods in Denmark. *International Journal of Food Microbiology*, **53**, 195–203.
- Pontello, M., Sodano, L., Nastasi, A., Mammina, C., et al. 1998. A community-based outbreak of *Salmonella enterica* serotype Typhimurium associated with salami consumption in northern Italy. *Epidemiology and Infection*, **120**, 209–214.
- Porto-Fett, A.C.S., Hwang, C.-A., Call, J.E., Juneja, V.K., Ingham, S.C., Ingham, B.H., and Luchansky, J.B. 2008. Viability of multi-strain of *Listeria monocytogenes*, *Salmonella typhimurium*, or *Escherichia coli* O157:H7 inoculated into the batter or onto the surface of a soudjouk-style fermented semi-dry sausage. *Food Microbiology*, **25**, 793–801.
- Ramsaran, H., Chen, J., Brunke, B., Hill, A., Griffiths, M.W. 1998. Survival of bioluminescent *Listeria monocytogenes* and *E. coli* O157:H7 in soft cheese. *Journal of Dairy Science*, **81**, 1810–1817.
- Reitsma, C.J. and Henning, D.R. 1996. Survival of enterohaemorrhagic *E. coli* O157:H7 during the manufacture and curing of cheddar cheese. *Journal of Food Protection*, **59**, 460–464.
- Riordan, D.C.R., Duffy, G., Sheridan, J.J., Eblen, B.S., Whiting, R.C., Blair, I.S., and McDowell, D.A. 1998. Survival of *Escherichia coli* O157:H7 during manufacture of pepperoni. *Journal of Food Protection*, **61**, 146–151.
- Roberts, D. 1990. Sources of infection: food. *Lancet*, **336**, 859–861.
- Ross, T. 1996. Indices for performance evaluation of predictive models in food microbiology. *Journal of Applied Bacteriology*, **81**, 501–508.
- Ryser, E.T. and Marth, E.H. 1987a. Behavior of *Listeria monocytogenes* during the manufacture and ripening of Cheddar cheese. *Journal of Food Protection*, **50**, 7–13.

- Ryser, E.T., and Marth, E.H. 1987b. Fate of *Listeria monocytogenes* during manufacture and ripening of Camembert cheese. *Journal of Food Protection*, **50**, 372–378.
- Saad, S.M.I., Vanzin, C., Oliveira, M.N., and Franco, B.D.G.M. 2001. Influence of lactic acid bacteria on survival of *E. coli* O157:H7 in inoculated Minas cheese during storage at 8.5 °C. *Journal of Food Protection*, **64**, 1151–1155.
- Shay, B.J. 1993. Factors affecting growth and survival of *Salmonella* in fermented salami manufactured under Australian conditions. In: *Proceedings of the Gold Coast Scientific Program*, pp. 23–32. Australian Veterinarians in Public Health and Australian Veterinarians in Industry.
- Smith, J.L., Huhtanen, C.N., Kissinger, J.C., and Palumbo, S.A. 1975. Survival of salmonellae during pepperoni manufacture. *Applied Microbiology*, **30**, 759–763.
- Spahr, U. and Schafroth, K. 2001. Fate of *Mycobacterium avium* subsp. *paratuberculosis* in Swiss hard and semi-hard cheese manufactured from raw milk. *Applied and Environmental Microbiology*, **67**, 4199–4205.
- Sung, N. and Collins, M.T. 2000. Effect of three factors in cheese production (pH, salt, and heat) on *Mycobacterium avium* subsp. *paratuberculosis* viability. *Applied and Environmental Microbiology*, **67**, 1334–1339.
- Taplin, J. 1982. *Salmonella newport* outbreak – Victoria. *Communicable Diseases Intelligence*, **1**, 3–5.
- Teo, A., and Schlessner, J. 2000. Survival of naturally occurring *Escherichia coli* and a streptomycin-resistant strain of *E. coli* K12 (ATCC 35695) during the aging period of hard cheeses made from raw milk. *Journal of Dairy Science*, **83** (Suppl. 1), 109.
- Thevenot, D., Delignette-Muller, M.L., Christeans, S., and Vernozy-Rozand, C. 2005. Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. *International Journal of Food Microbiology*, **102**, 85–94.
- van Boekel, M.A.J.S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology*, **74**, 139–159.
- Vestergaard, E.M. 2001. Building product confidence with challenge studies. *Dairy Food and Environmental Sanitation*, **21**, 206–209.
- Warburton, D.W., Weiss, K.F., Purvis, U., and Hill, R.W. 1987. The microbiological quality of fermented sausage produced under good hygienic practices in Canada. *Food Microbiology*, **4**, 187–197.
- Withers, H. and Couper, J. 2012. Effect of microbial interactions on pathogen growth and survival during fermentation of raw milk – final report. MPI Technical Paper No. 2012/13. Accessed at 19 June 2014, <http://foodsafety.govt.nz/elibrary/industry/microbial-interactions-pathogen-raw-milk/index.htm>.
- Yousef, A.E. and Marth, E.H. 1990. Fate of *Listeria monocytogenes* during the manufacture and ripening of Parmesan cheese. *Journal of Dairy Science*, **73**, 3351–3356.

CHAPTER 12

Impact of forming and mixing operations on the microbial ecology of foods: focus on pathogenic microorganisms

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Forming operations may affect microbial kinetics through changes in the rheological properties of the product or because of the use of physical treatments such as heating, application of pressure, molding, etc. In addition, it is likely that these food processes can lead to cross-contamination phenomena as microorganisms can transfer from contaminated equipment surfaces to foods as a consequence of the mechanical processes taking place in these unit operations. Mixing is the combination of different food materials. This unit operation, in addition to modifying the physical–chemical properties of the product, can affect microorganisms from a physical and biological standpoint: reducing or increasing their concentration and/or prevalence due to the dilution or additive effect produced when different ingredients are added; modifying their spatial distribution by homogenization (i.e., redistribution of microorganisms); or affecting microbial kinetics due to the incorporation of inhibitory or antimicrobial substances during mixing. The development and use of quantitative models can help us to understand better how these unit operations can impact the risk posed by food-borne pathogenic microorganisms. The present chapter carries out a thorough review of the main

factors involved in forming and mixing operations determining pathogenic microbial concentration and prevalence along the food chain. Experimental models are also presented as tools to study such phenomena and generate data for modeling purposes.

12.1 Forming

12.1.1 Description and types

Forming usually involves manipulation of deformable rheological materials, performed manually or automatically by machines. There are multiple processes intended to change food shape, which can be used for hamburger patties, chocolates, jellies, tablets, butter, sausages, variety breads, margarine bars, and cheeses. Forming operations may also affect microbial kinetics through changes in the rheological properties of the product or because of the use of physical treatments such as heating, application of pressure, molding, etc.

Here a list of the most widely used forming methods is presented:

- Compacting
- Pressure
- Extrusion
- Molds

- Powders and binding agents
- Heat and pressure
- Extrusion cooking

Among them, molding and extrusion are the most important forming processes. Generally speaking, extrusion consists of forcing different food materials (mixed) through a perforated plate or die systems designed specifically for the food product. The cooking extrusion is a specific kind of extrusion in which food materials are submitted to a heat treatment together with the extrusion process. This process is usually applied for pasta and cereals (Potter and Hotchkiss, 2012). There are other more specific extrusion processes such as thermoplastic extrusion, which is considered an HTST (high-temperature, short-time) process in the food industry providing a valuable characteristic to products (color, texture, etc.) while nutritional aspects can be better preserved (Steel *et al.*, 2012).

12.1.2 Effect on pathogens/spoilage microorganisms

In most of the cases, the forming process involves pressure application, which can be combined, in some cases, with relatively high heating temperatures (e.g., extrusion cooking). These mechanical and physical phenomena can significantly affect microbial cell viability and reduce microbial load during the forming processes (Georget *et al.*, 2015).

Extrusion usually applies to high temperatures (>85°C), this being effective in destroying vegetative forms of most mesophilic bacteria. The study by Ukuku *et al.* (2012) tested the effect of low-temperature extrusion treatments on *E. coli* at 35, 55, and 75°C in corn meal (CM) and whey protein concentrate (WPC80). Inactivation of *E. coli* bacteria on CM and WPC80 extruded at 35 and 55°C reached reductions of 4.8 log, and 6.9 log and 1.8 log, and 4.3 log, respectively. High temperatures resulted in levels below the detection limit (20 cfu/g) in both extrudates (Ukuku *et al.*, 2012). A similar result was found for *Salmonella typhimurium* by Okelo *et al.* (2006), reporting a total elimination of the pathogen under moderate extrusion conditions (83°C and 7 s)

when samples were inoculated at a maximum level of 8 log cfu/g. By selecting optimum extruder conditions (time, residence time, etc.), most thermophilic bacteria can be eliminated including members of the mesophilic *Bacillus cereus* group, but spores require higher temperatures to get a more significant reduction, although a complete elimination cannot be achieved (Steel *et al.*, 2012). However, extruded products are usually dried, which means water activity is lower than 5%. Thus snack foods and breakfast cereals are safe to eat because the raw materials are subjected to high temperatures (higher than 130°C) and the water activity of the product is low because the product is dried to a moisture content of less than 5%.

The operating conditions under which spores are inactivated are not well understood (Guy, 2001). It has been proved that extrusion can reach maximum spore reductions of 5–8 log at temperatures between 150 and 180°C. However, lower temperatures (e.g., 100°C) can result in injured spores, which can recover if conditions are adequate due to presence of certain nutrients (e.g., L-alanine) and optimum temperatures (Likimani and Sofos, 1990).

The mechanical forces exerted during extrusion can also play an important role in bacteria and spore inactivation due to these forces might cause cell rupture (Fraiha *et al.*, 2011). In this sense, Bulut *et al.* (1999) concluded that the physical forces generated in the reverse screw element and the extruder die (e.g., shear forces) were more determinant than heat in cell destruction.

Furthermore, other factors related to food can affect microbial inactivation as a consequence of the pressure exerted during forming processes. There are solvents in foods that can act as baroprotectants (NaCl, sucrose) (Georget *et al.*, 2015) or synergistic bactericides, reducing or enhancing the effectiveness of microbial inactivation process.

12.1.3 Predictive models

Pathogenic and spoilage bacteria can survive different forming processes and therefore models could be useful validation tools to assess

microbial food safety in this type of processes. As stated above, forming processes, and specifically extrusion, can exert a significant effect on the fate of microorganisms, as a consequence of heat and pressure. Therefore models should be developed by considering both phenomena. Few models have been developed for forming processes, extrusion being the most modeled process. In this sense, the study by Bianchini *et al.* (2012) developed a response surface model (e.g. polynomial model) to describe the effect of moisture (24.9–31.1%) and temperature (67.5–85°C) on inactivation by extrusion of *Enterococcus faecium* NRRL B-2354 in a carbohydrate–protein mix. The model obtained was as follows:

$$y = 185.04 - 3.11X_1 - 4.23X_2 + 0.02X_{12} - 0.004X_1X_2 + 0.08X_{22} \quad (12.1)$$

By applying the model, reduction of *E. faecium* can be estimated at different combinations of moisture content and extrusion temperature. For example, at the temperature of 81.1°C and moisture content of 28.1%, the model predicted a reduction of about 5 log.

Other studies treated the phenomenon as a typical inactivation process by applying the well-known *D*-value concept and assuming a first-order kinetic as presented below (Bigelow, 1921; Bigelow and Esty, 1920):

$$\frac{\partial N_t}{\partial t} = -k'(T)N(t) \quad (12.2)$$

where N_t defines the survival cells at time t and k as the first-order rate constant dependent on temperature (T).

Equation (12.2) can be later developed to result in a more explicit function as described by Equation (12.3), and then related to the *D*-value concept (decimal reduction time) by arranging it to be expressed in logarithms with base 10 (Eq. 12.4):

$$\log S_t = -k(T)t \quad (12.3)$$

where S_t defines the ratio N_t/N_0 (N_0 is the initial number of cells) and $k = k'/\ln(10)$.

$$\log S_t = -\frac{t}{D} \quad (12.4)$$

where S_t defines the ratio N_t/N_0 (N_0 is the initial number of cells), the *D*-value corresponds to the reciprocal of the rate constant, and t stands for treatment time.

Likimani *et al.* (1990) estimated the *D*-value and *z*-values for *Bacillus globigii* at mass temperatures higher than 95°C, using average time, and referred to this as the extrusion *D*-value or extrusion death rate. In this study, the authors also stated that the destruction of spores in the extruder was a function of temperature and residence time at constant moisture.

In line with the *D*-value concept, the work by Ukuku *et al.* (2012) evidenced the existence of a linear relationship between temperatures and pressures applied in extrusion and log-reduction of *E. coli*. On the contrary, Bulut *et al.* (1999) observed a non-linear relationship between the log reduction of *Microbacterium lacticum* and shear stress, even though die pressure and shear rate did show a linear relationship. In fact, most microbial survival curves have a non-log-linear behavior and therefore suitable predictive models should be applied such as biphasic models or the well-known Weibull function, which can reflect the existence of shoulder or asymptote in the inactivation curve (e.g., resistant population). In the case of the Weibull model represented by

$$\log S_t = -\frac{1}{2.303} \left(\frac{t}{\alpha} \right)^\beta \quad (12.5)$$

the model is defined by two parameters, the scale parameter α and the dimensionless shape parameter β . The shape parameter accounts for upward concavity of a survival curve ($\beta < 1$), a linear survival curve ($\beta = 1$), and downward concavity ($\beta > 1$) (Corradini and Peleg, 2007). However, no application of this kind of non-linear model to extrusion or any other forming

process has been found in the reviewed scientific literature.

Bulut *et al.* (1999) proposed that the linear relationship found between time and log-reduction for the application of a “shear D -value” could be defined as the time for a decimal reduction at a specific temperature and shear rate. As an example of this proposal, the authors estimated a D -value for *M. lacticum* at 75°C and a shear rate of 804 s⁻¹ that was equal to 3.5 min approximately. This same D -value was also calculated by only considering heat as the unique factor. As an example, for 75°C in gelatin at 65% water content the D -value was equal to 20 min. As suggested by the authors in this study, a comparison between both types of D -value could provide an insight into the microorganism structure.

12.2 Homogenizing

12.2.1 Description and types

Grinding is a unit operation widely used in the food industry and designed to reduce the size of materials to provide a usable form or to separate their components. Perhaps the most extensive application of grinding in the food industry is in milling of grains to make flour, but it is used in many other processes, such as in the grinding of corn for manufacture of corn starch, the grinding of sugar and milling of dried foods, such as vegetables (Earle and Earle, 1983). These operations can be implemented separately, in combination (dehydration, crystallization followed by grinding), or associated with sorting, mixing agglomeration, or coating. The material is then subjected to mechanical stress leading to an increase in its free energy and fragmentation. The free energy generated can be transformed according to various processes in elastic energy, plastic energy, energy surface, and various other forms of energy. Depending on the nature of the crushed material and technology used, the phenomena of surface or mass amorphization of crystalline solids, of agglomeration, of polymorphic transition, and mechanochemical reactions can be found (Baudelaire, 2013).

12.2.2 Principles of grinding and applications

Three grinding principles known as the energy-size reduction principle, the population balance principle, and the dynamic principle have been formulated for industrial scale-up. Although it can be considered as a rudimentary unit operation, grinding is a complex process that depends on several parameters such as the type of material to be ground, the type of equipment and technology used, and the operating parameters. Grinding was initially used in conjunction with screening for the production of flour from grains and tubers thereafter. Over the last century, the practice has spread to spices and very recently to dehydrated fruits and vegetables (Baudelaire, 2013).

The main grinding applications for the production of food ingredients can be highlighted:

- **Grinding in flour production.** Powders are the most common food meal from grains (wheat, maize, sorghum, millet, etc.) and starches from tubers (potato, cassava, yam, etc.). The objective of grinding is to separate the edible parts of the grain (the endosperm or almond) or the tuber from those that are not (the lignocellulose envelopes). The starches are obtained by grinding cereal grains or dried tuber chips using hammer mills followed by sieving. Flours (wheat, maize, etc.) are obtained by fine grinding (smooth or ribbed metal cylinders well suited to wheat) grains made in stages separated by sieving, while semolina (wheat semolina, cornmeal) consisting of “hominy” (3–6 mm) and of “grits” (0.75–2 mm) result from coarser grinding by hammer mills followed by roller mills.
- **Grinding in spice production.** Some types of grinding are more sophisticated in the spice industry where the value added can be high. These include, for example, the cryogenic grinding of nutmeg (coriander, pepper) and grinding with two pin rotors in a wide chamber without sieving. In the spice industry, there is also “old” crushing with horizontal grinding stones to grind mustard

seeds previously diluted in a liquid of vinegar called “sour grapes”.

- **Grinding in fruit and vegetable powder production.** The grinding characteristics of some vegetables such as carrots (Chakkaravarthi *et al.*, 1993), coconut (Raghavendra *et al.*, 2006), and some legumes (Indira and Bhattacharya, 2005) have been studied. Several studies on the production of unripe mango powder have been carried out in India (Dabhade and Khedar, 1980), where powder obtained and commonly called “Amchur” is void of vitamins and is mostly consumed as a spice.

The result of a grinding operation depends both on the properties of the food material (hardness, plasticity, abrasion, adhesion, shape) and also the nature, the distribution, and the intensity of the stress applied by the grinding tool. The contact forces applied to a solid create, depending on its density and orientation, a network of cracks that would determine the size and shape of the fragments resulting from material breakdown. Studies on the deformation of materials (Klimpel and Austin, 1969) has distinguished two types of breakdown, that is, brittle breaking and ductile breaking, that describe several categories of materials (brittle, semi-brittle, plastic, or complex).

Another limitation in the milling process is that some foods grind easily while others have a poor grinding ability. The grinding ability can be limited by intrinsic (water content, sugar content, etc.) and extrinsic (the glass transitions and caking, etc.) factors. The control of these factors is essential to optimize grinding (Baudelaire, 2013).

12.2.3 Effect on pathogens/spoilage microorganisms

Provided dry foods remain dry after milling there are negligible changes caused by microorganisms (Fellows, 2009). Berghofer *et al.* (2003) found that the most frequently detected microorganisms in wheat and wheat flour were *Bacillus* spp., coliforms, yeasts, and molds. The most common molds isolated were *Aspergillus*

spp., *Penicillium* spp., *Cladosporium* spp., and *Eurotium* spp. When wheat grain components become separated during milling, contaminants are concentrated in the bran and wheat germ (the outer layers of the grain) while the inner endosperm fraction contains lower microbial counts. The quality of incoming wheat has an important influence on the microbiological quality of the products, but the authors found higher microbiological counts midway in the milling process, which indicated that contamination from equipment may also contribute to the observed contamination (Fellows, 2009).

The peel on intact fruits and vegetables is a physical and biochemical barrier to most microorganisms, but the damaged surfaces caused by size reduction release cellular nutrients that provide a suitable substrate for microbiological growth. Additionally, the microenvironment surrounding packed fresh sliced foods provides conditions that increase the numbers and types of microorganisms that develop, and can result in significant changes to the microflora. For example, the risk of pathogenic bacteria such as *Clostridium* spp., *Yersinia* spp., and *Listeria* spp. growing on minimally processed sliced fruit and vegetable products (e.g., cantaloupe, watermelon, cucumber, lettuce, and coleslaw vegetables) may increase as a result of the high relative humidity and lower oxygen concentrations inside film packaging, especially if products are stored above 5°C. Conversely, low temperatures may select psychrotrophic spoilage microorganisms such as *Pseudomonas* spp. There are also greater opportunities for contamination by pathogens from increased handling of fresh-cut products. Abdul-Raouf *et al.* (1993) reported the growth of *Escherichia coli* O157:H7, psychrotrophic, and mesophilic microorganisms on shredded lettuce, sliced cucumber, and shredded carrot stored under different storage temperatures simulating those routinely used in commercial practice.

Sliced cold meat products have a high risk of contamination by pathogenic and spoilage microorganisms unless food-handling guidelines are strictly observed. The slicing operation

may increase the microbial load on products via blades, as well as increasing nutrient availability as a result of tissue damage. Even with stringent hygienic conditions and chilling, extensive handling before and after slicing may cause significant contamination of cold meat products. Voidarou *et al.* (2006) found contamination by the bioindicators *E. coli*, *Staphylococcus aureus*, and *C. perfringens* on sliced turkey, pork ham, smoked turkey, and smoked pork ham.

12.3 Mixing

12.3.1 Description and types

The food processing industry relies heavily on mixing to ensure delivery of a product with constant properties in order to achieve characteristics that are important for the final product, such as texture, homogeneity, composition, and temperature. Nowadays, a large majority of processed ready-to-eat products are indeed multiphase dispersions: solids dispersed in liquids (e.g., canned), emulsions (e.g., soups, margarines, and spreads), or bubbly dispersions (merengue, ice cream, sponge cake, among others) and consumers expect to have the same amount of each ingredient to meet their nutritional needs (Niranjan, 1995).

Mixing is an unit operation used in the chemical, pharmaceutical and food process industries. Its main objectives are to increase homogeneity of a system by reducing non-uniformities or distribution of composite or proprieties or temperature, control heat and mass transfer rates, reactions and structural changes (Harnby *et al.* 2001; Cullen, 2009). Mixing is therefore recognized as one the most widely practiced food-processing operations (Muthukumarappan, 2009). Different types of system are required for the different requirements of the food industry, for example:

- **Gas-liquid mixing.** Ensuring that enough air is mixed into a fermenter liquid to ensure microbial growth is not oxygen-limited.
- **Liquid-liquid mixing.** The creation of liquid-liquid emulsions is key to the manufacture of margarines and spreads.

- **Solid-liquid mixing.** The addition of solids to liquids involved in the reconstitution of fluids such as the addition of coffee, milk, and sugar to hot water. The addition of liquid to solid systems is relevant to the production of many food batters, pastes, and doughs.
- **Solid-solid mixing.** Ensuring that all the ingredients in a cereal box are mixed uniformly to provide a consistent nutritional value according to labeling, for texture effects, or to provide a specific taste.

When food products are mixed there are a number of aspects that differ from other industrial processes:

- Mixing is often used primarily to develop desirable product characteristics, rather than simply ensure homogeneity.
- It is often multicomponent, involving ingredients of different physical properties and quantities.
- It very often involves high-viscosity or non-Newtonian liquids.
- Some components may be fragile and damaged by overmixing.
- There may be complex relationships between mixing parameters and product characteristics that may change as mixing proceeds.

The criteria for successful mixing have been described as first achieving an acceptable product quality (in terms of sensory, properties, functionality, homogeneity, particulate, and integrity) followed by adequate safety, hygienic design, legality (compositional standards for some foods), process, and energy efficiency, and flexibility to changes in processing (Campbell, 1995; Fellows, 2009).

12.3.2 Principles and applications

The mixing operations occur widely throughout the food processing industries and the primary objective in mixing is to achieve a homogeneous mixture; generally, this means attaining a nearly uniform distribution of the ingredients for improving food quality, such as texture and color development. Food mixtures involve many ingredients, including liquids, powders, and gases. Some important ingredients are

contained only in minor quantities, which should be dispersed evenly and efficiently in the final mixture (Levine and Behmer, 1997).

High viscosity and non-Newtonian fluids require special mixing equipment. Mixing patterns and product characteristics are related in a complex manner. Scale-up of food mixing is based more on constant food properties than on constant power/volume ratios. High shear stresses, induced by agitators, are required for making fine dispersions and emulsions, while low shears are used for mixing solid particles/pieces in solid or liquid phases. Segregation of particles from mixed products should be prevented (Saravacos and Maroulis, 2011).

The mixing of food pastes and doughs is accomplished in specialized equipment, developed empirically by equipment manufacturers and industrial users. Dough mixing and processing are important operations in the baking, pasta, and cereal process industries. Doughs are more solid-like and viscoelastic than those of usual pastes (Saravacos and Maroulis, 2011).

Mixing of protein doughs increases their viscoelasticity and gas-holding capacity, essential requirements for bread making. Gluten development during dough mixing (kneading) is based on the alignment, uncoiling, extension, and folding of the protein molecules. Dough mixers are usually batch units, and can be vertical (most common) or horizontal (Matz, 1989).

12.3.3 Equipment

A wide variety of equipment for mixing is used in the food processing industry, and new mixing technologies are constantly emerging on the industrial scenario. The selection of the correct type of mixer for a particular application depends on the type and amount of food being mixed and the speed of operation needed to achieve the required degree of mixing (Fellows, 2009; Jakubczyk and Nirajan, 2006).

The performance of a mixer can be tailored to a particular process by adjusting different combinations of agitators, speeds, and shear

rates (Ames, 2000). Alternatively, the sequence and rate in which ingredients are added in a process can be altered to enhance the capabilities of a mixer. For example, the lowest-viscosity ingredient is added first because it takes less power to mix it (Anonymous, 2004).

Mixers can be grouped into types that are suitable for:

- dry powders or particulate solids;
- low- or medium-viscosity liquids;
- high-viscosity liquids and pastes;
- dispersion of powders in liquids.

12.3.4 Effect on pathogens/spoilage microorganisms

The main effect of mixing is to increase the uniformity of products by evenly distributing ingredients throughout the bulk. Changes in nutritional value are therefore dependent on the amount and types of ingredient that are added rather than the mixing action. Mixing has no direct effect on the shelf life of a food, but it may have an indirect effect by intimately mixing added components and causing a reaction among them (Fellows, 2009). When food products are mixed, various units are joined together (e.g., when milk from different cows is assembled in a milk tank or cuts of different carcasses are joined for grinding). As a consequence of this process, microorganisms are redistributed, resulting in an increase in prevalence and a decrease in the mean concentration in contaminated food units (Bassett *et al.*, 2012). This is likely to lead to a more random spatial distribution and a changing of the number of cells per portion, for example, per unit of weight or volume (Figure 12.1). In general, mixing will disperse the microbial populations. Mixing can be an active process or it can be a result of, for instance, spontaneous movements caused by temperature or concentration differences in liquids (Bassett, 2010).

There is little published information on the effect of mixing operations on microorganisms. It is unlikely that the shearing conditions or temperature in a mixer would reduce the number of contaminating microorganisms and

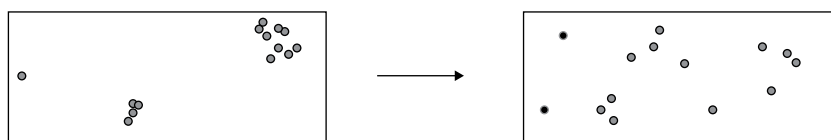


Figure 12.1 Mixing food ingredients results in rearrangement of the distribution of microorganisms in a food product.

hence mixing does not have a preservative effect. In some instances, especially where the temperature of the food is allowed to rise during mixing, there may be an increase in numbers of microbial contaminants, caused in part by the greater availability of nutrients as a result of the mixing action (Fellows, 2009).

Although these processes do not result in an increase or decrease in the number of microorganisms in the total amount of food produced, they change the distribution of microorganisms over food items. This has an impact on the variability of doses between servings, and therefore may have an impact on the risk assessment (Bassett *et al.*, 2012).

References

- Abdul-Raouf, U.M., Beuchat, L.R., and Ammar, M.S. 1993. Survival and growth of *Escherichia coli* 0157: H7 on salad vegetables. *Applied and Environmental Microbiology*, **59**, 1999–2006.
- Ames, G. 2000. High shear mixing advances for foods, pharmaceuticals, cosmetics. *Mixing, Blending and Size Reduction Handbook*, pp. 2–4. Admix Inc.
- Anonymous. 2004. *IFST Current Hot Topics: Genetic Modification and Food*. Institute of Food Science and Technology, UK.
- Basset, J. 2010. *Impact of Microbial Distributions on Food Safety*, pp. 7–11. International Life Sciences Institute, Brussels, Belgium.
- Bassett, J., Nauta, M., Linqvist, R., and Zwietering, M. 2012. *Tools for Microbial Risk Assessment*, pp. 13–18. International Life Sciences Institute, Brussels, Belgium.
- Baudelaire, E.D. 2013. Grinding for food powder production. In: *Handbook of Food Powders: Process and Properties* (eds Bhandari, B., Bansal, N., Zhang, M., and Schuck, P.), pp. 132–149. Woodhead Publishing Limited, Philadelphia, PA, USA.
- Berghofer, L.K., Hocking, A.D., Miskelly, D., and Jansson, E. 2003. Microbiology of wheat and flour milling in Australia. *International Journal of Food Microbiology*, **85**, 137–149.
- Bianchini, A., Stratton, J., Weier, S., Hartter, T., *et al.* 2012. Validation of extrusion as a killing step for *Enterococcus faecium* in a balanced carbohydrate-protein meal by using a response surface design source. *Journal of Food Protection*, **9**, 1548–1720.
- Bigelow, W.D. 1921. The logarithmic nature of thermal death time curves. *Journal of Infectious Diseases*, **27**, 528–536.
- Bigelow, W.D. and Esty, J.R. 1920. The thermal death point in relation to typical thermophilic organisms. *Journal of Infectious Diseases*, **27**, 602–617.
- Bulut, S., Waites, W.M., and Mitchell, J.R. 1999. Effects of combined shear and thermal forces on destruction of *Microbacterium lacticum*. *Applied Environmental Microbiology*, **65**, 4464–4469.
- Campbell, G.M. 1995. New mixing technology for the food industry. In: *Food Technology International Europe* (ed. Turner, A.), p. 119–122. Sterling Publications International, London, UK.
- Chakkaravarthi, A., Math, R.G., Walde, S.G., and Rao, D.G. 1993. Grinding characteristics of carrots (*Daucus carota*, L.). *Journal of Food Engineering*, **20**, 381–389.
- Corradini, M.G. and Peleg, M. 2007. A Weibullian model for microbial injury and mortality. *International Journal of Food Microbiology*, **119**, 319–328.
- Cullen, P.J. 2009. Food mixing: principles and applications. In: *Mixing in the Food Industry: Trends and Challenges* (Cullen, P.J. and O'Donnel, C.P.), pp. 1–5. Wiley-Blackwell, UK.

- Dabhade, R.S. and Khedar, D.M. 1980. Studies of drying and dehydration of raw mangoes for preparation of mango powder VI. Changes in chemical constituents of raw mango powder during storage. *Indian Food Packer*, **34**, 42–47.
- Earle, R.L. and Earle, M.D. 1983. *Unit Operations in Food Processing*, 2nd edn. Pergamon Press, Oxford, UK. Available at: <http://www.nzifst.org.nz/unitoperations/sizereduction1.htm#grinding>. Accessed: 18 October 2015.
- Fellows, P.J. 2009. *Food Processing Technology: Principles and Practice*, 3rd edn, pp. 157–187. CRC Press, UK.
- Fraiha, M., Ferraz, A., and Biagi, J. 2011. Pre-treatment of thermophilic spores in CO₂ modified atmosphere and their survivability during food extrusion. *Ciência e Tecnologia de Alimentos*, **31**, 167–171.
- Georget, E., Sevenich, R., Reineke, K., Mathys, A., et al. 2015. Inactivation of microorganisms by high isostatic pressure processing in complex matrices: a review. *Innovative Food Science and Emerging Technology*, **27**, 1–14.
- Guy, R. 2001. *Extrusion Cooking: Technologies and Applications*. Woodhead Publishing, Cambridge, UK.
- Harnby, M., Edwards, M.F., and Nienow, A.W. 2001. *Mixing in the Process Industries*. Butterworth-Heinemann, Oxford, UK.
- Indira, T.N. and Bhattacharya, S. 2005. Grinding characteristics of some legumes. *Journal of Food Engineering*, **76**, 113–118.
- Jakubczyk, E. and Niranjana, K. 2006. Transient development of whipped cream properties. *Journal of Food Engineering*, **77**, 79–83.
- Klimpel, R. and Austin, L.G. 1969. The statistical theory of primary breakage distributions for brittle materials. *Trans. AIME*, **284**, 88–94.
- Levine, L. and Behmer, E. 1997. Dough processing systems. In: *Handbook of Food Engineering Practice* K. J. (eds Valentas, K.J., Rotstein, E., and Singh, R.P.). CRC Press, New York, USA.
- Likimani, T.A. and Sofos, J.N. 1990. Bacterial spore injury during extrusion cooking of corn/soybean mixtures. *Journal of Food Microbiology*, **11**, 243–250.
- Likimani, T.A., Sofos, J.N., Maga, J.A., and Harper, J.M. (1990). Methodology to determine destruction of bacterial spores during extrusion cooking. *Journal of Food Science*, **55**, 1388–1393.
- Matz, S.A. 1989. *Equipment for Bakers*. Elsevier Science Publications, London, UK.
- Muthukumarappan, K. 2009. Mixing fundamentals. In: *Food Mixing: Principles and Applications* (ed. Cullen, P.J.). Blackwell, UK.
- Niranjan, K. 1995. An appraisal of the characteristics of food mixing. In: *Food Process Design and Evaluation* (ed. Singh, R.K.), pp. 47–67. Technomics, Lancaster, PA, USA.
- Okelo, P.O., Wagner, D.D., Carr, L.E., Wheaton, F.W., Douglass, L.W., and Joseph, S.W. 2006. Optimization of extrusion conditions for elimination of mesophilic bacteria during thermal processing of animal feed mash. *Animal Feed Science and Technology*, **129**, 116–137.
- Potter, N. and Hotchkiss, J.H. (eds). 2012. *Food Science*, 5th edn. Springer Science.
- Raghavendra, S.N., Ramachandra, S.S.R., Rastogi, N.K., Raghavarao, K.S.M.S., Sourav, K., and Tharanathan, R.N. 2006. Grinding characteristics and hydration properties of coconut residue: a source of dietary fiber. *Journal of Food Engineering*, **72**, 281–286.
- Saravacos, G.D. and Maroulis, Z.B. 2011. *Food Process Engineering Operations*, pp. 184–190. CRC Press, Boca Raton, FL, USA.
- Steel, C., Leoro, M.G.V., Schmiele, M., Ferreira, R.E., and Chang, Y.K. 2012. Thermoplastic extrusion in food processing. In: *Thermoplastic Elastomers* (ed. El-Sonbati, A.), pp 265–290. InTech, Croatia.
- Ukuku, D., Onwulata, C., and Mukhopadhyay, S. 2012. Behavior of *Escherichia coli* bacteria in whey protein concentrate and corn meal during twin screw extrusion processing at different temperatures. *Journal of Food Process and Technology*, **3**, 1–4. Doi: 10.4172/2157-7110.1000150.
- Voidarou, C., Tzora, A., Alexopoulos, A., and Bezirtzoglou, E. 2006. Hygienic quality of different ham preparations. In: *IUFoST 13th World Congress of Food Sciences Technology*, 17–21 September, Nantes, France. Available at: <http://dx.doi.org/10.1051/IUFoST:20060771>. Access: 21 October 2015.

CHAPTER 13

Impact of specific unit operations on food-borne microorganisms: curing, salting, extrusion, puffing, encapsulation, absorption, extraction, distillation, and crystallization

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13.1 Introductory remarks

Food-borne diseases represent a major cause of morbidity and mortality in the general population, particularly in vulnerable groups. Food-borne illnesses result from consumption of food containing pathogens or by food contaminated by poisonous chemicals or biotoxins. Although most of the food-borne illness cases are mild and self-limiting, severe incidents can occur in high risk groups, such as infants, young children, and the elderly (Fleury *et al.*, 2008). Food-borne disease outbreaks have become difficult to control due to the globalization of the food market and changing patterns of human consumption, with fresh and minimally processed foods currently being preferred (Schelin *et al.*, 2011). Since food is a biological material it is capable of supporting the growth of microorganisms, and food-borne diseases result from the ingestion of contaminated foods and food products (Guyader and Atmar, 2008). Although more than 250 different types of viruses, bacteria, parasites, toxins, metals, and prions are associated with food-borne diseases in humans (Schmidt *et al.*, 2009), in general terms hospitalizations and deaths associated with food-borne infections are due to bacterial

agents. Food intoxication results from consumption of toxins (or poisons) produced in food by bacterial growth. The toxins, and not the bacteria, are those responsible for illness. Toxins may not alter the appearance, odor or flavor of food. The most common types of toxin-producing bacteria include *Staphylococcus aureus* and *Clostridium botulinum*.

13.2 Burden of food-borne illnesses

Over 200 different diseases are known to be transmitted via food and the incidence of food-borne illnesses is increasing worldwide (Bryan, 1982). In developing countries, diarrheal diseases affect more than 1.5 billion individuals annually with a death toll of 1.8 million (WHO/FOS, 2000). Beyond the direct adverse health impacts, food-borne illnesses cause huge financial burdens. The 2006 United States outbreak of *Escherichia coli* O157:H7 in spinach led to US\$100 million in losses for the spinach industry due to product recalls and lost sales (Porter and Lister, 2006). A single outbreak ($n=43$) of Hepatitis A caused by a single infected food handler would cost

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US\$800,000 (Koopmans *et al.*, 2002). The United Kingdom spends £300–700 million while Australia spends AU\$2.6 billion, annually, on food-borne illnesses (WHO/FOS, 2000). Increasing incidences of food-borne illnesses are attributed to adjustments needed to accommodate for globalization of the food supply and variations on consumer demographics. Changes in production, processing, packaging, and distribution systems are mainly due to globalization. Geographically expansive distribution systems allow for the dissemination of food products as well as the hazards that they may contain, potentially leading to large-scale outbreaks of food-borne illnesses.

13.3 Food safety and food quality

There is a need of assuring that foods will not cause harm or include harmful substances within, or on, them prior to consumption (Alli, 2004). It is generally assumed that all food available for human consumption has met safety standards and requirements ensuring that consumption will not cause harm (Harrison and Hester, 2001). In most cases food safety is an implicit part of food quality. The safety and reliability of the food supply has been, however, affected in recent times by scares threatening human health. Outbreaks of food contamination and food poisoning result in long-lasting damage to market demand and credibility of food companies, brands, and regulatory bodies (Grunert, 2002). Food safety is integrated within food quality assurance and quality control programs. Quality control and quality assurance are schemes concerned with meeting standards, regulations, and maintaining consistency of predetermined products and may reflect quality at the manufacturing or production level (Harrison and Hester, 2001). Food quality assurance sets targets for policies, programs, systems, and procedures concerning foodstuffs (Muller and Steinhart, 2006). Quality assurance

is concerned with assurance and consistency within food products while food quality control is closely tied to the manufacturing processes, to ensure that raw materials meet quality specifications.

The presence of pesticides, allergens, contaminants, residues, physical hazards, biological and pathogenic organisms, as well as other undesirable contents, all disrupt quality perception and expectations (Muller and Steinhart, 2006). Food safety is not, however, the entire responsibility of the producer or manufacturer. Correct handling, preparation and storage of foods are also the responsibility of the intermediate consumer (restaurants and retailers), as well as the end consumer (Alli, 2004). Consumers can partially evaluate food safety prior to purchase by checking indirect indicators such as color, expiry date, smell, and condition of products (Grunert, 2002). These sensory and labeling features provide consumers with some guidance for choosing safe foods, but judgments of quality are subjective and based on personal experiences, expectations, and perceptions about food (Brunso *et al.*, 2005).

13.4 Prevention and control through processing

13.4.1 Introduction: unit operations in food processing

There are about forty unit operations commonly employed in the food industry (Brennan *et al.*, 1990; Fellows, 2000; Zeuthen and Bogh-Sorensen, 2003). Due to the complexities of food processes, a standardized classification of unit operations for the food industry would be difficult to establish. A proposed list of unit operations as normally utilized in the food industry is given in Table 13.1. These operations may be broadly divided into the following four categories: preliminary processes, conversion operations, preservation techniques, and separation techniques. While some of the listed unit operations are common to either chemical or

Table 13.1 Classification of unit operations in the food industry.

Unit operation	Example
Preliminary processes	
Classification	Sorting, grading
Depuration	Cleaning, peeling, de-skinning, de-husking
Preparation	Coring, filleting, trimming, stemming
Physical conversions	
Size reduction	Cutting, slicing, dicing, shredding, milling, grinding, pulverizing
Size enlargement	Agglomeration, granulation, instantiation, coagulation, flocculation
Shaping and re-shaping	Sheeting, tableting, flaking, puffing
Mixing and emulsification	Blending, dispersing, aerating, kneading, homogenization
Mass transfer operations	Extraction, leaching, rehydration, absorption
Others	Encapsulation, coating
Chemical conversions	
Biochemical	Fermentations, enzymatic conversions
Purely chemical	Caramelization, alkalinization, acidification (pickling)
Preservation techniques	
Elevated temperature	Blanching, pasteurization, sterilization, canning, dehydration
Room temperature	Salting/brining, irradiation, high-pressure processes, high-intensity fields processes
Low temperature	Chilling, freezing, freeze-concentration, freeze drying
Others	Packaging, coating, waxing
Separation techniques	
Solid-solid	Screening, leaching
Solid-gas	Cyclonic separation, air filtration, air classification
Solid-liquid	Sedimentation, centrifugation, filtration
Liquid-liquid	Distillation, extraction
Liquids with dissolved solids	Membrane separations, crystallisation

food processes (e.g., distillation or extraction), differing only in the raw materials used, some others can be considered specific to the food industry, such as blanching.

13.4.2 Unit operations for preservation

Food preservation normally involves preventing the growth of microorganisms (bacteria, molds, yeasts, or other), as well as inhibiting or retarding chemical reactions, which cause undesirable effects on its safety, quality, and palatability. The need for providing satisfying, nutritious, and economical food to a growing population has caused the development of a huge food preservation industry worldwide. Thermally processed foods constitute a large part of this industry and, as such, they are

often referred as conventional food preservation technologies.

13.4.2.1 Use of heat: sterilization and pasteurization

Thermal processing implies the controlled use of heat to increase or reduce the rates of reactions in foods. As shown in Figure 13.1, the effects of temperature range in inhibiting growth of pathogens in food are well defined. In general terms there are two types of processes: sterilization and pasteurization. The object of sterilisation is to destroy all microorganisms, i.e. bacteria, yeasts, and molds, in the food material to prevent decomposition of the food, which makes it unattractive or inedible, as well as to prevent any pathogenic organisms from surviving and being eaten with the food.

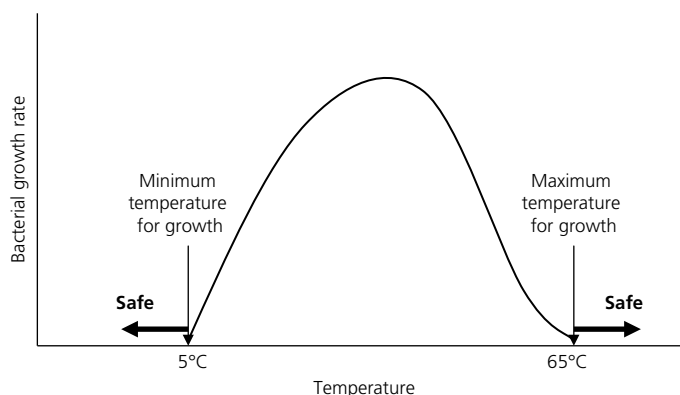


Figure 13.1 Temperature range of growth for most human pathogenic bacteria.

Pathogenic toxins may be produced during storage of the food if certain organisms are still viable. Microorganisms are destroyed by heat, but the amount of heating required for the killing of different organisms varies. Also, many bacteria can exist in two forms, the vegetative or growing form and the spore or dormant form. The spores are much harder to destroy by heat treatment than the vegetative forms. Sterilization is generally conducted at temperatures over 100°C. Commercial sterility is defined as the condition where microorganisms capable of growing in the food product during storage and distribution at room temperatures are inactivated during processing. Commercially sterile canned foods are safe and stable when stored on a dry shelf. These products are high-moisture foods packaged in hermetically sealed containers. Containers can be made of glass, metal, or multilayer polymers and laminates with adequate mechanical resistance to abuse and adequate oxygen barrier properties.

Heat processing below 100°C is usually termed pasteurization and is generally designed to kill all pathogenic organisms and some of the spoilage organisms that would be capable of growing in foods under defined storage conditions. Pasteurization is a common way of treatment of liquid foods, such as dairy products, fruit, and vegetable juices, as well as different brewing products. Both batch and

continuous methods of pasteurization can be used for fluid foods, and the treatment may be carried out before or after placing the product in the container. In batch pasteurization, individual volumes are treated in jacketed stainless steel vessels that may be used both for heating (with steam or hot water) and cooling (with chilled water or brine). Continuous pasteurization may be carried out by passage through plate heat exchangers, which usually comprise the stages of pre-heating, heating, holding, and cooling (Figure 13.2). Traditional pasteurization methods for food drinks include thermal processing either as a low temperature–long time (LTLT) method or a high temperature–short time (HTST) technique. The method known as ultra high temperature (UHT), or aseptic processing, has also been employed for treatment of beverages. Currently, HTST pasteurization is the mode commonly used for heat treatment of beverages. HTST pasteurization is a continuous process that shows several advantages over batch pasteurization. UHT processing involves the production of a sterile product by rapid heating to high temperatures followed by a short holding time and ending with rapid cooling. The processed product is filled into a pre-sterilized container within a sterile environment, to provide a prolonged shelf life.

Heat treatment of foodstuffs is often accompanied by undesirable changes such as loss of nutritional quality and sensory attributes. The

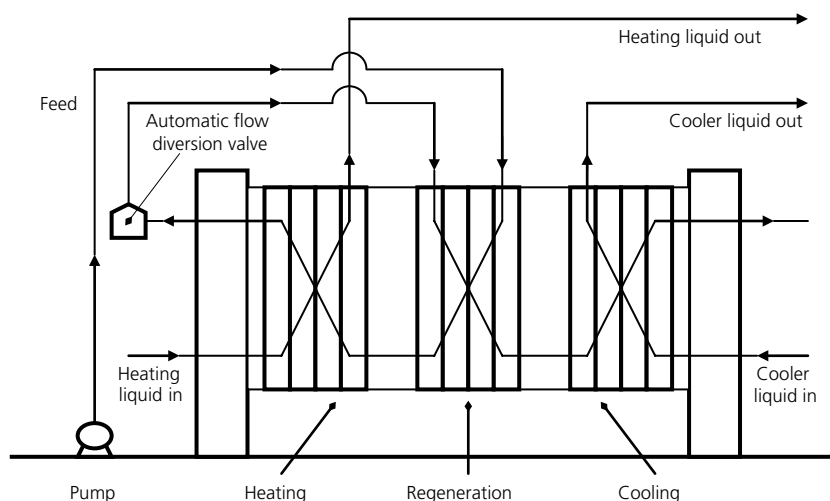


Figure 13.2 Diagram of continuous pasteurization using a plate heat exchanger.

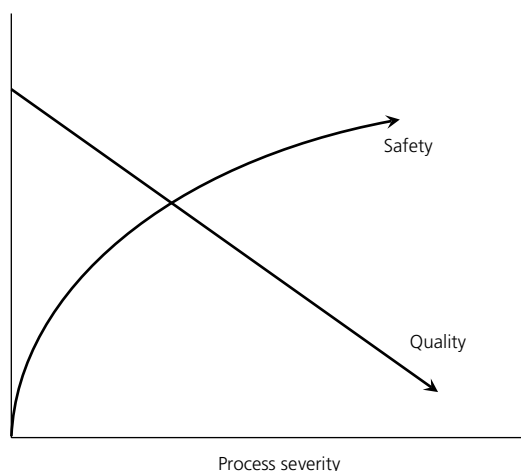


Figure 13.3 Effects of increasing process severity on safety and quality.

severity of thermal processing, normally expressed as temperature and holding time, is directly proportional to safety, but indirectly proportional to quality as a whole (Figure 13.3). Principles of chemical kinetics dictate that, in general, reaction velocity duplicates roughly with increasing temperature by 10°C . A number of biochemical reactions triggered by a temperature increase in food processing will cause irreversible effects in nutritional and sensory aspects of the foods being processed. Heat

treatment should therefore be carefully optimized, i.e., a compromise needs to be reached between temperature intensity and holding time. The best combination of heating and holding time would lead to a safe, stable product, with minimum sensory and nutritive impairment. Bacterial and enzyme inactivation, as well as biochemical reactions, follow well-established kinetics, which are the basis to thermal processing calculations, in order to obtain the well-balanced product.

The preservative effect of heat processing is due to the denaturation of proteins, which destroys enzyme activity and enzyme-controlled metabolism in microorganisms. The rate of destruction is a first-order reaction, so when food is heated to a temperature high enough to destroy contaminating microorganisms, the same percentage die in a given time interval regardless of the numbers present initially. This is known as the logarithmic order of death and can be represented by a death rate curve. The time necessary to destroy 90% of the microbial population, i.e., that time needed to reduce cell numbers by a factor of 10, is referred as the decimal reduction time or D value. This value differs for different microbial species, with a higher value of D indicating greater heat resistance. Figure 13.4 shows a death rate curve.

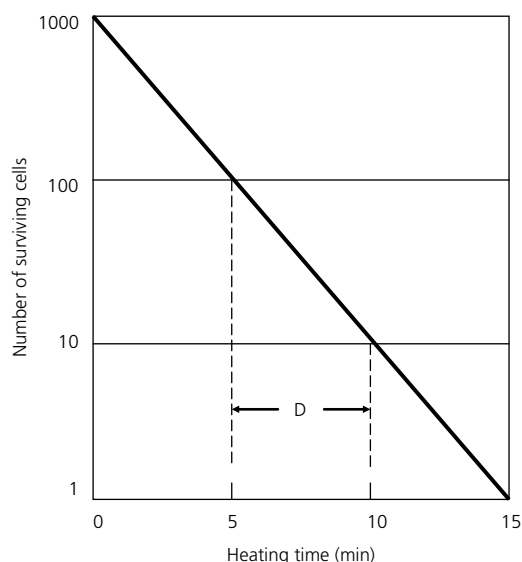


Figure 13.4 Death rate curve.

By collating D values at different temperatures, a thermal death time (TDT) curve is derived. The slope of the TDT curve is termed the z value and is defined as the number of degrees Celsius required to bring about a tenfold change in decimal reduction time. The D and z values are used to characterize the heat resistance of enzymes, microorganisms, or chemical components of a food. Figure 13.5 illustrates a thermal death time curve.

The food-borne illness associated with canned foods is botulism caused by toxin production when the anaerobic spore-forming microorganism *Clostridium botulinum* grows in the food prior to consumption. Food products at risk for botulism are those where the water activity is above 0.85 and the pH values of the contents of a container prior to thermal processing are above 4.6. Some products acidified to a pH of 4.6 and below, but originally at a high pH before acidification may also carry some risk for botulism. These food products are considered low acid or acidified foods, respectively. The former require thermal processing at temperatures above 120°C in high-pressure steam or water to inactivate spores of bacteria. The latter require relatively

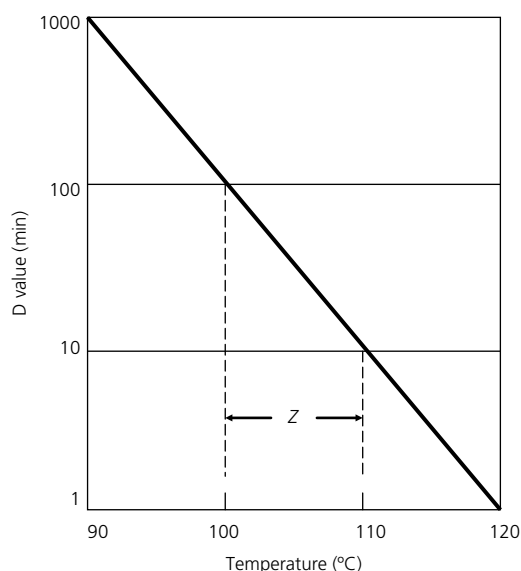


Figure 13.5 Thermal death time curve.

mild temperatures around 100°C but care must be taken to ensure that the product pH inside the container must be at 4.6 or below within 24 hours after thermal processing. Advances in thermal processing technology have resulted in virtual elimination of food poisoning outbreaks from commercial thermally processed foods. Although there were still about 20 to 30 cases of botulism outbreaks per year in the United States from 1978 to 1994, these outbreaks were mainly due to foods prepared in the kitchen and improperly handled after cooking or home-canned foods that were inadequately processed (Bell and Kyriakides, 2000). The last botulism outbreak in the United States from commercially processed canned soup that resulted in fatalities occurred in 1982. Two nationwide recalls of canned products necessitated by the threat of botulism occurred in 2000 and 2007 (FDA/CFSAN, 2008). The 2000 recall involved canned creamed soups and the 2007 recall involved several types of canned chilli and canned French cut green beans.

Pertaining to pasteurized products, acidic foods (pH <4.6) such as fruit juices are stable at ambient conditions with shelf lives in the order

of months, and are not traditionally considered vehicles for food-borne pathogenic bacteria after pasteurization. There have been, however, three pathogens (*Salmonella enterica*, *Escherichia coli* O157:H7, and *Cryptosporidium parvum*) associated with food-borne illnesses in fruit juices. The main outbreaks involving *S. enterica* and *E. coli* O157:H7 occurred in apple juice and orange juice. *E. coli* O157:H7 was confirmed, in 1991, as the epidemiological agent in apple juice. Fruit juice processors in the United States are required to have pasteurization treatments that result in 5 log reductions of the most resistant microorganism of public health concern (Mazzotta, 2001). The National Advisory Committee on Microbial Criteria for Foods has recommended *E. coli* O157:H7 and *L. monocytogenes* to be used as appropriate target microorganisms for pasteurization of fruit juices. For the case of low-acid (pH >4.6) food products, such as milk, a shorter shelf life (days) is obtained after pasteurization and refrigerated storage is needed to maintain them safe by restricting the growth of surviving pathogens. Low-acid pasteurized foods, such as milk, dairy products, vegetable products, sauces, and some carbonated drinks, are stored, transported, and sold under refrigerated conditions and with a specifically restricted shelf life, in order to minimize the outgrowth of pathogenic microbes. Table 13.2 summarizes pasteurization characteristics for food products of different pH ranges.

13.4.2.2 Treatment at room temperature: curing, salting

Curing is a preservation method that was originally developed for treatment of foods by addition of sodium chloride, so it was referred to as either curing or salting. Curing has been generally applied to certain foods, such as meat, fish and vegetables, by adding, apart from salt, nitrates, nitrites, or sugar. Curing is also used for the purpose of flavor or color enhancement. Some parts of the literature distinguish the use of salt alone as salting, corning, or salt curing, while other parts reserve the term curing for the use of salt with nitrates/nitrites. The cure ingredients can be rubbed on to the food surface, mixed into foods, or dissolved in water (brine, wet, or pickle curing). In the latter processes, the food is submerged in the brine until completely covered. With large cuts of meat, brine may also be injected into the muscle. The term pickle in curing has been used to mean any brine solution or a brine cure solution that has sugar added.

Salt addition inhibits microbial growth by plasmolysis as water is drawn out of the microbial cell by osmosis due to the higher concentrations of salt outside the cell. A cell loses water until it reaches a state where it cannot grow and cannot survive any longer. The concentration of salt outside of a microorganism needed to inhibit growth by plasmolysis depends on its genus and species. The growth of some bacteria is inhibited by salt concentrations as low as 3%, e.g.,

Table 13.2 Pasteurization characteristics for food products of different pH values.

Food product	pH	Target microorganism	Log reductions
Apple juice	<3.5	<i>Escherichia coli</i> O157:H7	5
Egg in shell	>7	<i>Salmonella</i>	5
Egg products	>7	<i>Salmonella enteritidis</i> , <i>Salmonella typhimurium</i>	7
Milk and milk products	~6.5–7	<i>Mycobacterium tuberculosis</i> , <i>Coxiella burnetii</i>	5
Orange juice	<3.5	<i>Escherichia coli</i> O157:H7, <i>Salmonella</i>	5
Ready-to-eat meals	>4.6	<i>Listeria</i>	5–7

Salmonella, whereas other types are able to survive in much higher salt concentrations. For example, up to 20% salt concentration is needed to inhibit growth of *Staphylococcus* or up to 12% salt concentration is required to prevent growth of *Listeria monocytogenes*. Fortunately the growth of many undesirable organisms normally found in cured meat and poultry products is inhibited at relatively low concentrations of salt.

The use of sodium nitrite as curing salt plays an important role in inhibiting the growth and toxin production of *Clostridium botulinum* in cured products (Giese, 1994). Treatment concentration in excess of 100 mg/kg may be sufficient for protection against typical microflora. For example, it has been reported that a concentration of 200 mg/kg of sodium nitrite was capable of inhibiting strains of *Achromobacter*, *Aerobacter*, *Escherichia*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas* species in cured products (Woods *et al.*, 1990). Since the 1970s there has been concern about a possible link between nitrite and cancer. There is no conclusive evidence that nitrite is directly carcinogenic (Cantor, 1997), but in high doses it has been implicated as a co-carcinogen (Schweinsberg and Burkle, 1985). It has been shown to induce mutations in some bacterial strains of *Salmonella typhimurium* used for detection of base-pair substitutions (Balimandawa *et al.*, 1994). Some epidemiological studies have suggested a link between dietary nitrates and nitrites, and the incidence of cancer. Different studies, at different times, have failed to show a correlation between dietary nitrite and gastric cancer (Forman *et al.*, 1985; van Loon, 1998).

13.4.3 Unit operations of physical conversions

13.4.3.1 Shaping: extrusion, puffing

Extrusion cooking is a high-temperature, short-time process in which moistened, expansive, starchy, and/or protein-rich food materials are plasticized and cooked in a tube by a combination of moisture, pressure, temperature, and mechanical shear, resulting in molecular transformation and chemical reactions (Castells

et al., 2005). This technology has some unique positive features compared with other heat processes because the material is subjected to intense mechanical shear. In extrusion cooking covalent bonds in biopolymers are broken with a consequent intense structural disruption and mixing, which facilitate the modification of functional and textural properties of food ingredients (Carvalho and Mitchelle, 2000). In addition, the extrusion process denatures undesirable enzymes, inactivates some antinutritional factors, sterilizes the finished product, and retains natural colors and flavors of foods (Bhandari *et al.*, 2001). The process has found numerous applications, including ready-to-eat (RTE) cereals, salty and sweet snacks, indirect expanded products, croutons for soups and salads, textured meat-like materials from defatted high-protein flours, nutritious precooked food mixtures for infant feeding, and confectionery products (Eastman *et al.*, 2001). As previously mentioned, extrusion cooking generally renders sterilized finished products with a low moisture content (10–15%), which is often lowered even more (2–3%) by drying in order to ensure the typical crispness of many extruded products. For this reason, microbial contamination of extruded products may not be considered an issue. There have been, however, reported studies on the use of extrusion processing to reduce microbial counts in food ingredients. Likimani *et al.* (1990) proposed a methodology for determining the destruction of bacterial spores in a single-screw extruder under varying processing conditions. The *D* value for extrusion was calculated on the basis of average time at a mass temperature of >95°C. They stated that destruction of spores in the extruder was a function of mass temperature and residence time at constant moisture levels (18%) in a corn–soybean (70%/30% wt/wt) mixture. Increasing mass temperature resulted in greater lethality, while increasing screw speed, which reduced residence time, resulted in lesser lethality. Using the same system as mentioned above, Likimani and Sofos (1990) examined the potential for injury of *Bacillus globigii* spores

during extrusion cooking. Their results showed that extrusion processing at low barrel temperatures (80 to 100°C) resulted in injury to spores of *B. globigii*, whereas at higher temperatures spore injury was not detectable.

Puffed cereal refers to grains like wheat, millet, corn, or rice, which are made by exploding cooked cereal from a pressure chamber, thus expanding the grain to several times its original size. The principle of the puffing process is heating the cereal, and sometimes other vegetable products, in a pressure chamber in the presence of steam. When the containment seal of the vessel is suddenly broken, the entrained steam flashes and bloats the endosperm of the kernel, increasing its volume (Fast, 2000). Rice and wheat are the two main cereals whose granules can be processed individually in order to expand or inflate them, in order to obtain RTE products. Puffed cereals are also consumed as snack foods and as a popular street food in some parts of the world. Similarly to the case of extruded products, puffed cereals are obtained with a low moisture content and are not considered, generally, at great risk of post-process microbial contamination. Over the past several decades, however, a number of outbreaks of salmonellosis have been associated with the consumption of RTE low-moisture products, including children's snacks made of puffed rice. These outbreaks emphasize the difficulty of eradicating *Salmonella* from the environment of dry product manufacturing facilities, while highlighting the need to reinforce industry preventive control measures through guidance based on the best available information (Scott *et al.*, 2009).

13.4.3.2 Encapsulation

Encapsulation is a process employed for entrapping active agents within a carrier material and is a useful tool to improve the delivery of bioactive molecules and living cells into foods. It involves the incorporation of food ingredients, enzymes, flavoring agents, acids, bases, artificial sweeteners, coloring agents, preservatives, leavening agents, antioxidants, cells, or other materials,

into small capsules. Applications for this technique have increased in the food industry since encapsulated materials can be protected from moisture, heat, or other extreme conditions, thus enhancing their stability and maintaining viability. Materials used for the design of a protective shell of encapsulates must be of a food grade, biodegradable, and able to form a barrier between the internal phase and its surroundings. Among all materials, the most widely used for encapsulation in food applications are polysaccharides. Proteins and lipids are also appropriate for encapsulation. Different technologies are used to form encapsulates and include spray drying, spray chilling, spray cooling, extrusion coating, and fluidized bed coating. Spray drying is the most extensively applied encapsulation technique in the food industry because it is flexible and continuous, but more importantly because it represents an economical operation.

Encapsulation has not been widely used as a food preservation technology. An example of such use is the inactivation of *Listeria* in processed foods. *Listeria* represents a high risk in processed meat. An encapsulation technique consisting of a core containing two substrates (glucose and thiocyanate), coated first with two enzymes and then with protecting polymers, has been developed (Jacquot, 2000). Under a dry form, the mix is stable for a long period, but as soon as it is in contact with moisture, it starts to release an end product with high bacteriostatic properties against *Listeria*. The system allows not only a long-term action, but also easier handling and dosage of the complex substrate–enzyme mix.

13.4.3.3 Mass transfer: gas absorption and extraction

Mass transfer operations involve the diffusion of a component to be separated through a stationary phase and include gas absorption and liquid extraction. Gas absorption is a process of removal of small amounts of impurities in a vapour phase by contacting a liquid phase, which dissolves them. The absorbed gas is removed from the solvent and such solvent is

recovered and returned to the system by a process called stripping. The stripping agent is either a gas or a superheated vapour. Absorption and stripping are achieved by bringing gas and liquid phases into intimate contact using units in which the solvent enters at the top of the absorber/stripper and flows down while a rising gas stream flows countercurrently. The two phases mix and contact one another in plates or packing elements and the solute is transferred from the gas phase to the solvent. Applications of absorption technology in food processing are limited. Stripping is used in the food industry for deodorization of vegetable oils. Free fatty acids and other organic materials (aldehydes or ketones) may cause undesirable flavors and aromas in edible oils. Typically, a steam-stripping process is used to selectively remove those undesirable chemicals from the oil. Steam stripping is based on the differences in concentration between free fatty acids and the main triglycerides of vegetable oils. Free fatty acids possess some finite solubility in steam while triglycerides are essentially insoluble. When steam contacts the vegetable oil, free fatty acids and other volatiles are removed, leaving essentially pure triglycerides in the liquid oil.

Extraction is a unit operation used to recover valuable soluble components from raw materials by primarily dissolving them in a liquid solvent and, subsequently, removing them from such a liquid. Extraction works according to the principle that soluble components can be separated from insoluble or less soluble components by dissolving them in a suitable solvent. Liquid-liquid extraction, or simply extraction, is the term used when both streams in the extraction are liquid. Solid-liquid extraction is sometimes called leaching. Commonly, the extract is the product or intermediate product and the residue is a waste or by-product. It is not always the objective to recover one particular compound in a pure form from a raw material, i.e., sometimes extraction is intended to separate all the soluble compounds from the residue. The efficiency of the extraction process depends on the selectivity of the solvent. Common solvents

include water, alcohol, organic solvents, and supercritical carbon dioxide. Examples of extraction in food processing include the extraction of sugar from sugar-beets or sugar-cane, of oil from oilseeds and from virgin pomace, of coffee extract from coffee beans, and of various other compounds, such as proteins, pectins, vitamins, pigments, essential oils, aroma compounds, or flavour compounds, from many different materials.

Washing is almost identical to extraction with the main distinction being that in washing the inert material is the required product and the solvent used is water, which is cheap and readily available. Washing could be considered a special leaching process, when an undesirable component is removed from a solid with water. Examples of washing in food processing are butter and casein, as well as cheese washing, in the dairy industry.

Apparently, antimicrobial or food safety effects of gas absorption, extraction, and washing in food processing are topics not extensively discussed in the literature.

13.4.4 Unit operations based on separation techniques

13.4.4.1 Liquid-liquid separations: distillation

Distillation is a method of separating mixtures based on differences in their volatilities in a boiling liquid mixture. Distillation is a unit operation or a physical separation process, and not a chemical reaction. Commercially, distillation has a number of applications. It is used to separate crude oil into more fractions for specific uses such as transport, power generation, and heating. Water is distilled to remove impurities, such as salt from seawater. Distillation of fermented solutions has been used since ancient times to produce distilled beverages with a higher alcohol content. In the food industry distillation can be used to separate flavors or essential oils, but is mainly used either for the production of potable alcohol or spirits, or for the industrial production of alcohol from agricultural raw materials

(e.g., fruit, grain), which can then be used in alcoholic beverages. Distillation is intended to concentrate volatile components of foods and, therefore, to alter their flavour and aroma. Distillation is not a preservation operation but destroys microorganisms partly as a result of heating during the process and partly by the high concentrations of alcohol or essential oils (e.g., terpenes, such as citral, β -pinene, and β -cymene), which have antimicrobial properties (Belletti *et al.*, 2004; Smith-Palmer *et al.*, 1998).

13.4.4.2 Liquids with dissolved solids: crystallization

The process of forming crystals from solution is known as crystallization. It constitutes a physical method of separation to obtain the solid in a pure form. Crystallization is a term that describes several different phenomenon related to the formation of a crystalline lattice structure. In the food industry, crystallization is used for two specific purposes: firstly, to separate out a solid phase of different composition from the liquid phase when one or both the fractions may be valuable and, secondly, to control or bring about desirable changes to the texture of the solid product without separating fractions. Crystallization is often a four-step process that includes the generation of a supersaturated state, the formation of nuclei or nucleation, the growth of crystals or propagation, and the perfection of crystals or maturation. Controlling crystallization in food processing requires control of the relative rates of nucleation and growth. Crystallization may serve for the recovery of crystalline products, such as sugar, glucose, lactose, citric acid, or salt, or for the removal of certain undesirable components or modifications of certain food products in order to obtain a desirable structure.

The manufacture of sucrose, from sugar-cane or sugar-beet, is an important example of crystallization in food technology. Crystallization is also used in the manufacture of other sugars, such as glucose and lactose, in the manufacture of food additives, such as salt, and in the processing of foodstuffs, such as ice cream. Most

of the crystallized foods are dried as end products and, as such, they present a low risk of pathogenic microbial contamination. Sugar can be considered, undoubtedly, as one of the main food products obtained by crystallization. Cane-sugar has never been associated with food-borne poisoning outbreaks, so reference to *Salmonella* spp. in connection to microbial criteria (Chen and Chung, 1993) should be considered as part of the verification applied to raw materials used in different food formulations. The processing and refining of sugar eliminate vegetative pathogenic microorganisms present in the raw materials.

13.5 Conclusions and prospects for the future

As stated earlier, changing patterns in global food production have resulted in food being distributed over large distances. Such a fact, combined with increasing integration and consolidation of agriculture and food production, can result in a contaminated food rapidly causing a geographically widespread outbreak. This chapter reviewed the main impact of some unit operations used in food processing and preservation, focusing on the considered conventional or predominant food processing technologies. Alternatively, non-predominant food technologies not relying on heat (which may also cause undesirable effects) have been recently the subject of research and study. These technologies include ultra-high hydrostatic pressure and high-voltage pulsed electric fields, and could have an enormous impact on the food industry. The evaluation of processing effects on safety of preserved food materials is a task needed to determine viability of novel techniques, as feasible alternatives to traditional processes in order to meet consumer demands. Another important measure aimed at preventing outbreaks of food-borne related illnesses is the establishment of food safety programs in the food and related industries. A food safety program is a set of actions and systems designed

to minimize the occurrence of food safety hazards within a food production, processing, and/or preparation facility. The program is documented through a written food safety plan that addresses all possible hazards, establishes control measures, and specifies corrective actions.

References

- Alli, I. 2004. *Food Quality Assurance: Principles and Practices*. CRC Press, Boca Raton, FL, USA.
- Balimandawa, M., Demeester, C., and Leonard, A. 1994. The mutagenicity of nitrite in the *Salmonella*/Microsome test system. *Mutation Research*, **321**, 7–11.
- Bell, C. and Kyriakides, A. 2000. *Clostridium botulinum: A Practical Approach to the Organism and Its Control in Foods*. Blackwell Science, Oxford, UK.
- Belletti, N., Ndaguimana, M., Sisto, C., Guerzoni, M.E., Lanciotti, R., and Gardini, F. 2004. Evaluation of the microbial activity of citrus essences on *Saccharomyces cerevisiae*. *Journal of Agriculture and Food Chemistry*, **52**, 6932–6938.
- Bhandari, B., D'Arcy, B., and Young, G. 2001. Flavour retention during high temperature short time extrusion cooking process: a review. *International Journal of Food Science and Technology*, **36**, 453–461.
- Brennan, J.G., Butters, J.R., Cowell, N.D., and Lilly, A.E.V. 1990. *Food Engineering Operations*. Elsevier, London, UK.
- Bryan, F.L. 1982. *Diseases Transmitted by Foods*, 2nd edn. US Department of Health and Human Services, Center for Professional Development and Training, Atlanta, GA, USA.
- Cantor, K.P. 1997. Drinking water and cancer. *Cancer Causes and Control*, **8**, 292–308.
- Carvalho, C.W.P., Mitchelle, J.R. 2000. Effect of sugar on the extrusion of maize grits and wheat flour. *International Journal of Food Science and Technology*, **35**, 569–576.
- Castells, M., Marin, S., Sanchis, V., and Ramos, A.J. 2005. Fate of mycotoxins in cereals during extrusion cooking: a review. *Food Additives and Contamination*, **22**, 150–157.
- Chen, J.C.P. and Chung, C.C. 1993. *Cane Sugar Handbook. A Manual for Cane Sugar Manufacturers and Their Chemists*. John Wiley & Sons Ltd, Chichester, UK.
- Eastman, J., Orthoefer, F., and Solorio, S. 2001. Using extrusion to create breakfast cereal products. *Cereal Foods World*, **46**, 468–471.
- Fast, R.B. 2000. Manufacturing technology of ready-to-eat breakfast cereals. In: *Breakfast Cereals and How They Are Made* (eds Fast, R.B. and Caldwell, E.F.), pp. 29–34. American Association of Cereal Chemists, St Paul, MN, USA.
- FDA/CFSAN 2008. US Food and Drug Administration, Center for Food Safety and Applied Nutrition. Available online at: <http://www.fda.gov/cfsan/subject/canned food recalls>.
- Fellows, P.J. 2000. *Food Processing Technology: Principles and Practice*. Woodhead Publishing Ltd, Cambridge, UK.
- Flcury, M.D., Stratton, J., Tinga, C., Charron, D.F., and Aramini, J. 2008. A descriptive analysis of hospitalization due to acute gastrointestinal illness in Canada, 1995–2004. *Canadian Journal of Public Health*, **99**, 489–493.
- Forman, D.A., Al-dabbagh, S., and Doll, R. 1985. Nitrates, nitrites and gastric cancer in Great Britain. *Nature*, **313**, 620–625.
- Giese, J. 1994. Antimicrobials: assuring food safety. *Food Technology*, **48**, 101–110.
- Grunert, K.G. (2002) Current issues in the understanding of consumer food choice. *Trends in Food Science and Technology*, **13**, 275–285.
- Guyader, F.S. and Atmar, R.L. 2008. Binding and inactivation of viruses on and in food, with a focus on the role of matrix. In: *Foodborne Viruses: Progress and Challenges* (eds Koopmans, M.P.G., Clive, D.O., and Bosch, A.), pp. 189–208. ASM Press, Washington DC, USA.
- Harrison, R.M. and Hester, R.E. 2001. *Food Safety and Food Quality: Issues in Environmental Science and Technology*. Royal Society of Chemistry, Cambridge, UK.
- Jacquot, M., Revol-Junelles, A.M., Millière, J.B., Miclo, A., and Poncelet, D. 2000. Anti *Listeria monocytogenes* activity of the lactoperoxidase system (LPS) using encapsulated substrate. *Minerva Biotechnologica*, **12**, 301–304.

- Koopmans, M., von Bonsdorff, C.-H., Vinjé, J., de Medici, D., and Monroe, S. 2002. Foodborne viruses. *FEMS Microbiology Reviews*, **26**, 187–205.
- Likimani, T.A. and Sofos, J.N. 1990. Bacterial spore injury during extrusion cooking of corn/soybean mixtures. *International Journal of Food Microbiology*, **11**, 243–250.
- Likimani, T.A., Sofos, J.N., Maga, J.A., and Harper, J.M. 1990. Methodology to determine destruction of bacterial spores during extrusion cooking. *Journal of Food Science*, **55**, 1388–1393.
- Mazzotta, A.S. 2001. Thermal inactivation of stationary-phase and acid-adapted *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *Journal of Food Protection*, **64**, 315–320.
- Muller, A. and Steinhart, H. 2006. Recent developments in instrumental analysis for food quality. *Food Chemistry*, **102**, 436–444.
- Porter, D.V. and Lister, S.A. 2006. *CRS Report for Congress: Food Safety: Federal and State Response to the Spinach E. coli Outbreak*. Available online at: <http://www.nationalaglawcenter.org/assets/crs/RL33722.pdf>.
- Schelin, J., Wallin-Carlquist, N., Cohn, M.T., Lindqvist, R., Barker, G.C., and Rådström, P. 2011. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence*, **2**, 580–592.
- Schmidt, R.H., Goodrich, R.M., Archer, D.L., and Schneider, K.R. 2009. *General Overview of the Causative Agents of Foodborne Illness*, pp. 1–5. Food Science and Human Nutrition Department, Florida Cooperative Extension Service, IFAS, University of Florida. Publication: FSHN033.
- Schweinsberg, F. and Burkle, V. 1985. Nitrite: a co-carcinogen? *Journal of Cancer Research and Clinical Oncology*, **109**, 200–202.
- Scott, V.N., Chen, Y., Freier, T.A., et al. 2009. Control of *Salmonella* in low-moisture foods I: Minimizing entry of *Salmonella* into a processing facility. *Food Protection Trends*, **29**, 342–353.
- Smith-Palmer, A., Stewart, J., and Fyfe, L. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Applied Microbiology*, **26**, 118–122.
- van Loon, A.J. 1998. Intake of nitrate and nitrite and the risk of gastric cancer: a prospective cohort study. *British Journal of Cancer*, **78**, 129–135.
- WHO/FOS (World Health Organization Food Safety Programme). 2000. *Foodborne Illness: An Increasing Public Health Concern*. Available online at: http://www.wpro.who.int/fsi_guide/files/foodborne_illness.ppt.
- Woods, L.F.J., Wood, J.M., and Gibbs, P.A. 1990. Nitrite. In: *Mechanisms of Action of Food Preservation Procedures* (ed. Gould, G.W.), pp. 225–246. Elsevier Applied Science, London.
- Zeuthen, P. and Bogh-Sorensen, L. 2003. *Food Preservation Techniques*. Woodhead Publishing Ltd, Cambridge, UK.

CHAPTER 14

Impact of food unit operations on virus loads in foods

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14.1 Introduction

Food-borne viruses are enteric viruses (18 to 70 nm in diameter) able to infect human hosts. They are defined by several features that result in different detection methodologies and survival and inactivation characteristics compared to food-borne bacterial pathogens. Since (food-borne) viruses need specific host cells to replicate (in contrast to bacteria), infection of the host and replication of the food-borne viruses occur in the intestinal tract of the host organism. Viral particles are thereafter excreted in stool or emesis, mostly in high concentrations.

Most common food-borne viruses were discovered in the 1970s (noroviruses (NoV), hepatitis A virus (HAV), rotaviruses (RoV), sapoviruses (SaV)), followed by less frequent observed food-borne viruses such as hepatitis E virus (HEV) and aichiviruses (Oh *et al.*, 2006). NoVs and HAV are recognized as the two most important food-borne viruses, of which NoVs are by far associated with the highest number of cases of food-borne microbial infectious disease (Scallan *et al.*, 2011) and therefore will be the primary focus in this chapter.

The structure of food-borne viruses is quite diverse, although all have a non-enveloped protein capsid protecting a nucleic acid genome. Although food-borne viruses cannot replicate in the environment, they are resistant to environmental stressors, such as heat, high

or low pH, drying, light, and UV exposure (Baert *et al.*, 2009; Vasickova *et al.*, 2010). These resistances allow most food-borne viruses to pass the gastric fluids in the gut and the alkaline/proteolytic activity in the duodenum, permitting infection of the lower digestive tract. Moreover, the high persistence allows most food-borne viruses to retain their infectivity in foods such as lettuce, shellfish, frozen soft red fruits, herbs, and turkey meat for periods ranging between two days up to four weeks (Bidawid *et al.*, 2001; Butot *et al.*, 2008; Hewitt and Greening, 2004). In this chapter we will discuss the impact of food unit operations on virus loads in foods.

14.2 The use of surrogate viruses to assess inactivation processes

As mentioned above, since food-borne viruses need specific host cells to replicate, it is difficult to determine the infectivity of food-borne viruses with cell or animal models. Therefore, molecular methods, in particular reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is still the most commonly used detection method of food-borne viruses. However, the correlation between viral particles and genomic copies is not clear. So far, the prediction for viral infectivity has been attempted

by investigating the integrity of a viral RNA molecule and capsid protein, which are the two essential parts for an intact and infectious virus particle. For the genome integrity evaluation, although it is possible to amplify near full-length human NoV genomes, amplification efficiency decreases with fragment size, thus making amplification of full-length genomic RNA relatively insensitive. Wolf *et al.* (2009) suggested that one important factor for assessing genomic integrity of RNA viruses is the dependency of the reverse transcription (RT) reaction on high-quality, non-fragmented template RNA. This fact can be exploited by separating the PCR amplification site and RT priming site within the virus genome. On the other hand, multiple studies have been conducted to assess the number of infectious NoVs based on the capsid integrity/function such as RNase, propidium monoazide (PMA), or cell binding pretreatments prior to the RT-qPCR (Li *et al.*, 2011a; Nuanualsuwan and Cliver, 2002; Parshionikar *et al.*, 2010; Topping *et al.*, 2009).

Surrogates that share pathological and/or biological features with human NoVs have been used to study the stability of human NoVs. Feline calicivirus (FCV), murine norovirus 1 (MNV-1), Tulane virus, as well as bacteriophages such as Coliphage MS2 and *Bacterioides fragilis* phage B40-8 have been proposed and used as indicator systems for enteric viral pathogens and as tools for studying viral disinfection. The sensitivity of the surrogates to certain inactivation treatments can be different. For instance, in complex matrices for inactivation at temperatures $\geq 50^{\circ}\text{C}$, the following ranking of heat sensitivity among frequently used (surrogate) viruses was observed: poliovirus > FCV > MNV > HAV > *Bacterioides fragilis* phage > Simian rotavirus > PhiX174 > F-specific RNA phages (e.g., MS2) (Bertrand *et al.*, 2012).

In general, the reduction of viral infectivity of these surrogates are far higher than the reduction of corresponding RT-qPCR signals after inactivation treatments. In a recent systematic review study (Knight *et al.*, 2016), the data extracted from a number of studies and

different treatments, e.g., temperature, sodium hypochlorite, and UV suggested that RT-qPCR signals for human NoVs were more persistent following treatments compared with RT-qPCR signals from surrogate viruses. Therefore, the data generated from different studies should be interpreted with care considering the methodologies used.

14.3 Virus contamination in food processing

Figure 14.1 illustrates the major transmission routes of food-borne viruses. Food processing refers to the procedures in between primary food production and storage/distribution. Virus contamination in food processing can occur either from the incoming (raw) materials or during processing, of which the contamination can come directly from infected food handlers or indirectly via food contact materials (working benches, conveyer belts, utensils, equipment, or contaminated water). In the following the three most probable sources for virus contamination in food processing will be discussed.

14.3.1 Raw materials

In recent years, NoV positive signals generated by RT-qPCR have been obtained during NoV prevalence studies covering food and the environment. Shellfish, in particular bivalves, could bioaccumulate viral particles from a large quantity of water and are quite often consumed uncooked. Fresh produce (leafy greens, berries, etc.) are generally consumed without effective inactivation treatment to get rid of the contaminated viruses. Therefore these two categories are two main at-risk food types for NoV transmission (Ethelberg *et al.*, 2010; Le Guyader *et al.*, 2008).

Observed prevalence ranged from 3.9 to 76.2% in shellfish (Loutreul *et al.*, 2014; Lowther *et al.*, 2012; Nishida *et al.*, 2003; Woods and Burkhardt, 2010), from 6.7 to 55.5% in fresh produce (Baert *et al.*, 2011; Loutreul *et al.*, 2014), and from 15.1 to 45% in drinking water

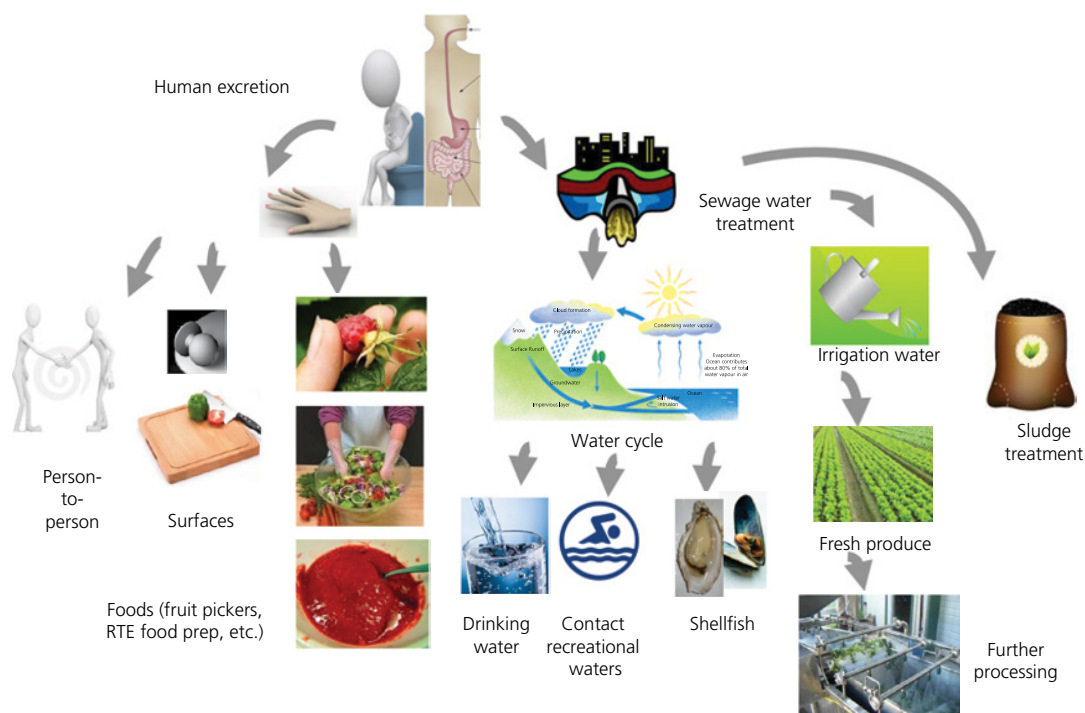


Figure 14.1 Transmission routes of food-borne viruses.

sources and surface water (El-Senousy *et al.*, 2013; Lodder *et al.*, 2010; Steyer *et al.*, 2011). In our laboratory, a convenience study was performed detecting NoVs from various batches of shellfish and fishery products from a Belgian seafood company over a 6 month period (Li *et al.*, 2014). For the intact shellfish (oysters, mussels, and clams), 21 of 65 samples from 12 of 34 batches were positive for NoVs; 9 samples contained quantitative NoV levels at 3300 to 14 300 genomic copies per gram. For the semiprocessed fishery products (scallops and common sole rolls with scallop fragments), 29 of 36 samples from all eight batches were positive for NoVs; 17 samples contained quantitative NoV levels at 200 to 1800 copies per gram. De Keuckelaere *et al.* (2015) collected a total of 130 samples of frozen raspberries from 26 batches in four different raspberry processing companies for NoV detection. As a result, six out of 70 frozen raspberry samples serving raspberry puree production (14 batches) provided a

NoV RT-qPCR signal above the limit of quantification confirmed by sequencing. The mean NoV level in 20 g of these samples was 4.3 log genomic copies of NoV GI/20 g. For the individually quick-frozen raspberries, one batch out of 12 tested NoV positive, but only 1 out of the 5 samples analyzed in this batch showed a positive RT-qPCR GI NoV signal confirmed by sequencing. The RT-qPCR signal was below the limit of quantification of the assay used (<3.7 log genomic copies/20 g). These results indicate that the raw materials are an important contamination source of virus load in food industries.

To ensure compliance with relevant food safety management systems that fully take into account the risk posed by NoVs, and notwithstanding the limitations of current NoV detection methods, it is likely to be a useful parameter to be used for verification of GMP and HACCP applied to berries during processing. In spite of the studies mentioned above, currently data on detection of NoV in foods in non-outbreak

situations is still scarce, making it highly uncertain in terms of type and number of corrective actions at processing as well as of the benefits for public health. However, sampling and testing might be useful as a tool for verification and to raise awareness on good hygienic practices during handling of foods at individual processing premises, e.g., where rapid alerts, observational audits, or epidemiological studies indicated a higher risk of infection or any other information on potential breaches in “good practices” at the discretion of the food business operator.

14.3.2 Food handlers

NoV-infected food handlers, who can shed large quantities of viruses, play an important role in NoV transmission, especially for cooked or semi-cooked products. The risk of contamination posed by an infected food handler can depend on personal factors specific to a food handler, including, for example, the phase of clinical infection that impacts the degree of virus shedding, personal hygiene habits, and a variety of behavioral factors such as the willingness to work when feeling ill. Currently, there seem to be still large gaps in food handlers’ education on the prevention of NoV transmission. Verhoef *et al.* (2013) investigated possible associations between reported behavior, knowledge and awareness of NoV, and the environmental presence of NoV, by conducting face-to-face interviews using structured questionnaires in 1023 catering companies, 101 non-hospital health care centers, 52 hospital central kitchens, and in 102 hospital in-patient units. Three surface swabs were taken at each setting as well. Several gaps in education and training were identified, demonstrating that knowledge on NoV was low, although awareness of NoV was significantly higher among food handlers in institutional settings than in catering companies.

In reality, the introduction routes of virus load to food products can be complicated. A recent study in our laboratory developed a quantitative exposure model simulating human NoV transmission during preparation of deli

sandwiches (Stals *et al.*, 2015). This model simulated the preparation of the deli sandwiches and contained the NoV reservoirs, locations allowing the accumulation of NoV and the working of intervention measures. NoV shedding food handlers were found to play a more important role in the NoV contamination in the sandwiches than the initial NoV contaminated lettuce used on the sandwiches. High compliance of hand washing after a restroom visit did reduce the NoV presence substantially on all reservoirs.

In addition, it should be well noted that infected food handlers can involve both symptomatic as well as asymptomatic food handlers and asymptomatic food handlers can also shed similar high viral loads (Ozawa *et al.*, 2007). Moreover, asymptomatic infections with NoV are quite common. For instance, up to 14% of analyzed feces samples of asymptomatic food handlers working at a non-outbreak related facility in Japan tested positive for GII NoV (Okabayashi *et al.*, 2008). Furthermore, asymptomatic food handlers have been implicated in food-borne outbreaks (Barrabeig *et al.*, 2010; Daniels *et al.*, 2000).

14.3.3 Cross-contamination during food processing

Cross-contamination occurs when viruses are transferred from a contaminated surface/material to one that is not contaminated in food processing. The viruses can come from people, work surfaces or equipment, other foods, and other food-contacting materials.

Equipment such as knives used for trimming, conveyor belts, or utensils used for processing may act as vehicles for cross-contamination. For example, a study using MNV-1 as a model demonstrated that knives and graters processing contaminated fresh produce items including cucumbers and tomatoes can become contaminated by the virus and cross-contaminate subsequently processed items (Wang *et al.*, 2013).

Although water is a useful tool for reducing potential contamination, several publications both in real life and lab-scale showed that washing water of inadequate quality has the

potential to be a direct source of contamination and a vehicle for spreading localized bacterial and viral contamination. Holvoet *et al.* (2014) conducted a quantitative study of microbial cross-contamination in a simulated commercial fresh-cut lettuce washing process. Two cross-contamination processes (from lettuce to water and from water to lettuce) were included. As a result, it was found that the microbial load of the water in the washing baths, initially starting off with potable water, increased rapidly during the washing process of the contaminated lettuce if no sanitizers were used. Furthermore, when the washing bath was inoculated with 6.5 log pfu/100ml MS2 phages or NoV surrogate MNV-1, the contamination of the subsequently washed lettuce can be as high as 3.7 log pfu/g lettuce for MS2 and 4.4 log pfu/g lettuce for MNV-1. These data further highlighted the vulnerability of fresh produce to cross-contamination during the washing stage and stresses that notwithstanding the use of initial potable water and partial refreshment of water but without the use of sanitizers, microbial pathogens (or indicator organisms) may easily be introduced and reside for prolonged periods in washing baths, enabling cross-contamination to the final fresh-cut product.

14.4 Survival of virus in the food processing chain

As mentioned above, food-borne viruses are capable of infecting a host following oral ingestion, and need to be able to pass the stomach and those proximal parts of the small intestine with contents that are rich in digestive enzymes and bile salts. Therefore, food-borne viruses are generally stable outside the host and remain infectious in the environment or on food. Once the virus contamination is introduced in the food processing line, it is difficult to get rid of unless specific intervention measurements have been taken accordingly.

Table 14.1 summarizes some recently reported studies on the survival of food-borne

viruses and surrogates under different conditions, indicating that these viruses prefer cold, dark, and wet conditions, while sensitive to alkaline pH and some natural foods such as pomegranate juice (Horm and D'Souza, 2011; Wei *et al.*, 2010) with unknown mechanisms. Organic components, especially proteinaceous matrix, could offer a protection effect for the viruses from the adverse conditions to remain infectious for a long time (Dawson *et al.*, 2005; Takahashi *et al.*, 2011).

14.5 Effect of food preservation techniques on the virus load

14.5.1 Reduction of pH

Reduction of pH is one of the most commonly used strategies in food preservation. It can be achieved by directly adding food acidulants or indirectly by fermentation (lactic acid fermentation). Due to the survival of food-borne viruses in gastric fluids in the gut, it can be assumed that most food-borne viruses are quite acid resistant. It was reported that HAV infectious units were present after 5 h of exposure to a pH of 1 at room temperature. At 38°C, HAV remained infectious for up to 90 min at pH 1 (Scholz *et al.*, 1989). For the surrogates of human NoVs, less than 1 log reduction of MNV-1 was observed when exposed to a pH of 2 at 37°C for 30 min while FCV was reduced by 4.4 log after exposure to the same conditions (Cannon *et al.*, 2006). Therefore MNV-1 was recognized to be more appropriate as a human NoV surrogate than FCV in this regard. Similarly, Lee *et al.* (2012) investigated the survival of FCV and MNV-1 during *Dongchimi* fermentation. At the end of the fermentation of 20 days, populations of FCV and MNV-1 decreased about 4.12 and 1.47 log units, respectively.

14.5.2 Reduction of a_w

The a_w of a food product can be lowered by evaporation of H₂O and/or by adding solutes like salt and sugar.

Table 14.1 Survival of food-borne viruses and surrogates under different conditions.

Condition	Virus type	Detection methods	Viral survival description	References
Surface water and groundwater, at 25 and 4 °C	MNV-1, FCV, MS2, PV, NV	Plaque assay (to test infectivity reduction) for MNV-1, FCV MS2, PV; real-time RT-PCR (to test RNA reduction) for MNV-1, FCV, MS2, PV, and NV	MNV-1 was suggested as a promised NoV surrogate with an infectivity reduction rate of 0.16 log pfu/day in surface water and 0.04 log pfu/day in groundwater at 25 °C. FCV infectivity reduction rates were significantly higher than those of the other surrogates. Infectivity reduction rates were significantly higher than RNA reduction rates at 25 °C but not significant at 4 °C.	Bae and Schwab, 2008
Groundwater at room temperature in the dark	NV	Volunteer study and RNase treated real-time RT-PCR	NV remained infectious for at least 61 days. NV RNA within intact capsids was detected with no significant reduction throughout 427 days and a significant 1.10 log reduction by day 1266.	Seitz <i>et al.</i>, 2011
Milk, orange, and pomegranate juice, and juice blends at refrigeration (4 °C)	MNV-1, FCV, MS2	Plaque assay	MNV-1: no reduction in milk and orange juice, 1.4 log pfu/ml reduction in pomegranate juice after 21 days, >5 log pfu/ml reduction in orange and pomegranate juice blend after 7 days. FCV: 3 log pfu/ml reduction in in milk after 21 days, >6 log pfu/ml reduction in orange and pomegranate juice after 14 days, >6 log pfu/ml reduction in juice blend after 1 day. MS2: 1.28 log pfu/ml reduction in orange juice, <1 log pfu/ml reduction in milk, pomegranate juice, and juice blend.	Horm and D'Souza, 2011
Buffer at 4, 8, and 22 °C, fresh produce (lettuce, carrot, cabbage, onion, parsley, pepper, tomato, cucumber, raspberries and strawberries) washing with water and storage at 4, 8, and 22 °C	MS2	Plaque assay	Buffer: 4 and 8 °C: <1 log reduction after 50 days; 22 °C: >1 log reduction by day 9. Fresh produce: Washing with water: 0.08 to 0.79 log reduction; 4 °C: <1 log reduction for all produce types by day 7, <2 log reduction in cabbage and carrots by day 87; 8 °C: <1 log reduction for all produce types by day 7, ~1 log reduction in tomato, cabbage, carrots, and lettuce by day 39; 22 °C: 1 log reduction on lettuce and <1 log reduction on tomato and parsley by day 7.	Dawson <i>et al.</i>, 2005

Fresh produce (onions and spinach), washing with water, frozen at -21 °C.	MNV-1	Plaque assay	Washing with water: 0.4 and 1.0 log reduction on onion and spinach after 5 min. Frozen: No reduction during storage for 6 months.	Baert <i>et al.</i> , 2008a
Marinated mussels at 4 °C	HAV, FCV, NoV	TCID ₅₀ for HAV and FCV, real-time RT-PCR for HAV, FCV, NoV	There was a 1.7 log reduction in HAV TCID ₅₀ titer but no reduction in NoV or HAV RT-PCR titer after 4 weeks. FCV was inactivated in acid conditions although still detectable by RT-PCR.	Hewitt and Greening, 2004
Foods and stainless steel at refrigeration or room temperature	FCV	Plaque assay	FCV remains infectious on lettuce, ham, and stainless steel but not on strawberries by day 7. Statistically higher FCV titers were recovered from ham under all conditions than from lettuce, strawberries, or stainless steel.	Mattison <i>et al.</i> , 2007
Viral suspension and dried stainless steel surfaces at 4 °C and room temperature	MNV-1 and FCV	Plaque assay	4 °C: ~1 log reduction for MNV-1 and FCV both in dry and wet conditions at day 7; Room temperature: ~4 log reduction for FCV both in dry and wet conditions and MNV-1 in dry condition, ~1 log reduction for MNV-1 in wet condition at day 7.	Cannon <i>et al.</i> , 2006
Stainless steel coupons attached with lettuce, cabbage, or ground pork residuals at 25 °C	MNV-1	Most probable number (MPN) method	Residue-free coupons: 6.2 log MPN/ml infectivity reduction at day 30; Coupons with residues: 1.4 log MPN/ml reduction at day 30.	Takahashi <i>et al.</i> , 2011

The reported effects of relative humidity (RH) on HAV was controversial. Mbithi *et al.* (1991) reported that HAV decay rates were higher at a high RH (80%), revealing 34% survival, than at a low RH (25%), showing 52% survival, on non-porous inanimate surfaces during 4 h at 20°C. However, Abad *et al.* (1994) reported enhanced HAV survival when the RH was high (90%) compared to moderate RH (50%) on non-porous materials during 60 days at 20°C. Similarly, Stine *et al.* (2005) investigated the impact of relative humidity (RH) on viruses on the surface of cantaloupe, lettuce, and bell peppers. Survival of HAV, FCV, and PRD1 (ds-DNA coliphage) differed between produce types and was not uniformly affected by relative humidity (Stine *et al.*, 2005).

Park and Ha (2014) investigated the influence of NaCl on the inactivation of MNV-1 and HAV in the Korean traditional salted oyster product during 72 h of storage at 10°C. Both MNV-1 and HAV titers significantly decreased by stepwise increases in NaCl and storage time; MNV-1 was more resistant to NaCl than HAV. A 1.23 log reduction of MNV-1 and 2.45 log reduction of HAV survival occurred in 10% NaCl-containing oysters at 72 h of storage, suggesting that NaCl concentrations of $\geq 10\%$ could be potentially used for inactivation of NoV and HAV in traditional Korean foods, including those containing raw oysters such as *Eoriguljeot* and fermented *Jeotgals*.

14.5.3 Modified atmosphere packaging (MAP)

Modified atmosphere packaging (MAP) can inhibit spoilage causing bacterial and fungal microorganisms to extend the shelf life of foods. In food products packed under modified atmosphere, the growth and survival of pathogenic microorganisms is of major importance. Bidawid *et al.* (2001) reported that MAP did not influence HAV survival on lettuce incubated at 4°C. Improvement in virus survival (43% HAV survival) was observed in the presence of higher CO₂-levels (70%) compared to storage in air or lower CO₂ levels (ca. 6% HAV survival) after 12

days at room temperature, which may be attributed to the inhibitory effect of CO₂ on the enzymes or microflora present in lettuce. MAP conditions would therefore not be a suitable strategy to reduce the number of viruses present on food.

14.5.4 Use of temperature

14.5.4.1 Chilling and freezing

Experimental studies performed on chilled fresh produce and other chilled foods ascertained survival of a considerable number of viruses before deterioration of the specified food.

Mattison *et al.* (2007) investigated the survival of FCV inoculated on lettuce and strawberry disks stored at 4°C. Approximately 2 log reduction was observed on lettuce after 7 days at 4°C. Strawberries showed more than 2.5 log reduction after 6 days at 4°C. Although viral reductions could be induced during the food chilling storage, survival of a significant number of viruses was noted on minimal processed fresh produce during the short shelf life. Dawson *et al.* (2005) used MS2 as surrogate for NoV to follow up the survival on different types of fresh produce. At 4°C, less than 1 log reduction was observed after 7 days storage on produce (tomato, cabbage, carrots, pepper, strawberry, and lettuce) and less than 2 log reduction was established before the deterioration of the produce types.

Besides the persistence on fresh produce, Mattison *et al.* (2007) examined the survival of FCV on ham disks and observed approximately 1 log reduction after 7 days storage at 4°C. The better survival on ham compared to fresh produce remained undetermined but the authors suggested that ham protected the virus better against dryness. Contrary to fresh produce, ham is rich in proteins and fats. The presence of these compounds might offer protection for the virus particles.

Similarly, freezing will not ensure an adequate reduction of the viral load on foods as well. FCV and canine calicivirus (CaCV) revealed a decline in infectivity of respectively

0.34 ± 0.18 log and 0.44 ± 0.12 log after 5 cycles of freeze-thawing (Duizer *et al.*, 2004). No significant reduction of MNV-1 infectivity was observed in deep-frozen onions or deep-frozen spinach during a period of 6 months (Baert *et al.*, 2008a). Butot *et al.* (2008) reported that the number of rotavirus and HAV remained stable during 90 days on frozen raspberries, blueberries, strawberries, parsley, and basil with the exception of rotavirus, which was reduced by 1 log on blueberries and basil after 2 days of frozen storage. In contrast with HAV and rotavirus, FCV showed a 1 to 2 log reduction after 2 days storage on frozen strawberries and raspberries, probably due to the acid environment.

14.5.4.2 Heating

Among different treatments for NoV inactivation, the use of high temperature has been proven to be the most direct and effective strategy, which is also most widely used in the food industries area for food safety control and preservation. However, excessive heating may cause undesirable organoleptic changes to foods. Due to the customers' demands for minimally processed foods to maintain nutrition and flavor, it is of interest to focus on the inactivation of food-borne pathogens with rather short times and low temperatures.

Table 14.2 summarizes some heat inactivation studies of food-borne viruses and surrogates. FCV was shown to be more heat resistant than MNV-1 or phage B40-8 measured by infectivity-based plaque assay. Human NoVs were observed to be more heat resistant than both MNV-1 and FCV measured by the nucleic acid-based real-time RT-PCR with or without enzyme pretreatment (Hewitt *et al.*, 2009; Topping *et al.*, 2009). Besides temperature and processing time, the matrix of viruses subjected to heat is also an important factor influencing the inactivation effect (Hewitt *et al.*, 2009). In a comprehensive review of the effect of temperature on inactivation of enteric viruses in food and water, an overall lower virus sensitivity to temperature change was noted in complex matrices (e.g., dairy and food products) com-

pared to simple matrices (e.g., drinking water and synthetic media) at the high-temperature range ($>50^{\circ}\text{C}$) (Bertrand *et al.*, 2012).

The application and the comparison of different models to determine thermal inactivation kinetics of NoV surrogates in produce matrices is rather uncommon. In other simple matrices, such as viral suspension (from stool or medium), more studies are available that compare different thermal inactivation models such as first-order kinetics (monophasic reduction), biphasic models, and Weibull models. In most of these studies, models selected as most appropriate for heat inactivation deviated from the classic monophasic reduction model, especially for heat inactivation at lower temperatures (Tuladhar *et al.*, 2012). The Weibull model, for which the reduction rate(s) need not be constant over time, but may continuously change under progressing treatment, was chosen as the most appropriate model in several studies (on FCV, MNV-1, and MS2) (Bozkurt *et al.*, 2013, 2014).

The reduction of viral genomic copies lagged far behind the reduction of infectivity, even with a pretreatment with RNase and/or proteinase before RT-PCR detection, suggesting the alteration of viral capsid composition as the primary target of heat inactivation instead of a complete capsid destruction or nucleic acid damage.

The effectiveness of heat treatment in produce to prevent food-borne outbreaks has been illustrated recently in a large outbreak due to NoV contaminated frozen strawberries in Germany in 2012. Some elderly who consumed strawberries from the same virally contaminated batch as those served to the infected school children did not experience infection, since their strawberries were heated before consumption (Mäde *et al.*, 2013). However, as mentioned above, since the infectivity of human NoVs cannot be measured due to a lack of cell culture systems, little is generally known about the exact time/temperature combination to inactivate NoVs in foods. Based on the existing data mostly obtained by the use of surrogate viruses, which are more or less closely related to the human NoVs, it can be concluded that NoVs

Table 14.2 Some heat inactivation studies on food-borne viruses and surrogates.

Virus type	Matrix	Detection methods	Viral reduction	Reference
FCV and MNV-1	Cell culture medium	Plaque assay	FCV: 56°C, 6.7 min/63°C, 0.4 min/72°C, 0.1 min, 1 log reduction. MNV-1: 56°C, 3.5 min/63°C, 0.4 min/72°C, 0.2 min, 1 log reduction.	Cannon et al., 2006
MNV-1, <i>B. fragilis</i> Phage B40-8	Raspberry puree	Plaque assay	MNV-1: 65°C, 0.5 min, 1.86 log reduction; 75°C, 0.25 min, 2.81 log reduction. B40-8: 65°C, 0.5 min, 2.77 log reduction; 75°C, 0.25 min, 3.44 log reduction.	Baert et al., 2008b
MNV-1, NoV GI and GII, HAV	Water and milk	Plaque for MNV-1, TCID ₅₀ for HAV, real-time RT-PCR for MNV-1, NoVs, HAV	MNV-1 infectivity: In water: 63°C 2 min, 3.43 log reduction; 72°C 1 min, >3.5 log reduction. In milk: 63°C 2 min, 1.85 log reduction; 72°C 1 min, >3.5 log reduction. HAV infectivity: In water: 63°C 1 min, 1.26 log reduction; 72°C 1 min, >3.5 log reduction. In milk: 63°C 2 min, 1.6 log reduction; 72°C 1 min, 2.22 log reduction. MNV-1, NoVs and HAV RNA: 63 and 72°C, 0–10 min, ≤1 log reduction.	Hewitt et al., 2009
MNV-1	Cell culture medium	Plaque assay and enzyme-pretreated real-time RT-PCR (Proteinase K and RNase A)	Infectivity: 80°C, 2.5 min, 6.5 log reduction. RNA: 80°C, 60 min, <2 log reduction.	Baert et al., 2008c
FCV and NoV GII.4	Cell culture medium for FCV, stool suspension for NoV	Plaque assay and enzyme-pretreated real-time RT-PCR (RNase One)	FCV Infectivity: 60°C, 2 min, >4.5 log reduction. FCV RNA: 60°C, 2 min, ~1.5 log reduction. NoVs RNA: 80°C, 2 min, ~1.5 log reduction.	Topping et al., 2009

tolerate a low pH value and that in a temperature range above 70°C they lose infectivity in dependence of the holding time applied. Heating strawberry compote to core temperatures of above 90°C and/or long holding times in the temperature range above 70°C seem to be a suitable way to completely deactivate the virus. However, it is to be expected that stirring large quantities of deep-frozen strawberries in boiling water or the uneven heating resulting from brief boiling of a large quantity of compote cannot safely inactivate NoVs present on the strawberries (BfR, 2012).

14.5.5 Washing and decontamination processes

14.5.5.1 Washing with water

Generally, washing results in a decrease of approximately ≤ 1 logarithm in the quantity of viruses detected (Baert *et al.*, 2008a; Butot *et al.*, 2008; Dawson *et al.*, 2005; Li *et al.*, 2011b). Minor adaptations to the classic washing step by immersion such as usage of bubbling (Fraisie *et al.*, 2011) or warm water (43°C) (Butot *et al.*, 2008; Lukasik *et al.*, 2003), or the inclusion of hand rubbing (Lukasik *et al.*, 2003) did not significantly improve viral reduction on the produce. Besides, a disadvantage of washing produce with water is the presence of viruses in the residual washing water, which can lead to cross-contamination between contaminated and non-contaminated foods, as discussed above.

14.5.5.2 Chlorine solutions

Chlorine is the most commonly used chemical treatment in water disinfection worldwide. In the food industry, chlorine can be used as a surface disinfectant as well as sanitizer in fresh produce processing. The forms of chlorine commonly used include chlorine gas (Cl_2), sodium hypochlorite (NaClO), and calcium hypochlorite ($\text{Ca}(\text{ClO})_2$). Chlorine solutions can be either applied by immersion of the foods or by spraying.

The viral inactivation of chlorine (e.g., 3% sodium hypochlorite, 5 min contact) has been

proven under different conditions (Table 14.3). MNV-1 was more resistant to chlorine than FCV and MS2 detected by plaque assay (D'Souza and Su, 2010), while human NoV was observed to be even more resistant than MNV-1 detected by enzyme pretreated RT-PCR (Girard *et al.*, 2010). The effectiveness of chlorine on viral disinfection depends on several factors. In order to obtain certain effectiveness, the required amount of free chlorine multiplied by the virus contact time were shown to be higher at pH 8 than at pH 6, higher at 5°C than at 15°C, and higher for aggregated viruses than dispersed viruses (Thurston-Enriquez *et al.*, 2003b). The presence of organic matters could negatively influence the effectiveness of chlorine on NoVs. This may explain why chlorine resistance of NoVs was observed in some studies.

Meanwhile, the disadvantages of chlorine being caustic to processing equipment, chemical residual changing sensorial quality of foods, as well as the toxic by-product generation have to be noted. The interaction between chlorine and natural organic matter results in the formation of so-called disinfection by-products (DBPs), among which trihalomethanes (THMs) are the most relevant. Chronic exposure to THMs could result in cancer. Although formation of THMs in process wash water occurs, no residue can be found in vegetable tissue after rinsing with tap water (Gomez-Lopez *et al.*, 2013; Lopez-Galvez *et al.*, 2010). Hence, when good practices are applied, chlorine-based sanitizers can be safely used to wash fresh produce, in spite of the formation of THMs in the washing water.

In a mechanistic study on the impact of disinfectants on MS2 phages, free chlorine has been shown to act on both genome and proteins as free chlorine treatment caused losses in both genome- and protein-mediated functions, namely replication and injection (Wigginton *et al.*, 2012). However, the mechanism of action of hypochlorite or the susceptibility to hypochlorite might be dependent upon the type of viruses (Wigginton *et al.*, 2012).

Table 14.3 Some decontamination studies of food-borne viruses and surrogates by chemical sanitizers.

Chemical sanitizers	Virus type	Matrix	Detection methods	Viral reduction	Reference
Chlorinated tap water, ClO ₂	FCV, NoVs, HAV	Blueberries, raspberries, strawberries, basil, parsley	Plaque assay for FCV and HAV, real-time RT-PCR for FCV, NoVs, HAV	Chlorinated tap water, 200 ppm, 30s (on the five matrix): NoV GI RNA: >3.4, 0, 3.1, >3.4, 0.9 log reduction NoV GII RNA: 3, 0.9, 1.4, 1.6, 1.8 log reduction FCV infectivity: >3.5, >3.5, >3.5, >3.5, >2.7 log reduction FCV RNA: 4, >3, 3.4, 2.5, 2 log reduction HAV infectivity: 2.4, 0.6, 1.8, 2.4, 1.4 log reduction HAV RNA: 2.2, 0.7, 1.9, 1.4, 1.1 log reduction ClO ₂ , 10 ppm, 30s: NoV GI RNA on raspberries and parsley: 0.5, 0.62 log reduction NoV GII RNA on raspberries and parsley: 0.98, 1.19 log reduction FCV infectivity on parsley: 1.31 log reduction FCV RNA on parsley: 0.81 log reduction HAV infectivity on raspberries and parsley: 0.79, 0.72 log reduction HAV RNA on raspberries and parsley: 1.75, 0.3 log reduction	Butot <i>et al.</i> , 2008
Chlorine, glutaraldehyde (GDA), alcohol, trisodium phosphate (TSP)	MNV-1, FCV, MS2	Formica coupon	Plaque assay	Sodium hypochlorite (0.6%), 5000 ppm available chlorine, 1 min contact: MNV-1, FCV, MS2: 2.73, 6.90, 6.98 log reduction GDA (2%), 1 min contact: MNV-1, FCV, MS2: >6, >6, 3.74 log reduction Ethanol (70%), 1 min contact: MNV-1, FCV, MS2: 0, 0, 0.09 log reduction TSP (2%), 1 min contact: MNV1, FCV, MS2: 1.05, 6.90, 6.98 log reduction TSP (5%), 1 min contact: MNV-1, FCV, MS2: 7.10, 6.93, 6.89 log reduction	D'Souza and Su, 2010
Chlorine, ethoxylated alcohol, quaternary ammonium compound	MNV-1, human NoV	Stainless steel fomite	Plaque assay for MNV-1, enzyme pretreated real-time RT-PCR for MNV-1 and human NoV	Sodium hypochlorite 3%, 5 min contact: MNV-1 infectivity and RNA: >4 log reduction; human NoV: ~2 log reduction 2-(1-Butoxy) propanol and ethoxylated alcohols, 5 min contact: MNV-1 infectivity and RNA: <1 log reduction; human NoV: 0 log reduction 0.08% <i>N</i> -alkyl dimethyl benzyl ammonium chloride, 0.02% <i>N</i> -alkyl dimethyl benzyl ammonium chloride, 5 min contact: MNV-1 infectivity and RNA: ~1 log reduction	Girard <i>et al.</i> , 2010

Chlorine, peroxyacetic acid (PAA)	MNV-1, B40-8	Lettuce	Plaque assay	Sodium hypochlorite, 200 ppm available chlorine, 250 ppm PAA, 5 min contact: MNV-1: 1 log reduction compared with washing with water B40-8: no reduction compared with washing with water	Baert <i>et al.</i> , 2009
PAA, GDA, alcohol	MNV-1	Stainless steel disk	End point titration	PAA (1000 ppm), GDA (2500 ppm), ethanol (50% (v/v)), and 1-propanol (30% (v/v)), 5 min contact: >4 log reduction under both clean and dirty conditions	Magulski <i>et al.</i> , 2009
Alcohol	MNV-1	Fingerpads	TCID ₅₀	60% (v/v) of ethanol, 1-propanol, 2-propanol, 30s, room temperature, 6.32, 4.24, 1.66 log reduction. Ethanol concentrations of 60–90% (v/v) were superior to 1-propanol and 2-propanol	Paulmann <i>et al.</i> , 2011
Ozone (O ₃)	MNV-1, HAV	Green onion	Plaque assay for MNV-1, integrated cell culture/ RT-PCR MPN for HAV	Ozone 6.25 ppm, 10 min, 20 °C: MNV-1: 1.5 ± 0.1 log reduction for internalized viruses, 2.5 ± 0.9 log reduction for surface viruses HAV: 1.5 ± 0.3 log reduction for internalized viruses, 2.9 ± 0.6 log reduction for surface viruses	Hirneisen and Kniel, 2013
H ₂ O ₂	MNV-1, bacteriophages (phiX174 and B40-8)	Stainless steel disc	Plaque assay	H ₂ O ₂ 2.1%, 10 min: MNV-1, phiX174: ~4 log reduction H ₂ O ₂ 15%, 10 min: B40-8: ~4 log reduction	Li <i>et al.</i> , 2011b

14.5.5.3 Other sanitizers

Other commonly used components of disinfectants and sanitizers were also studied against food-borne viruses and surrogates (Table 14.3). Alcohols including ethanol and propanol were observed to have even bigger variances in the effectiveness of viral inactivation than chlorine (D'Souza and Su, 2010; Girard *et al.*, 2010). The effect of quaternary ammonium compounds on NoVs were proven to be rather limited (Girard *et al.*, 2010). Peroxyacetic acid (PAA), glutaraldehyde (GDA), and trisodium phosphate (TSP) were reported to be effective against human NoV surrogates on surfaces (D'Souza and Su, 2010; Magulski *et al.*, 2009). PAA (250 ppm, 5 min contact) was also evaluated as taken up in the washing solutions of fresh produce, which was as effective (1 log reduction for MNV-1 compared with washing with water) as chlorine (200 ppm available chlorine, 5 min contact) but was not influenced by the presence of organic matter (Baert *et al.*, 2009).

Chlorine dioxide (ClO_2) and ozone (O_3) are both strong oxidizing agents and effective in inactivating a wide range of bacteria, fungi, viruses, and protozoa (Hirneisen *et al.*, 2010). However, both ClO_2 and O_3 have to be generated on-site and remain unstable towards multiple factors such as pH and organic matter. Their high oxidation potential may lead to undesirable reaction with food components and safety issues for the operating personnel. Therefore, the application of ClO_2 and O_3 on NoV disinfection can be comparatively limited in the food industry.

Hydrogen peroxide (H_2O_2) can be used as a liquid or as a mist/vapor during decontamination and sanitizing processes. The usage of a spray instead of a commonly used wash process would have the potential benefit of reducing the necessary large amount of potable water and disinfectant needed when using the latter option.

14.5.6 Radiation

Food irradiation is a physical means of food processing that involves exposing the pre-packed or bulk foodstuffs to gamma rays, X-rays, or

electrons. Both ionizing and non-ionizing radiation have been tested as a wide range of germicidal effect on microorganisms. Table 14.4 summarizes some radiation inactivation study of food-borne virus surrogates.

The most widespread used form of non-ionizing radiation for decontamination is the use of UV light. UV disinfection primarily occurs due to the germicidal action of UV-B (280 to 315 nm) and UV-C light (200 to 280 nm) on microorganisms (USEPA, 2006). Most studies use low-pressure mercury lamps with a major wavelength output (85%) at 253.7 nm (monochromatic UV radiation). It is generally believed that UV light induces damage on the viral genetic materials and only affects the capsid at higher doses. The use of UV light as a disinfection strategy has many benefits, including a relatively low cost, lack of irritating or toxic by-products, and ease of handling and use (Fino and Kniel, 2008). However, consideration should be given to a practical UV delivery system to be used in large-scale processing, which limits the occurrence of shadowing.

Another disinfection strategy using non-ionizing radiation is the use of pulsed light (PL) treatment. PL is a modified and claimed improved version of delivering UV-C to bodies, using xenon lamps to deliver short time pulses of an intense broad spectrum rich in UV-C light. In a study of Belliot *et al.* (2013) MS2 phages were spiked and treated with 10–30 pulses. On pepper and garlic, only very small reductions in viral titer were observed (<0.5 log PFU after 20 pulses), while 1.3 log reduction was obtained for chopped mint. This is in contrast to the high reductions obtained in viral suspensions after merely 4 pulses (>8 log reduction). Therefore, the downside of PL treatment is still the superficial character of UV treatment.

Ionizing radiation is radiation that carries enough energy to liberate electrons from atoms or molecules, thereby ionizing them. Ionizing radiation used in food processing can be electromagnetic radiation (gamma rays and X-rays) or particulate radiation (electron beam). Viruses, having relatively little nuclear material

Table 14.4 Some inactivation studies by radiation on food-borne virus surrogates.

Radiation type	Virus type	Matrix	Detection methods	Viral reduction	Reference
UV radiation	MS2, FCV	Buffer and groundwater	MPN	Buffer: MS2: 119 ml/cm ² , 2 log reduction FCV: 36 ml/cm ² , 2 log reduction Groundwater: FCV: 29 ml/cm ² , 2 log reduction	Thurston-Enriquez <i>et al.</i> , 2003a
UV radiation	MS2, FCV	Tap water for MS2, virus stock solution with low or high protein content	Plaque assay for MS2, TCID ₅₀ for FCV	MS2: 650 ml/cm ² , 3 log reduction FCV: 120 ml/cm ² , 3 log reduction No significant difference between FCV solutions with low or high protein content	De Roda Husman <i>et al.</i> , 2004
UV radiation	FCV	Green onion, lettuce, and strawberries	TCID ₅₀	Lettuce: 20, 120, 240 ml/cm ² , 3.48, 3.82, 4.62 log reduction Green onion: 20, 120, 240 ml/cm ² , 2.46, 3.92, 3.88 log reduction Strawberries: 20, 120, 240 ml/cm ² , 1.13, 1.57, 2.28 log reduction	Fino and Kniel, 2008
Gamma radiation	MS2, FCV	Tap water for MS2, virus stock solution for FCV	Plaque assay for MS2, TCID ₅₀ for FCV	MS2: 100 Gy, 3-log reduction FCV: 500 Gy, 3-log reduction	De Roda Husman <i>et al.</i> , 2004
Gamma radiation	MNV-1	Strawberries, lettuce, and spinach	Plaque assay	5.6 kGy, 1.7 to 2.4 log reduction 11.2 kGy, 3.6 to 4.1 log virus reduction 22.4 kGy, >7 log virus reduction in romaine lettuce and strawberries, while 5 log virus reduction in spinach	Feng <i>et al.</i> , 2011

and being small “targets,” are relatively resistant to radiation compared to most vegetative bacteria. Reported D_{10} values for enteric viruses/surrogates are, for example, 2.97 kGy for HAV on strawberries (γ -rays) (Bidawid *et al.*, 2000) and 2.95 kGy for FCV on lettuce (e-beam) (Zhou *et al.*, 2011). However, D_{10} values are affected by a number of factors including temperature, water activity, and chemical composition of the food (EFSA, 2011). In Europe, the Scientific Committee on Food (SCF) has expressed several opinions on irradiated foods and acceptable doses for specific food classes/commodities (e.g., in 1986, 1992, and 1998). For vegetables and for fruits, overall average radiation doses (kGy) of up to 1 and up to 2 kGy, respectively, were evaluated as acceptable. However, this irradiation dose seems impractical for the inactivation of viruses on fresh produce as only <2 log virus reduction of MNV-1 (4 kGy, γ -rays) was achieved on spinach, romaine lettuce, and strawberries in a study by Feng *et al.* (2011) and only a reduction of ≤ 0.70 log of MNV-1 was achieved on cabbage and strawberries in a study by Sanglay *et al.* (2011) (4 kGy, e-beam). As such, to achieve meaningful reductions of viruses, the doses required are typically above legally approved doses and what most produce will tolerate in terms of changes in food appearance, flavor, color, and texture (Fan *et al.*, 2008).

14.5.7 High hydrostatic pressure (HHP)

High-pressure processing (HPP) is a non-thermal process that inactivates pathogenic and spoilage microorganisms as well as endogenous enzymes and has been used as a “cold pasteurization” method. Pressures up to 1000 MPa are used that are instantaneously and uniformly transmitted throughout a sample, thus making this process independent of the shape or size of the food. As for the mechanism for inactivation of enteric viruses by HPP, experiments suggest that HPP inactivates viruses by targeting the capsid and not the virus genome. HPP induces denaturation of capsid proteins, which renders the virus incapable of binding to its receptor on the surface of its host cell.

Table 14.5 summarizes some HHP inactivation study of NoVs and its surrogates. For NoVs, the effectiveness of high pressure was not only proven by surrogates FCV and MNV-1, but also by volunteer study of NV, the prototype of human NoVs (Leon *et al.*, 2011). However, among enteric viruses a high variability in pressure resistance has been noted; even different virus strains can behave differently under pressure. In addition, some parameters, such as temperature and pH, were shown to influence the HPP inactivation of different types of viruses, in a contradictory way. For example,

Table 14.5 Some inactivation studies by HHP on NoV and its surrogates.

Virus type	Matrix	Detection methods	Viral reduction	Reference
FCV	Cell culture medium	TCID ₅₀	200 MPa, 270 s, 18–20°C, 1 log reduction, 265 and 300 MPa, 180 s, 18–20°C, >5 log reduction, 450 and 600 MPa, 30 s, 18–20°C, >5 log reduction.	Grove <i>et al.</i> , 2008
MNV-1	Cell culture medium and shellfish	Plaque assay	Cell culture medium: 350 MPa, 5 min, 20°C, 1.15–log reduction 350 MPa, 5 min, 5°C, 5.56–log reduction Shellfish: 400 MPa, 5 min, 5°C, 4.05 log reduction	Kinsley <i>et al.</i> , 2007
MNV-1	Shellfish	Plaque assay	400 MPa, 5 min, 0°C, >2 log reduction	Li <i>et al.</i> , 2009
NV	Shellfish	Volunteer study	600 MPa, 5 min, 6 or 25°C, reduction to non-infectious level	Leon <i>et al.</i> , 2011

colder initial temperatures of the product enhanced the inactivation of human NoV GI.1 (Leon *et al.*, 2011) and surrogates MNV-1, TV, and FCV (Chen *et al.*, 2005; Li *et al.*, 2013c). In contrast, HAV is more resistant to HPP at a lower temperature than at room temperature (Kingsley and Chen, 2009). Similarly, NoV surrogates tend to be more sensitive to HPP at neutral pH than at acidic pH (Li *et al.*, 2013b; Lou *et al.*, 2011), while for HAV the opposite is the case (Kingsley and Chen, 2009). As such, given the complexity of food matrices and the variable response of different viruses, direct validation of HPP conditions within the food or food matrix will be required.

The cost of HPP is comparatively high for large-scale application in the food industry, thus so far this technique has only been commercialized in some developed locations for foods such as shellfish, which are highly associated with virus contamination that remains difficult to get rid of.

14.5.8 Treatment with natural biochemical substances

As reviewed by Li *et al.* (2013a), the exploration of natural sources for antimicrobial components has become a new trend worldwide. The reported natural bioactive substances with antiviral effects on food-borne viruses can be grouped into several categories including polyphenols and proanthocyanins, saponin, polysaccharides, organic acid, protein and polypeptides, and essential oils. These substances, in the form of pure compounds or crude extracts, can be obtained from commonly consumed foods, food additives in use, or traditional medicinal herbs. Within these categories, plant polyphenols and proanthocyanins are the most well studied group, with promising effects shown on a wide range of food-borne viruses. For instance, the research group of Su and D'Souza reported the time dependent effects of cranberry juice (proanthocyanins as the functioning components), pomegranate juice (polyphenols as the functioning components), and grape seed extract on the infectivity of a series of NoV surrogates

(Su *et al.*, 2010a, 2010b, 2010c, 2011; Su and D'Souza, 2011). More recently, the research group of Oh *et al.* performed studies on black raspberry juice, mulberry juice, and red wine, identifying also antiviral effects on NoV surrogates (Lee *et al.*, 2014; Oh *et al.*, 2012, 2013, 2015).

In general, although not fully understood, the mechanism of action for the antiviral efficacy of the natural components is usually determined to be the prevention of the viral attachment to host cells, causing block/damage either on the viral capsids or the receptors on the cell membranes (Li *et al.*, 2013a).

The research on the antiviral effect of the natural bioactive substances is still at a preliminary stage. First of all, the compositions of the natural compounds from different producers can vary considerably, causing difficulties for the food additive safety evaluation as well as direct comparisons of their antiviral effects. Secondly, most of the antiviral studies were performed in tissue culture medium or sterile buffer solutions, which cannot simulate the practical scenarios. It is generally believed that food components such as proteins and fat may have a protective effect on the viral particles from various physical and chemical inactivation (Li *et al.*, 2011b). It was reported that the specific binding ability of human NoVs to salivary HBGA receptors could be effectively enhanced or decreased by food extracts/juices including shellfish, fruits, and vegetables (Li *et al.*, 2012a). In the antiviral study of Li *et al.* (2012b), the efficacy of grape seed extract in reducing viral loads was tested on stainless steel surfaces with proteins as interfering substances (only a marginal reduction of 0.56 log pfu/ml was observed with a high dose of grape seed extract at 2 mg/ml) and in fresh-cut lettuce wash water (promising effect of ca. 1.5 to 2 log pfu/ml reduction with grape seed extract at 2 mg/ml). Therefore, studies should be performed mimicking practical scenarios for their potential use in applications in the food industry either as an antiviral additive to the food itself or as a sanitizing agent for reconditioning of water or disinfecting food contact surfaces in the food processing environment.

14.5.9 Hurdle technologies

In order to obtain a better inactivation while retaining the least adverse effect, the combination of a few different treatments was investigated as the application of the concept “hurdle technology”.

Xie *et al.* (2008) evaluated the inactivating efficacy of MS2 inoculated on to 5 cm² iceberg lettuce sections and found a reduction of 0.5 to 1.0 log pfu by UV light for 20 to 60 s, but a 3 log reduction with 2% H₂O₂ spray at 50°C. A higher reduction (4.12 log) was obtained with 2% H₂O₂ spray for 10 s followed by UV illumination for 20 s. Compared to the 1.67 log reduction obtained by 200 ppm of calcium hypochlorite, this combination represents an alternative to chlorine-based washes for decreasing the virus load on fresh produce with a less detrimental effect on the organoleptic and nutritional quality of foods.

The reduction potential of a combination of vaporized H₂O₂ and UV light was also the subject in a study on the decontamination of lettuce with MNV-1, ϕ X174, and B40-8 (Li *et al.*, 2011b). In this study 500 g of fresh-cut lettuce was treated using a tumbling drum at a speed of 77 rpm. Liquid H₂O₂ that was vaporized during one treatment of 5 min contained 80 ml of a 2.52% H₂O₂ solution. Even though the treatment of the current study was much longer compared to the treatment parameters used by Xie *et al.* (2008) and the concentration of the H₂O₂ mist was comparable, significant lower reductions were obtained in this study by Li *et al.* (2011b). In this study only a 1.7, 2.0, and 1.0 log₁₀ reductions of respectively MNV-1, ϕ X174, and B40-8 phages were obtained. This is considerably lower than the 4.1 log reduction using the protocol of Xie *et al.* (2008). However, in this study the H₂O₂ vapor was administered at room temperature, and real industrial practices were mimicked by treating 500 g of lettuce in a drum. In the study by Xie *et al.* (2008) it was also observed that simultaneous treatment of the lettuce with UV light and H₂O₂ (50°C, 2%, 20 s, 3.53 log₁₀ reduction) was less efficient than to perform the treatment in two consecutive steps: e.g., 10 s of H₂O₂ and 30 s of UV light (4.12 log

reduction). The authors suggested that the lack of a synergistic effect of UV light when combined simultaneously with H₂O₂ may have been due to the formation of reactive radicals in the head-space of the treatment chamber as opposed to the lettuce surface (Xie *et al.*, 2008).

Predmore and Li (2011) evaluated the effectiveness of a panel of surfactants, including sodium dodecylsulfate (SDS), Nonidet P-40 (NP-40), Triton X-100, and polysorbates on removal of MNV-1 from fresh produce. While tap water alone and chlorine solution (200 ppm) gave only <1.2 log reductions in virus titer, a reduction of approximately 3 log was observed in all the tested fresh produce after sanitization with a solution containing a combination of 50 ppm of each surfactant and 200 ppm of chlorine, indicating the combination of a surfactant with a commonly used sanitizer to be a feasible approach for reduction of the virus load in fresh produce.

14.6 Conclusion and perspectives

As further reading material besides this chapter, there are still multiple review articles available in the literature on the effect of food processing techniques (Hirneisen *et al.*, 2010; RIVM, 2013; Sanchez *et al.*, 2013; Zuber *et al.*, 2013) and preservation methods (Baert *et al.*, 2009) on food-borne viruses. In general, food-borne viruses are stable and persistent to most currently used food preservative strategies. Researchers have been continuously committed to exploring novel techniques to control virus contamination in food products. However, due to the complicated properties of food products, multiple factors have to be considered such as safety (e.g., chemical residuals), feasibility (e.g., radiation penetration), and the maintenance of food sensory and nutritional quality. Especially for the foods mostly related with virus contamination risk, such as shellfish, berries, and leafy greens, minimal food processing treatments are commonly preferred and applied.

Specifically, current treatment regimes for shellfish products placed live on the market (depuration and relaying) as commonly practiced do not effectively reduce NoV. Depuration and relaying may be improved by optimizing process parameters to enhance NoV reduction (e.g., depuration times, water temperature). However, limited data are currently available. Alternative treatments such as commercial heat treatment and high pressure may be effective for NoV inactivation, but give rise to organoleptic changes that may be unacceptable to consumers. The most effective public health measure to control human NoV infection from oyster consumption is to produce oysters from areas that are not faecally contaminated, particularly given the ineffectiveness of current control regimes.

For fresh produce, berries may be subjected to minimal processing such as cleaning, cutting, washing and mashing as well as freezing. Leafy greens may be further processed to obtain ready-to-eat products, and these steps include: selection, elimination of external leaves, cutting, washing, rinsing, dewatering, packaging, and storage. During such minimal processing, contamination and cross-contamination via equipment, water (if washing is applied), and particularly via food handlers are the main risk factors. Attention should be paid to the selection of the water sources for food production and in particular to the avoidance of the use or the ingress of water contaminated by sewage. Production areas should be evaluated for hazards that may compromise hygiene and food safety, particularly to identify potential sources of faecal contamination. Another main mitigation option for reducing the risk of virus contamination is adherence to hand hygiene by food handlers at all stages of the supply chain.

Thus, as recommended by EFSA (2012, 2014a, 2014b), the most important strategy to control food-borne virus contamination in food products should be the implementation of food safety management systems including Good Agricultural Practices (GAP), Good Hygiene Practices (GHP), and Good Manufacturing Practices (GMP) along the farm-to-fork continuum.

Lastly, it should be noted that the current NoV related studies cited above were exclusively conducted by the use of RT-qPCR based on methods of which the results may have large gaps with the actual viral infectivity or by the use of surrogates of which the applicability has always been and is still under question due to the different clinical manifestations, infected cell types, host receptors, etc. Therefore, in the future the efforts on *in vitro* cultivation of human NoVs should never be stopped.

References

- Abad, F.X., Pinto, R.M., and Bosch, A. 1994. Survival of enteric viruses on environmental fomites. *Applied and Environmental Microbiology*, **60**, 3704–3710.
- Bae, J. and Schwab, K.J. 2008. Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Applied Environmental Microbiology*, **74**, 477–484.
- Baert, L., Uyttendaele, M., Vermeersch, M., Van Coillie, E., and Debevere, J. 2008a. Survival and transfer of murine norovirus 1, a surrogate for human noroviruses, during the production process of deep-frozen onions and spinach. *Journal of Food Protection*, **71**, 1590–1597.
- Baert, L., Uyttendaele, M., Van Coillie, E., and Debevere, J. 2008b. The reduction of murine norovirus 1, *B. fragilis* HSP40 infecting phage B40-8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. *Food Microbiology*, **25**, 871–874.
- Baert, L., Wobus, C.E., Van Coillie, E., Thackray, L.B., Debevere, J., and Uyttendaele, M. 2008c. Detection of murine norovirus 1 by using plaque assay, transfection assay, and real-time reverse transcription-PCR before and after heat treatment. *Applied Environmental Microbiology*, **74**, 543–546.
- Baert, L., Debevere, J., and Uyttendaele, M. 2009. The efficacy of preservation methods to inactivate foodborne viruses. *International Journal of Food Microbiology*, **131**, 83–94.

- Baert, L., Mattison, K., Loisy-Hamon, F., Harlow, J., Martyres, A., Lebeau, B., Stals, A., Van Coillie, E., Herman, L., and Uyttendaele, M. 2011. Review: norovirus prevalence in Belgian, Canadian and French fresh produce: a threat to human health? *International Journal of Food Microbiology*, **151**, 261–269.
- Barrabeig, I., Rovira, A., Buesa, J., Bartolome, R., Pinto, R., Prellezo, H., and Dominguez, A. 2010. Foodborne norovirus outbreak: the role of an asymptomatic food handler. *BMC Infectious Diseases*, **10**.
- Belliot, G., Loutreul, J., Estienne, M., Cazeaux, C., Nicorescu, I., Aho, S., Gervais, P., Orange, N., Pothier, P., and Morin, T. 2013. Potential of pulsed light to inactivate bacteriophage MS2 in simple liquid medium and on complex food-stuffs. *Food and Environmental Virology*, **5**, 176–179.
- Bertrand, I., Schijven, J.F., Sanchez, G., Wyn-Jones, P., *et al.* 2012. The impact of temperature on the inactivation of enteric viruses in food and water: a review. *Journal of Applied Microbiology*, **112**, 1059–1074.
- BfR (Federal Institute for Risk Assessment, Belgium). 2012. Tenacity (resistance) of noroviruses in strawberry compote. BfR Opinion No. 038/2012.
- Bidawid, S., Farber, J.M., and Sattar, S.A. 2000. Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. *International Journal of Food Microbiology*, **57**, 91–97.
- Bidawid, S., Farber, J.M., and Sattar, S.A. 2001. Survival of hepatitis A virus on modified atmosphere packaged (MAP) lettuce. *Food Microbiology*, **18**, 95–102.
- Bozkurt, H., D'Souza, D.H., and Davidson, P.M. 2013. Determination of the thermal inactivation kinetics of the human norovirus surrogates, murine norovirus and feline calicivirus. *Journal of Food Protection*, **76**, 79–84.
- Bozkurt, H., D'Souza, D.H., and Davidson, M. 2014. Thermal inactivation of human norovirus surrogates in spinach and measurement of its uncertainty. *Journal of Food Protection*, **77**, 276–283.
- Butot, S., Putallaz, T., and Sanchez, G. 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *International Journal of Food Microbiology*, **126**, 30–35.
- Cannon, J.L., Papafragkou, E., Park, G.W., Osborne, J., Jaykus, L.A., and Vinjé, J. 2006. Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *Journal of Food Protection*, **69**, 2761–2765.
- Chen, H.Q., Hoover, D.G., and Kingsley, D.H. 2005. Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection*, **68**, 2389–2394.
- Daniels, N.A., Bergmire-Sweat, D.A., Schwab, K.J., Hendricks, K.A., *et al.* 2000. A foodborne outbreak of gastroenteritis associated with Norwalk-like viruses: first molecular traceback to deli sandwiches contaminated during preparation. *Journal of Infectious Diseases*, **181**, 1467–1470.
- Dawson, D.J., Paish, A., Staffell, L.M., Seymour, I.J., and Appleton, H. 2005. Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. *Journal of Applied Microbiology*, **98**, 203–209.
- De Keuckelaere, A., Li, D., Deliëns, B., Stals, A., and Uyttendaele, M. 2015. Batch testing for noroviruses in frozen raspberries. *International Journal of Food Microbiology*, **192**, 43–50.
- de Roda Husman, A.M., Bijkerk, P., Lodder, W., Van Den Berg, H., *et al.* 2004. Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (gamma) radiation. *Applied And Environmental Microbiology*, **70**, 5089–5093.
- D'Souza, D.H. and Su, X. 2010. Efficacy of chemical treatments against murine norovirus, feline calicivirus, and MS2 bacteriophage. *Foodborne Pathological Disease*, **7**, 319–326.
- Duizer, E., Bijkerk, P., Rockx, B., de Groot, A., Twisk, E., and Koopmans, M. 2004. Inactivation of caliciviruses. *Applied and Environmental Microbiology*, **70**, 4538–4543.
- EFSA Panel on Biological Hazards (BIOHAZ). 2011. Scientific opinion on irradiation of food

- (efficacy and microbiological safety). *EFSA Journal*, **9**, 88.
- EFSA Panel on Biological Hazards (BIOHAZ). 2012. Norovirus (NoV) in oysters: methods, limits and control options. *EFSA Journal*, **10**, 2500 (39 pp.). doi: 10.2903/j.efsa.2012.2500. Available online at: www.efsa.europa.eu/efsa_journal.
- EFSA Panel on Biological Hazards (BIOHAZ). 2014a. Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and norovirus in berries). *EFSA Journal*, **12**, 3706 (95 pp.). doi: 10.2903/j.efsa.2014.3706.
- EFSA Panel on Biological Hazards (BIOHAZ). 2014b. Scientific opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (*Salmonella* and norovirus in leafy greens eaten raw as salads). *EFSA Journal*, **12**, 3600 (118 pp.). doi: 10.2903/j.efsa.2014.3600.
- El-Senousy, W.M., Costafreda, M.I., Pintó, R.M., and Bosch, A. 2013. Method validation for norovirus detection in naturally contaminated irrigation water and fresh produce. *International Journal of Food Microbiology*, **167**, 74–79.
- Ethelberg, S., Lisby, M., Böttiger, B., Schultz, A.C., et al. 2010. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. *Euro Surveillance*, **15**, pii=19484. Available online at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19484>.
- Fan, X., Niemira, B.A., and Prakash, A. 2008. Irradiation of fresh fruits and vegetables. *Food Technology*, **62**, 36.
- Feng, K., Divers, E., Ma, Y.M., and Li, J.R. 2011. Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*, **77**, 3507–3517.
- Fino, V.R. and Kniel, K.E. 2008. UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. *Journal of Food Protection*, **71**, 908–913.
- Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., et al. 2011. Comparison of chlorine and peroxyacetic-based disinfectant to inactivate feline calicivirus, murine norovirus and hepatitis A virus on lettuce. *International Journal of Food Microbiology*, **151**, 98–104.
- Girard, M., Ngazoa, S., Mattison, K., and Jean, J. 2010. Attachment of noroviruses to stainless steel and their inactivation, using household disinfectants. *Journal of Food Protection*, **73**, 400–404.
- Gomez-Lopez, V.M., Marin, A., Medina-Martinez, M.S., Gil, M.I., and Allende, A. 2013. Generation of trihalomethanes with chlorine-based sanitizers and impact on microbial, nutritional and sensory quality of baby spinach. *Postharvest Biology and Technology*, **85**, 210–217.
- Grove, S.F., Forsyth, S., Wan, J., Coventry, J., et al. 2008. Inactivation of hepatitis A virus, poliovirus and a norovirus surrogate by high pressure processing. *Innovative Food Science and Emerging Technology*, **9**, 206–210.
- Hewitt, J. and Greening, G.E. 2004. Survival and persistence of norovirus, hepatitis A virus, and feline calicivirus in marinated mussels. *Journal of Food Protection*, **67**, 1743–1750.
- Hewitt, J., Rivera-Aban, M., and Greening, G. 2009. Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis A virus in heat inactivation studies. *Journal of Applied Microbiology*, **107**, 65–71.
- Hirneisen, K.A. and Kniel, K.E. 2013. Inactivation of internalized and surface contaminated enteric viruses in green onions. *International Journal of Food Microbiology*, **166**, 201–206.
- Hirneisen, K.A., Black, E.P., Cascarino, J.L., Fino, V.R., Hoover, D.G., and Kniel, K.E. 2010. Viral inactivation in foods: a review of traditional and novel food-processing technologies. *Comprehensive Review of Food Science and Food Safety*, **9**, 3–20.
- Holvoet, K., De Keuckelaere, A., Sampers, I., Van Haute, S., Stals, A., and Uyttendaele, M. 2014. Quantitative study of cross-contamination with *Escherichia coli* O157, MS2 phage and murine norovirus in a simulated fresh-cut lettuce wash process. *Food Control*, **37**, 218–227.
- Horm, K.M. and D'Souza, D.H. 2011. Survival of human norovirus surrogates in milk, orange, and pomegranate juice, and juice blends at refrigeration (4°C). *Food Microbiology*, **28**, 1054–1061.

- Kingsley, D.H. and Chen, H.Q. 2009. Influence of pH, salt, and temperature on pressure inactivation of hepatitis A virus. *International Journal of Food Microbiology*, **130**, 61–64.
- Kingsley, D.H., Guan, D.S., and Hoover, D.G. 2005. Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. *Journal of Food Protection*, **68**, 1748–1751.
- Kingsley, D.H., Hollinian, D.R., Calci, K.R., Chen, H.Q., and Flick, G.J. 2007. Inactivation of a norovirus by high-pressure processing. *Applied and Environmental Microbiology*, **73**, 581–585.
- Knight, A.I., Haines, J., Stals, A., Li, D., Uyttendaele, M., Knight, A.D., and Jaykus, L.-A. 2016. A systematic review of human norovirus survival reveals a greater persistence of human norovirus RT-qPCR signals compared with surrogate viruses. *International Journal of Food Microbiology*, **216**, 40–49.
- Lee, M.H., Yoo, S.-H., Ha, S.-D., and Choi, C. 2012. Inactivation of feline calicivirus and murine norovirus during Dongchimi fermentation. *Food Microbiology*, **31**, 210–214.
- Lee, J.-H., Bae, S.Y., Oh, M., Kim, K.H., Chung, M.S. 2014. Antiviral effects of mulberry (*Morus alba*) juice and its fractions on foodborne viral surrogates. *Foodborne Pathogens and Disease*, **11**, 224–229.
- Le Guyader, F.S., Le Saux, J.-C., Ambert-Balay, K., Krol, J., *et al.* 2008. Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *Journal of Clinical Microbiology*, **46**, 4011–4017.
- Leon, J.S., Kingsley, D.H., Montes, J.S., Richards, G.P., *et al.* 2011. Randomized, double-blinded clinical trial for human norovirus inactivation in oysters by high hydrostatic pressure processing. *Applied and Environmental Microbiology*, **77**, 5476–5482.
- Li, D., Tang, Q.J., Wang, J.F., Wang, Y.M., Zhao, Q., and Xue, C.H. 2009. Effects of high-pressure processing on murine norovirus-1 in oysters (*Crassostrea gigas*) in situ. *Food Control*, **20**, 992–996.
- Li, D., Baert, L., Van Coillie, E., and Uyttendaele, M. 2011a. Critical studies on binding based RT-PCR detection of infectious noroviruses. *Journal of Virology Methods*, **177**, 153–159.
- Li, D., Baert, L., De Jonghe, M., Van Coillie, E., Ryckeboer, J., Devlieghere, F., and Uyttendaele, M. 2011b. Inactivation of murine norovirus 1, coliphage phi X174, and *Bacillus fragilis* phage B40-8 on surfaces and fresh-cut iceberg lettuce by hydrogen peroxide and UV light. *Applied and Environmental Microbiology*, **77**, 1399–1404.
- Li, D., Baert, L., Xia, M., Zhong, W.M., Jiang, X., and Uyttendaele, M. 2012a. Effects of a variety of food extracts/juices on the specific binding ability of norovirus GII.4 P particles. *Journal of Food Protection*, **75**, 1350–1354.
- Li, D., Baert, L., Zhang, D., Xia, M., Zhong, W., Van Coillie, E., Jiang, X., Uyttendaele, M., 2012b. The effect of grape seed extract on human noroviruses GII. 4 and murine norovirus-1 in viral suspensions, on stainless steel discs, and in lettuce wash water. *Applied and Environmental Microbiology*, **78**, 7572–7578.
- Li, D., Baert, L., and Uyttendaele, M. 2013a. Inactivation of food-borne viruses using natural biochemical substances. *Food Microbiology*, **35**, 1–9.
- Li, X.H., Chen, H.Q., and Kingsley, D.H. 2013b. The influence of temperature, pH, and water immersion on the high hydrostatic pressure inactivation of GI.1 and GII.4 human noroviruses. *International Journal of Food Microbiology*, **167**, 138–143.
- Li, X.H., Ye, M., Neetoo, H., Golovan, S., and Chen, H.Q. 2013c. Pressure inactivation of Tulane virus, a candidate surrogate for human norovirus and its potential application in the food industry. *International Journal of Food Microbiology*, **162**, 37–42.
- Li, D., Stals, A., Tang, Q.-J., and Uyttendaele, M. 2014. Detection of noroviruses in shellfish and semiprocessed fishery products from a Belgian seafood company. *Journal of Food Protection*, **77**, 1342–1347.
- Lodder, W.J., van den Berg, H., Rutjes, S.A., and de Roda Husman, A.M. 2010. Presence of enteric

- viruses in source waters for drinking water production in the Netherlands. *Applied Environmental Microbiology*, **76**, 5965–5971.
- Lopez-Galvez, F., Allende, A., Truchado, P., Martinez-Sanchez, A., Tudela, J.A., Selma, M.V. and Gil, M.I. 2010. Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology*, **55**, 53–60.
- Lou, F.F., Neetoo, H., Chen, H.Q., and Li, J.R. 2011. Inactivation of a human norovirus surrogate by high-pressure processing: effectiveness, mechanism, and potential application in the fresh produce industry. *Applied and Environmental Microbiology*, **77**, 1862–1871.
- Loutreul, J., Cazeaux, C., Levert, D., Nicolas, A., Vautier, S., Le Sauvage, A.L., Perelle, S., and Morin, T. 2014. Prevalence of human noroviruses in frozen marketed shellfish, red fruits and fresh vegetables. *Food and Environmental Virology*, **6**, 157–168.
- Lowther, J.A., Gustar, N.E., Hartnell, R.E., and Lees, D.N. 2012. Comparison of norovirus RNA levels in outbreak-related oysters with background environmental levels. *Journal of Food Protection*, **75**, 389–393.
- Lukasik, J., Bradley, M.L., Scott, T.M., Dea, M., Koo, A., Hsu, W.Y., Bartz, J.A., and Farrah, S.R. 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella Montevideo*, and *Escherichia coli* O157: H7 on strawberries by physical and disinfectant washes. *Journal of Food Protection*, **66**, 188–193.
- Mäde, D., Trübner, K., Neubert, E., Höhne, M., and Johne, R. 2013. Detection and typing of norovirus from frozen strawberries involved in a large-scale gastroenteritis outbreak in Germany. *Food and Environmental Virology*, **5**, 162–168.
- Magulski, T., Paulmann, D., Bischoff, B., Becker, B., Steinmann, E., Steinmann, J., and Goroncy-Bermes, P. 2009. Inactivation of murine norovirus by chemical biocides on stainless steel. *BMC Infectious Disease*, **9**, 107–114.
- Mattison, K., Karthikeyan, K., Abebe, M., Malik, N., Sattar, S.A., Farber, J.M., and Bidawid, S. 2007. Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *Journal of Food Protection*, **70**, 500–503.
- Mbithi, J.N., Springthorpe, V.S., and Sattar, S.A. 1991. Effect of relative-humidity and air temperature on survival of hepatitis-A virus on environmental surfaces. *Applied and Environmental Microbiology*, **57**, 1394–1399.
- Nishida, T., Kimura, H., Saitoh, M., Shinohara, M., et al. 2003. Detection, quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. *Applied and Environmental Microbiology*, **69**, 5782–5786.
- Nuanualsuwan, S. and Cliver, D.O. 2002. Pretreatment to avoid positive RT-PCR results with inactivated viruses. *Journal of Virology Methods*, **104**, 217–225.
- Oh, D.-Y., Silva, P., Hauroeder, B., Diedrich, S., Cardoso, D., and Schreier, E. 2006. Molecular characterization of the first Aichi viruses isolated in Europe and in South America. *Archives of Virology*, **151**, 1199–1206.
- Oh, M., Bae, S.Y., Lee, J.-H., Cho, K.J., Kim, K.H., and Chung, M.S. 2012. Antiviral effects of black raspberry (*Rubus coreanus*) juice on foodborne viral surrogates. *Foodborne Pathogens and Disease*, **9**, 915–921.
- Oh, M., Bae, S.Y., and Chung, M.S. 2013. Mulberry (*Morus alba*) seed extract and its polyphenol compounds for control of foodborne viral surrogates. *Journal of the Korean Society for Applied Biological Chemistry*, **56**, 655–660.
- Oh, M., Lee, J.-H., Bae, S.Y., Seok, J.H., Kim, S., et al. 2015. Protective effects of red wine and resveratrol for foodborne virus surrogates. *Food Control*, **47**, 502–509.
- Okabayashi, T., Yokota, S., Ohkoshi, Y., Ohuchi, H., et al. 2008. Occurrence of norovirus infections unrelated to norovirus outbreaks in an asymptomatic food handler population. *Journal of Clinical Microbiology*, **46**, 1985–1988.
- Ozawa, K., Oka, T., Takeda, N., and Hansman, G.S. 2007. Norovirus infections in symptomatic and

- asymptomatic food handlers in Japan. *Journal of Clinical Microbiology*, **45**, 3996–4005.
- Park, S.Y. and Ha, S.-D. 2014. Influence of NaCl on the inactivation of murine norovirus-1 and hepatitis A virus in the Korean traditional salted oyster product “Eoriguljeot” during storage. *Food Research International*, **62**, 382–387.
- Parshionikar, S., Laseke, I., and Fout, G.S. 2010. Use of propidium monoazide in reverse transcriptase PCR to distinguish between infectious and noninfectious enteric viruses in water samples. *Applied and Environmental Microbiology*, **76**, 4318–4326.
- Paulmann, D., Steinmann, J., Becker, B., Bischoff, B., and Steinmann, E. 2011. Virucidal activity of different alcohols against murine norovirus, a surrogate of human norovirus. *Journal of Hospital Infections*, **79**, 378–382.
- Predmore, A. and Li, J. 2011. Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. *Applied and Environmental Microbiology*, **77**, 4829–4838.
- RIVM. 2013. Efficacy of applied processing measures on virus reduction in food. RIVM Report 330371007/2013, pp. 1–36.
- Sanchez, A. 2013. *Hepatitis A Virus in Food: Detection and Inactivation Methods* (ed. Hartel, R.W.), p. 47. Springer.
- Sanglay, G.C., Li, J.R., Uribe, R.M., and Lee, K. 2011. Electron-beam inactivation of a norovirus surrogate in fresh produce and model systems. *Journal of Food Protection*, **74**, 1155–1160.
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., *et al.* 2011. Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases*, **17**, 7–22.
- Scholz, E., Heinricy, U., and Flehmig, B. 1989. Acid stability of hepatitis-A virus. *Journal of General Virology*, **70**, 2481–2485.
- Seitz, S.R., Leon, J.S., Schwab, K.J., Lyon, G.M., Dowd, M., McDaniels, M., Abdulhafid, G., Fernandez, M.L., Lindesmith, L.C., and Baric, R.S. 2011. Norovirus infectivity in humans and persistence in water. *Applied Environmental Microbiology*, **77**, 6884–6888.
- Stals, A., Jacxsens, L., Baert, L., Van Coillie, E., and Uyttendaele, M. 2015. A quantitative exposure model simulating human norovirus transmission during preparation of deli sandwiches. *International Journal of Food Microbiology*, **196**, 126–136.
- Steyer, A., Torkar, K.G., Gutiérrez-Aguirre, I., and Poljsak-Prijatelj, M. 2011. High prevalence of enteric viruses in untreated individual drinking water sources and surface water in Slovenia. *International Journal of Hygiene and Environmental Health*, **214**, 392–398.
- Stine, S.W., Song, I.H., Choi, C.Y., and Gerba, C.P. 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *Journal of Food Protection*, **68**, 913–918.
- Su, X. and D’Souza, D.H. 2011. Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology*, **77**, 3982–3987.
- Su, X., Howell, A.B., and D’Souza, D.H. 2010a. The effect of cranberry juice and cranberry proanthocyanidins on the infectivity of human enteric viral surrogates. *Food Microbiology*, **27**, 535–540.
- Su, X., Howell, A.B., and D’Souza, D.H. 2010b. Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates – a time dependence study *in vitro*. *Food Microbiology*, **27**, 985–991.
- Su, X., Sangster, M.Y., and D’Souza, D.H. 2010c. *In vitro* effects of pomegranate juice and pomegranate polyphenols on foodborne viral surrogates. *Foodborne Pathology and Disease*, **7**, 1473–1479.
- Su, X., Sangster, M.Y., and D’Souza, D.H. 2011. Time-dependent effects of pomegranate juice and pomegranate polyphenols on foodborne viral reduction. *Foodborne Pathology and Disease*, **8**, 1177–1183.
- Takahashi, H., Ohuchi, A., Miya, S., Izawa, Y., and Kimura, B. 2011. Effect of food residues on norovirus survival on stainless steel surfaces. *PloS ONE*, **6**, e21951. doi: 10.1371/journal.pone.0021951.

- Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., Riley, K., and Gerba, C.P. 2003a. Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied and Environmental Microbiology*, **69**, 577–582.
- Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., and Gerba, C.P. 2003b. Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Applied and Environmental Microbiology*, **69**, 3979–3985.
- Topping, J.R., Schnerr, H., Haines, J., Scott, M., *et al.* 2009. Temperature inactivation of feline calicivirus vaccine strain FCV F-9 in comparison with human noroviruses using an RNA exposure assay and reverse transcribed quantitative real-time polymerase chain reaction – A novel method for predicting virus infectivity. *Journal of Virology Methods*, **156**, 89–95.
- Tuladhar, E., Bouwknecht, M., Zwietering, M.H., Koopmans, M., and Duizer, E. 2012. Thermal stability of structurally different viruses with proven or potential relevance to food safety. *Journal of Applied Microbiology*, **112**, 1050–1057.
- USEPA (United States Environmental Protection Agency). 2006. *Ultraviolet Disinfection Guidance Manual for the Final Long Term. 2. Enhanced Surface Water Treatment Rule*. Office of Water, Washington, D.C. Available from: http://www.epa.gov/safewater/disinfection/lt2/pdfs/guide_lt2_uvguidance.pdf (accessed 12 February 2011).
- Vasickova, P., Pavlik, I., Verani, M., and Carducci, A. 2010. Issues Concerning Survival of Viruses on Surfaces. *Food and Environmental Virology*, **2**, 24–34.
- Verhoef, L., Jaramillo Gutierrez, G., Koopmans, M., and Boxman, I.L. 2013. Reported behavior, knowledge and awareness toward the potential for norovirus transmission by food handlers in Dutch catering companies and institutional settings in relation to the prevalence of norovirus. *Food Control*, **34**, 420–427.
- Wang, Q., Erickson, M.C., Ortega, Y., and Cannon, J.L. 2013. Physical removal and transfer of murine norovirus and hepatitis A virus from contaminated produce by scrubbing and peeling. *Journal of Food Protection*, **76**, 85–92.
- Wei, J., Jin, Y., Sims, T., and Kniel, K.E. 2010. Survival of murine norovirus and hepatitis A virus in different types of manure and biosolids. *Foodborne Pathology and Disease*, **7**, 901–906.
- Wigginton, K.R., Pecson, B., Sigstam, T., Bosshard, F., and Kohn, T. 2012. Virus inactivation mechanisms: impact of disinfectants on virus function and structural integrity. *Environmental Science and Technology*, **46**, 12069–12078.
- Wolf, S., Rivera-Aban, M., and Greening, G.G. 2009. Long-range reverse transcription as a useful tool to assess the genomic integrity of norovirus. *Food and Environmental Virology*, **1**, 129–136.
- Woods, J.W. and Burkhardt III, W. 2010. Occurrence of norovirus and hepatitis A virus in U.S. oysters. *Food and Environmental Virology*, **2**, 176–182.
- Xie, Y., Hajdok, C., Mittal, G.S., and Warriner, K. 2008. Inactivation of MS2 F(+) coliphage on lettuce by a combination of UV light and hydrogen peroxide. *Journal of Food Protection*, **71**, 903–907.
- Zhou, F.H., Harmon, K.M., Yoon, K.J., Olson, D.G., and Dickson, J.S. 2011. Inactivation of feline calicivirus as a surrogate for norovirus on lettuce by electron beam irradiation. *Journal of Food Protection*, **74**, 1500–1503.
- Zuber, S., Butot, S., and Baert, L. 2013. *Foodborne Viruses and Prions and Their Significance for Public Health* (eds Smulders, F.J.M., Norrung, B., and Budka, H.). Wageningen Academic Publishers.

CHAPTER 15

Impact of food unit operations on parasites in foods: focus on selected parasites within the fresh produce industry

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15.1 Background and introduction

15.1.1 Fresh produce as a transmission vehicle for parasites

Various parasites of public health significance can be transmitted to humans by the food-borne route. For some parasites, especially those associated with meat or fish, food-borne transmission is essential in the parasite lifecycle, and the meat animal or fish often acts as an intermediate host. Such parasites include *Trichinella spiralis*, often associated with pork, other species of *Trichinella*, often associated with horse meat or game, *Taenia solium* and *Taenia saginata*, associated with pork and beef, respectively, *Sarcocystis suis-hominis* and *Sarcocystis hominis* for pork and beef, respectively, and a range of helminths in fish such as *Opisthorchis* spp., *Clonorchis sinensis*, *Diphylllobothrium* spp., and anisakid nematodes. An important point with all these parasites is that adequate cooking of the meat or fish will also inactivate the parasite transmission stage.

As fresh produce, especially salad vegetables and fruit, is often eaten raw and with minimal preparation, it is a product group that is recognized as a growing cause of outbreaks of food-borne illness (Sivapalasingam *et al.*, 2004; Lynch *et al.*, 2009; Berger *et al.*, 2010; Kozak *et al.*,

2013). Such outbreaks are not only occurring more frequently but, with industrialization of the fresh produce industry and improvements in the cold chain for transporting fresh produce, is affecting greater numbers of people. For this transmission route, parasites with hardy transmission stages are a pathogen group of particular concern. However, most documented outbreaks associated with fresh produce are bacterial, possibly because of superior diagnostic capabilities for such infections and the shorter incubation period between infection and symptoms.

Fresh produce acts as a vehicle for food-borne parasitic infections when the transmission stages of the parasite contaminate the fresh produce, and these stages are not removed or inactivated during processing or preparation prior to consumption by another susceptible host. For infection to occur, the contaminating transmission stage must be consumed within the time frame of survival and infectivity for the specific parasite. Many types of fresh produce, especially salad vegetables, fruits that are not peeled, and berries, are particularly suited to acting as transmission vehicles as they are usually not cooked and have only minimal preparation before being eaten.

Contamination with infective parasite stages may occur at any stage along the fresh produce chain and the contamination may emanate from a variety of sources (see Table 15.1).

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Table 15.1 Contamination along the fresh produce chain; examples of contamination sources.

Contamination point	Contamination source	Examples	Comments and references
During cultivation Contaminated seed	Seed can be contaminated in the seed supply chain	Important for fresh produce where the seed is part of the end product that is consumed (e.g., sprouted seeds)	See Robertson <i>et al.</i> , 2002, in which <i>Cryptosporidium</i> oocysts and <i>Giardia</i> cysts were detected on unsprouted mung bean seeds
Direct contamination of the crop via feces of infected host, where the infectious stage is excreted in feces	Feces of infected hosts (human or other animal) –may be spread deliberately (manure or slurry) or contact with feces may be unintentional	Infectious stages of various parasites may be found in human or animal feces, including: Humans only: <i>Cyclospora</i> oocysts, <i>Entamoeba histolytica</i> cysts, <i>Taenia solium</i> eggs, <i>Trichuris trichiura</i> eggs; Canids: <i>Echinococcus</i> eggs, <i>Toxocara canis</i> eggs; Felids: <i>Toxoplasma</i> oocysts, <i>Toxocara cati</i> eggs; Humans and (perhaps) other mammals: <i>Ascaris</i> eggs, <i>Balantidium coli</i> cysts, <i>Cryptosporidium</i> oocysts, <i>Giardia</i> cysts	Direct contamination of a crop by feces may be difficult to prove – but is probably more common where fecal matter has direct contact with the crop. An outbreak of cryptosporidiosis in 2009 at a farm in the US was postulated to be due to contamination by feces from infected livestock (Collier <i>et al.</i> , 2011). Contamination of vegetables with <i>Giardia</i> cysts in the Philippines was speculated to be due to application of human and animal waste to agricultural land (De Leon <i>et al.</i> , 1992)
Direct contamination of the crop via infectious parasite stages, but not from feces	Active parasite contamination	<i>Fasciola hepatica</i> and other trematodes (e.g., other <i>Fasciola</i> species or <i>Fasciolopsis buski</i>) in which cercariae from snail intermediate hosts encapsulate to infectious metacystode stage on produce under cultivation (Ueno <i>et al.</i> , 1975); third stage infective larvae of the nematode <i>Angiostrongylus cantonensis</i> and <i>A. costaricensis</i> are usually transmitted to humans via ingestion of intermediate hosts (snails) or paratenic hosts (e.g., shrimp), but snails may be ingested inadvertently with fresh produce and snail slime contaminating fresh produce may contain infective larvae	Although primarily associated with aquatic plants, such as watercress, outbreaks of fascioliasis have occurred where extreme weather events have brought parasites, snails, and crops such as lettuce into contact (e.g., Rojas <i>et al.</i> , 2010). Although most cases of angiostrongyliasis are associated with intentional consumption of raw snails, individual cases occur where fresh produce (snail slime or unintentional ingestion of snails) seems to be the infection route (Cowie <i>et al.</i> , 2013)

(Continued)

Table 15.1 (Continued)

Contamination point	Contamination source	Examples	Comments and references
Contamination via application of water (irrigation water, pesticide, fungicide, etc.)	Contaminated water	All parasites that are excreted in faeces may contaminate water; contact between a crop under cultivation and contaminated water during irrigation or other agricultural practices may result in crop contamination	Contaminated irrigation water has been associated with contaminated produce (e.g., for <i>Cryptosporidium</i> oocysts and <i>Giardia</i> cysts; Amorós <i>et al.</i> , 2010). The potential for contamination is higher if the irrigation water is a recipient of wastewater (Srikanth and Naik, 2004; Lonigro <i>et al.</i> , 2006; Vuong <i>et al.</i> , 2007; Kaserue <i>et al.</i> , 2012; Ranjbar-Bahadori <i>et al.</i> , 2013)
During harvesting and post-harvesting procedures			
During harvesting and sorting	From hands of harvesters and sorters post-harvest that are contaminated with parasite transmission stages	Lack of hygiene and hygiene facilities, particularly for farm workers involved in harvesting of crops, may exacerbate chances of contamination of hand-picked crops. Intestinal helminths (<i>Ascaris</i> , <i>Trichuris</i> , <i>Taenia solium</i>) and protozoa (<i>Cryptosporidium</i> , <i>Giardia</i> , <i>Cyclospora</i> , etc.) with which farm workers or their close contacts may be infected, are particular risks	Children of seasonal farm workers are considered a high-risk group for intestinal parasites; a study from Turkey demonstrated over 50% prevalence of these pathogens, particularly <i>Giardia</i> (Koruk <i>et al.</i> , 2010)
Contamination via washwater	Contaminated water	All parasites that are excreted in faeces may contaminate water; contact between a crop and contaminated water during post-harvest washing to remove visual dirt may result in the crop becoming contaminated	A study from Pakistan demonstrated that unsanitary handling post-harvest (including washing in contaminated water) may cause greater contamination than wastewater irrigation (Ensink <i>et al.</i> , 2007)
Contamination from storage, transport, etc.	Environmental contamination	Parasites with robust transmission stages may contaminate harvested fresh produce; e.g., transport of fresh produce in a truck that has previously transported calves with cryptosporidiosis	In environments where soil is highly contaminated, this may be a particular risk. Contaminated soil has been postulated as a source of contamination post-harvest (Ensink <i>et al.</i> , 2007)
During processing prior to sale			
Contamination via washwater	Contaminated water	In many countries, fresh produce is collected from primary producers and processed for sale at a common facility. Handling is often minimal in such facilities, but washing and re-use of washwater may enable the spread of localized contamination through a produce lot. Decontamination of washwater is difficult due to high organic load, and is a recognized problem in the fresh produce industry (Gil <i>et al.</i> , 2009)	Investigation of water in wash-water tanks at selected packing houses in Mexico demonstrated that 16% of washwater tanks contained <i>Cryptosporidium</i> oocysts (up to 133 oocysts per 100 l) and 83% were positive for <i>Giardia</i> cysts (up to 533 cysts per 100 litres) (Chaidez <i>et al.</i> , 2005)

At the retailer or consumer		
At sale, preparation, or consumption	Usually from hands of retailer or food workers	Poor hygiene or lack of hygiene facilities may result in food handlers contaminating fresh produce, either at retail, at commercial or institutional premises for eating, or in their own homes. Food handlers are a common route for contamination (Greig <i>et al.</i> , 2007). Parasites most likely to be transmitted by this route are those for which the particular food handler (or close contact) is a host and excreting infectious stages. Intestinal helminths (<i>Taenia solium</i>) and protozoa (<i>Cryptosporidium</i> , <i>Giardia</i> , <i>Entamoeba</i> , etc.) are risks, but parasites requiring maturation (embryonation) in the environment (e.g., <i>Ascaris</i> , <i>Cyclospora</i>) before they become infective are less of a risk
Dilution of fruit juices	Contaminated water	If contaminated water is used in the processing or preparation of fresh produce, then the final food may also be contaminated
During preparation	Contamination from flies, other insects, or other reservoir hosts	Mechanical transmission of parasites from feces to fresh produce may occur at any stage of the food chain. Synanthropic filth flies are potential vectors of parasites from feces to food (Graczyk <i>et al.</i> , 2004). For the potentially food-borne parasite, <i>Trypanosoma cruzi</i> , the aetiological agent of Chagas disease, contamination of products from fresh produce (particularly fruit juices) has been associated with the reduviid bug vectors, or their feces, or the excretions of other hosts being incorporated into the juice
		Many documented outbreaks of food-borne giardiasis and food-borne cryptosporidiosis are associated with a food handler, or contamination is presumed to have occurred in the setting of food preparation or sale (e.g., Porter <i>et al.</i> , 1990; Mintz <i>et al.</i> , 1993; Quiroz <i>et al.</i> , 2000)
		In a study of different juices in Egypt, in which a large proportion were positive for <i>Cryptosporidium</i> oocysts and <i>Giardia</i> cysts, it is speculated that the likely source of contamination was the water used for dilution of the juice rather than the fruit itself (Mossallam, 2010)
		An experimental study demonstrated contamination of mango juice with <i>Cryptosporidium</i> oocysts by flies (Fetene <i>et al.</i> , 2011). Several outbreaks of Chagas disease have probably been caused by contamination from insects (reduviid bugs) or other hosts such as opossums (e.g., Alarcón de Noya <i>et al.</i> , 2010; Ríos <i>et al.</i> , 2011; Pereira <i>et al.</i> , 2012)

Given the number and variety of routes for contamination of fresh produce with a range of different potentially food-borne parasites, it is unsurprising that fresh produce is a known risk product for transmission of these pathogens. While food-borne transmission can be of medical importance to an individual, the real threat to public health and the economic importance of this transmission route lies in the potential for widespread outbreaks to occur.

Nevertheless, the number of outbreaks of parasitic disease in which contaminated fresh produce has been the proven transmission vehicle is relatively low. There are a number of reasons for this including:

- 1 Lack of diagnostic expertise. Many medical microbiology laboratories have relatively little expertise in diagnosing parasitic infections, and a substantial proportion of food-borne parasitic infections are not diagnosed.
- 2 Lack of association of parasitic infection with food-borne transmission. Even when a parasitic infection is diagnosed, the possibility of other transmission routes (e.g., via water or from a close contact) may prevent the infection being associated with consumption of fresh produce.
- 3 Relatively lengthy incubation period between infection and symptoms. The symptoms of most food-borne parasitic infections do not commence until several days (or weeks or even years for some parasites) following infection. Due to the lengthy incubation period, associating infection with a particular foodstuff may be difficult, even when epidemiological investigation may indicate this infection route. Furthermore, within the period between infection and diagnosis, the suspect food is likely to have been discarded or consumed; thus contamination of the suspected foodstuff cannot be demonstrated.
- 4 Methods for analysing fresh produce for contamination with parasite transmission stages are often inadequate. The lack of focus on food-borne parasites means that little effort has been directed towards development of

adequate methods for analysing fresh produce, or other food matrices, for contamination with parasite infective stages.

Although few outbreaks of parasitic infection are documented in which fresh produce has been proven to be the vehicle of infection, various biological factors for some parasites mean that they are particularly suited to food-borne transmission. These factors include:

- *High excretion rate.* High numbers of parasite transmission stages excreted from an infected host result in a greater chance of fresh produce being contaminated to such an extent that transmission occurs.
 - *Robust transmission stage.* Parasites with robust transmission stages are more likely to survive the period between contamination and ingestion by the next susceptible host. The increasing speed and sophistication in the fresh produce supply chain means that some fresh produce and attendant pathogens will be exposed to few environmental pressures. Although maintaining the cold chain is important for food safety as well as produce quality, as most bacteria do not grow as well at refrigeration temperatures, such temperatures may be ideal for keeping parasite transmission stages alive.
- Fresh produce is often exposed to a “sanitation” regimen, which may include physical or chemical processes (see later sections). Parasite transmission stages must be able to survive exposure to sanitizers in order to remain a health threat. For produce that is frozen, only parasites that survive freezing remain a threat.
- *Low infectious dose.* Even with a high excretion rate, post-harvesting procedures are likely to reduce the quantity of transmission stages of parasites on fresh produce to relatively low concentrations. If the infectious dose is low, only a few (or just one) parasite need to be ingested for successful transmission.
 - *Infective cycle.* Some parasites are infectious immediately upon excretion (e.g., *Cryptosporidium* spp., *Giardia duodenalis*, *Taenia*

solium), and this may facilitate onwards transmission if there is only a short period between product contamination and ingestion. For other parasites, a maturation period in the environment is necessary (e.g., *Toxoplasma gondii*, *Cyclospora cayetanensis*, *Ascaris lumbricoides*). Depending upon the duration of this period, along with factors such as contamination point, storage, and transport conditions, food-borne transmission may be facilitated or hindered.

- **Zoonotic potential and host specificity.** For parasites that infect animals other than humans, not only is fresh produce vulnerable to contamination from human sources (e.g., sewage discharge, parasites from food handlers) but it may also be contaminated from animal sources. This may be relevant where animal manure or slurry is used for improving the soil.

15.1.2 Ranking of food-borne parasites; occurrence and impacts

With so many different food-borne parasites it can be difficult to decide which are of most importance, globally, nationally, or locally. Furthermore, selecting criteria that reflect importance is also challenging and may depend upon the perspective of the entity posing the question. Is a pathogen that causes more infections (has a higher prevalence) of greater or lesser importance than a pathogen that causes fewer infections (has a lower prevalence), but for which symptoms of infection are more severe? Should global or regional distribution be considered, trade impacts, or potential for the pathogen to spread to new regions given current climate and globalization projections?

In order to address these questions, in 2010 the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) were requested by the Codex Committee on Food Hygiene (CCFH) to “review the current status of knowledge on parasites in food and their public health and trade impact in order to provide the CCFH with advice and guidance on the parasite–commodity

combinations of particular concern, the issues that need to be addressed by risk managers and the options available to them”.

An Expert Group on food-borne parasites was established that collaborated to provide a global ranking of 24 selected food-borne parasites (Robertson *et al.*, 2013) using a multicriteria decision analytic approach. The results of this consultation have been published and a full report is available (FAO and WHO, 2014). Of the 24 parasites ranked (Robertson *et al.*, 2013), over 50% of them have the potential to be transmitted to humans by ingestion of transmission stages as contaminants of fresh produce. Furthermore, the parasites ranked as being in the top 6 of global importance can all be transmitted to humans via ingestion of contaminated fresh produce.

15.1.3 Parasite selection for this chapter

Given the number of parasites that could be included in this review, a judicious selection of just four has been made based on perceived global importance and documented food-borne outbreaks. These are: *Cryptosporidium* spp. (protozoan), *Cyclospora cayetanensis* (protozoan), *Taenia solium* (cestode/tapeworm), and *Ascaris lumbricoides* (nematode/roundworm). These choices are made based on one or more of the following criteria: ranking position in the FAO/WHO global ranking, proven transmission via fresh produce (sometimes with outbreaks), high prevalence either globally or in specific regions, potentially very severe pathology.

Cyclospora and *Ascaris* are included, rather than *Toxoplasma gondii*, *Entamoeba histolytica*, *Echinococcus* spp., *Trypanosoma cruzi*, and *Giardia*, all of which scored as being “more important” than *Cyclospora* in the FAO/WHO global ranking and some of which scored as being “more important” than *Ascaris*, but the list is based upon the following:

- 1 Although *Toxoplasma* has the potential for severe consequences of infection (including abortion in pregnant women and encephalitis in the immunosuppressed), to date only one

- outbreak of toxoplasmosis associated with fresh produce has been reported (Ekman *et al.*, 2012). Furthermore, as the definitive host is the cat, for fresh produce to become contaminated with infectious oocysts then it must come into contact with oocysts excreted in cat faeces. In terms of general production of fresh produce this is less likely than contact with faeces of animals where their manure may be used as fertilizer, or human sewage.
- 2 Although the pathology of *E. histolytica* is potentially very severe, with the possibility of extraintestinal spread and development of amoebic liver abscess, there have, to date, been no reported outbreaks of food-borne amoebiasis (Theel and Pritt, 2012).
 - 3 Although the potential pathology associated with *Echinococcus* spp. infection is severe (hence the high ranking by FAO/WHO, 2014), evidence for infection associated with fresh produce is low and consumption of raw outdoor produce did not emerge as an important risk factor for infection in a German study (Kern *et al.*, 2004). As the effects of food unit operations on tapeworm eggs are likely to be highly similar regardless of species, much of the information provided in the following sections for effects of processes on *Taenia solium* eggs (ranked above *Echinococcus* spp. by FAO/WHO, 2014) will also be relevant for *Echinococcus* spp.
 - 4 Although *Giardia* is often considered to have similar transmission routes and epidemiology as *Cryptosporidium*, comparison of data on food-borne outbreaks associated with both parasites demonstrates that food-borne giardiasis outbreaks occur relatively rarely; fewer than 10 have been reported since 1960 and the most recent was over 10 years ago (Robertson, 2014a). Food-borne transmission of *Giardia* accounts for around only 200 cases of infection in total; presumably this difference between food-borne cryptosporidiosis and food-borne giardiasis is due to epidemiological and biological differences between the two parasites.
 - 5 Although *Trypanosoma cruzi* is definitely an important food-borne parasite, with significant outbreaks reported, including fatalities, it is geographically of limited importance (transmission generally occurring only where reduviid bugs are found; food-borne outbreaks have been exclusively reported from South American countries).
 - 6 Finally, there are two reasons for including *Cyclospora cayentanensis* and *Ascaris lumbricoide*, which are not ranked as being particularly important in the FAO/WHO ranking (2014), with *Ascaris* at position 9 of 24 and *Cyclospora* at position 13. *Ascaris* is a representative nematode, as the eggs of these parasites are often notoriously robust. Although *Ascaris* often has few acute symptoms, being often considered as an insidious infection, affecting the ability to reach full potential rather than resulting in acute disease, it is one of the most common intestinal parasites of humans globally. *Cyclospora* was included because of recent outbreaks of cyclosporiasis associated with fresh produce; at the time of writing this chapter (August 2014), 304 people with confirmed *Cyclospora* infection had been reported to CDC from various states of the United States, and some cases were linked to consumption of fresh cilantro imported from Puebla, Mexico (see: <http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2014/index.html>).
- In the following section a brief overview of each of the four selected parasites is provided, including some information on food-borne transmission.

15.1.4 Overview of selected parasites

15.1.4.1 *Cryptosporidium* spp.

15.1.4.1.1 Background

Over 25 different species of *Cryptosporidium* are recognized and these can infect a wide range of vertebrates, including humans. Of these, approximately twelve species (and several genotypes) are infectious to humans, but the overwhelming majority of human cases are associated with

two particular species: *Cryptosporidium hominis*, which has a predilection for humans as hosts but has very occasionally been identified in other animals, and *C. parvum*, a host-promiscuous species that can infect a wide range of vertebrates and is very common in domesticated and wild ruminants. It is particularly associated with cattle, especially calves. Other species of *Cryptosporidium* that have been more frequently associated with human infection include *C. ubiquitum* (commonly associated with deer and sheep infections) and *C. meleagridis* (commonly associated with infections of turkeys). It is important to be aware that although immunocompromised persons may be more susceptible to infection with these more unusual species, immunocompetent persons can also be infected. In recent years, *C. cuniculus* appears to be emerging as a human pathogen. Primarily associated with infections in rabbits, human infection potential was first properly realized in association with a water-borne outbreak in the United Kingdom (Robinson and Chalmers, 2010).

Cryptosporidium infection is a public health problem of global importance. Although data are not available for all countries, one estimate suggested that the prevalence of *Cryptosporidium* infection in patients with gastroenteritis was 1–4% in Europe and North America, and 3–20% in Africa, Asia, Australia, and Latin America (Current and Garcia, 1991). Laberge and Griffiths (1996) estimated that prevalence rates were 1–3% in industrialized countries and up to 10% in developing countries. Cryptosporidiosis has been reported in 106 countries worldwide (Fayer, 2008).

In healthy hosts, *Cryptosporidium* infection usually presents as either a self-limiting disease (cryptosporidiosis) or is asymptomatic, but it can be a life-threatening problem for the immunocompromised. In humans, *Cryptosporidium* primarily affects the gastrointestinal epithelium, resulting in production of acute watery diarrhoea. Other sites, such as the trachea, have been infected in immunocompromised persons.

There is usually around one week between ingestion of oocysts and development of symptoms. The diarrhoea is often acute, but can also be persistent, lasting several weeks, and may be accompanied by symptoms such as nausea, vomiting, abdominal pains, low-grade fever, and headache. The spectrum of symptoms depends on factors associated with the host (age, immunity, nutritional status), and also the species of *Cryptosporidium*. For immunocompetent patients, the symptoms of cryptosporidiosis usually resolve within 1 to 2 weeks, but a high rate of relapse following recovery has also been reported (40% of patients).

Cryptosporidiosis is likely to be more severe and persistent in immunocompromised patients. A daily output of diarrhoea of more than 15 l has been reported from immunocompromised patients, and this can contribute to mortality, particularly if the patient has other health problems such as inadequate nutrition or concomitant infections. A lack of effective treatment for cryptosporidiosis in immunocompromised patients means that infection with this parasite can be a serious condition.

15.1.4.1.2 Information on transmission of *Cryptosporidium* via fresh produce

The lifecycle of *Cryptosporidium* is complicated, with cycles of both asexual and sexual reproduction occurring within an infected host. This results in high numbers of the transmission stage (oocyst) of the parasite being produced. An excretion rate of over 10^9 oocysts daily has been reported from AIDS patients with symptomatic cryptosporidiosis (Goodgame *et al.*, 1993) and similar excretion rates have been reported from calves that are naturally infected with *C. parvum* (Uga *et al.*, 2000; Nydam *et al.*, 2001).

Cryptosporidium oocysts are small (3–5 µm in diameter) and immediately infectious upon excretion in the faeces of the infected host. However, the oocysts can also survive for prolonged periods (weeks or months) under cool, moist conditions, and are robust against various physical and chemical stresses (Robertson and

Gjerde, 2007). Various outbreaks of food-borne cryptosporidiosis have been documented, particularly from Nordic countries. However, this probably is due to superior infrastructure and resources in these countries enabling more directed investigation and reporting, rather than greater transmission or exposure (Robertson and Chalmers, 2013). Although fresh produce, or products from fresh produce such as juice, have been major vehicles of food-borne infection, some outbreaks have been associated with dairy products and meat. However, the majority of food-borne outbreaks are associated with salad and salad bar items (Robertson, 2014b). The largest outbreak of food-borne cryptosporidiosis occurred in the UK in 2012 (across four different regions of England and also Scotland) and affected over 300 individuals, mostly adults (Anonymous, 2013). The most likely vehicle of infection in this outbreak was “ready-to-eat”, pre-cut mixed salad leaves from a major supermarket chain. It should be noted that this was an epidemiological link and analysis of samples did not reveal contamination with *Cryptosporidium* oocysts and nor was the source of contamination identified.

15.1.4.2 *Cyclospora cayetanensis*

15.1.4.2.1 Background

Cyclospora cayetanensis is a coccidian protozoan in which sporulated oocysts ingested by susceptible hosts excyst in the gastrointestinal tract and invade the epithelial cells of the small intestine, where asexual and sexual multiplication occurs. Oocysts are excreted in the faeces, but are not immediately infectious upon excretion, taking around 1 to 2 weeks to sporulate and become infectious. *C. cayetanensis* seems to be anthroponotic, such that zoonotic infection is not a major concern. Although apparently endemic in different regions of South and Central America, the Caribbean, and Asia, the full geographic distribution of *Cyclospora* is probably unknown.

Usual symptoms of cyclosporiasis include watery diarrhoea, nausea, abdominal pain, and anorexia. Biliary disease, Guillain-Barré and

Reiter's Syndrome have been reported in association with previous *Cyclospora* infections (Ortega and Sanchez, 2010). In immunocompromised individuals, the symptoms of cyclosporiasis are more severe and may be prolonged. Various drug treatments are available, but due to lack of familiarity with the parasite in many countries, it may take several days for diagnosis. Prevalence of *Cyclospora* in developing countries ranges from 1% upwards, depending on country, study population, and season (Shields and Ortega, 2012); expatriates relocating to an endemic country seem particularly likely to become infected. Although *Cyclospora* infection is regularly diagnosed in developed countries, it is often as “traveller's diarrhoea” (therefore an imported infection, associated with travel to an endemic area) or associated with a food-borne outbreak, usually with imported fresh produce as the vehicle of infection. The apparent lack of establishment of endemic infection in developed countries may be due to the requirement for relatively warm conditions (23 to 27 °C) for optimal sporulation, which takes 1–2 weeks (Ortega and Sanchez, 2010). Other factors such as more limited geographical distribution of infection at present, diagnostic failure, infrastructure associated with sanitation and water supply, and apparent absence of zoonotic potential may also play a role.

15.1.4.2.2 Information on transmission of *Cyclospora cayetanensis* via fresh produce

Up until the mid-1990s, *Cyclospora* was considered to be of most public health significance in developing countries, and as an occasional cause of traveller's diarrhoea in industrialized countries. However, following several outbreaks of food-borne infection, it became recognized as an important aetiological agent of food-borne disease, particularly associated with the import of fresh produce, especially raspberries, from Central and South America to North America (Shields and Ortega, 2012). Our lack of understanding of the biology and ecology of *Cyclospora* means that we have extensive knowledge gaps

regarding transmission. However, we do know that *Cyclospora* oocysts leave the host unsporulated and take around 2 weeks to sporulate and become infective. We also know that, like *Cryptosporidium*, the infectious dose is low and that the oocysts are very hardy (Shields and Ortega, 2012). Multiple outbreaks of food-borne cyclosporiasis have been reported (between mid-1990s and 2010 over 40 outbreaks were reported; Shields and Ortega, 2012), with well over 1000 cases documented. Most of these outbreaks have been reported from North America, but outbreaks have also been identified in Europe, South America, and Asia. Several outbreaks from cruise ships have been reported, most recently from 2010 in which passengers and crew on two successive voyages of a cruise ship departing from, and returning to, Fremantle, Australia, were affected (Gibbs *et al.*, 2013). It is speculated that fresh produce taken onboard at a south-east Asian port during the first cruise was the vehicle of infection (Gibbs *et al.*, 2013). Although most outbreaks of food-borne cyclosporiasis are associated with fresh berries (particularly raspberries imported from Guatemala), other varieties of fresh produce have also been associated with outbreaks, including herbs, salads, sugar snap peas, and fruit juices. Apart from the current ongoing multistate outbreak of cyclosporiasis (<http://www.cdc.gov/parasites/cyclosporiasis/outbreaks/2014/index.html>), in which some cases in Texas seem to be associated with cilantro imported from Mexico, the most recent outbreak occurred in 2013 with around 650 cases reported; this was a multistate outbreak in the US where cases from two of the states involved (Iowa and Nebraska) were associated with salad mix produced in Mexico (Robertson *et al.*, 2014).

15.1.4.3 *Taenia solium*

15.1.4.3.1 Background

Taenia solium is a cestode (tapeworm) parasite in which the definitive hosts are humans and the intermediate hosts are pigs. As with other tapeworms, the scolex of *T. solium* attaches to

the intestinal wall of the definitive host, with four suckers and hooks assisting in holding on to the wall. The body of the worm is a chain of segments, known as proglottids. The sum of the proglottids is called a strobila and can grow to over 3 m in length. Humans acquire taeniosis (infection with adult tapeworm) by ingestion of the larval stages (cysticerci) in inadequately cooked pork. The distal proglottids detach from the worm when their eggs are mature and pass out into the environment with faeces; the eggs in these proglottids are infective to pigs and the larval stages that develop in pigs are infective to humans.

The tapeworm eggs that are infective to pigs are also infective to humans, and the resultant infection from ingestion of tapeworm eggs (cysticercosis) results in much greater pathology than infection with the adult worm (taeniosis). The cysticerci can develop in almost any tissue, but involvement of the CNS, resulting in a disease state known as neurocysticercosis, is the most important clinical manifestation in humans and may lead to epilepsy and death (Sorvillo *et al.*, 2007). This is one of the main reasons why this infection received the highest ranking in the FAO/WHO risk ranking exercise (FAO/WHO, 2014). Furthermore, although endemic cysticercosis is practically non-existent in Europe, apart from occasional cases in some countries, cysticercosis from consumption of *T. solium* eggs is considered as one of the more common parasitic diseases globally, with an estimated prevalence of over 50 million people (Psarros *et al.*, 2003; Hawk *et al.*, 2005). *T. solium* is endemic in several developing countries, including in Central and South America, sub-Saharan Africa, South East Asia, and Western Pacific (Schantz, 2002), and is considered as an emerging problem in the US and parts of Europe due to increased international travel (Schantz, 2002; Pal *et al.*, 2000).

Treatment options depend on the location, number, and stage of cysticerci, and clinical manifestations. Anthelmintic therapy, because it kills viable cysts and provokes an inflammatory response, may result in an acute increase in

symptoms and therefore co-administration with corticosteroids may be used to mitigate these effects. Thus, although anthelmintic therapy is generally indicated for symptomatic patients with multiple, live (non-calcified) cysticerci, administration of steroids is often indicated to suppress inflammatory responses. Conventional anticonvulsant therapy may be used for management of neurocysticercosis-associated seizure disorders, and intraventricular cysts should usually be treated by surgical removal.

15.1.4.3.2 Information on transmission of *Taenia solium* via fresh produce

The potential for contamination of vegetables with infectious *T. solium* eggs is due to the high excretion rate (mature worms in the human intestine may shed around 300 000 eggs daily, and they have a lifespan of up to 20 years or more, so shedding is prolonged) and their robustness in the environment, being able to survive weeks or months. Due to the long period between infection and symptoms, it is practically impossible to determine when cysticercosis from *T. solium* is due to food-borne transmission and when it is due to ingestion of eggs from other contaminated items; no food-borne outbreaks are recorded. In addition, auto-infection can occur by reverse peristalsis.

Nevertheless, food-borne transmission with products that are not cooked following contamination (such as fresh produce) is obviously a likely route. Some unrelated cases diagnosed amongst Orthodox Jews in New York, who had never knowingly eaten pork and did not have risk factors for contracting *T. solium*, are speculated to have been by food-borne infection (Schantz *et al.*, 1992). Consumption of raw vegetables has also been associated as a risk factor for cysticercosis (Prasad *et al.*, 2011). However, it should be remembered that in order for *T. solium* proglottids and eggs to be shed in a person's faeces, they must first be infected with the adult worm by eating undercooked pork. Therefore, many studies also show consumption of pork to be a risk factor, although this is not a transmission route for cysticercosis.

15.1.4.4 *Ascaris lumbricoides*

15.1.4.4.1 Background

Ascaris lumbricoides is the large intestinal nematode (roundworm) of humans, in which the adult worms occupy the small intestine and the female lays large numbers of eggs (O'Lorcain and Holland, 2000). *Ascaris* eggs are not infectious directly upon excretion, but embryonate in the environment after around 3 weeks (depending on environmental conditions). Following ingestion of embryonated eggs, the infective-larval stages of the parasite are released. These migrate through the body, via the liver and then the lungs. In the lungs, the larvae break through to the air spaces, migrate up the trachea, and are then swallowed and thereby return to the gastrointestinal tract. Here they mature to adult worms in the small intestine. Although *A. lumbricoides* is generally considered as infective only to humans, with no animal reservoir (that is, non-zoonotic), a related species in pigs, *A. suum*, may also infect humans (FAO/WHO, 2014).

The pathology from *Ascaris* infection in humans is mainly in the gut (small intestine and ileum) and the lungs, though larval migration through the liver and peritoneum may also cause damage (O'Lorcain and Holland, 2000). In the intestines, the worms can occur in such large numbers that blockage and rupture can occur in extreme cases. The parasites have also been known to cause death by blocking the pancreatic or bile ducts. The worms in the gut can cause malabsorption and anorexia, thereby contributing to malnutrition (O'Lorcain and Holland, 2000). Migration of larvae through the lungs can cause severe immune hypersensitivity responses (Loeffler's Syndrome) that may be life-threatening.

Ascaris is a very common intestinal parasite with an essentially global distribution, although prevalences tend to be low (but not zero) in countries with well-developed sanitation systems and very high in regions with poor sanitation. The association with tropical and subtropical countries probably reflects poorer overall sanitation systems and parasite control

programmes, and that development and survival of eggs is optimal under warm, moist conditions. Most recent estimates of global prevalence are between 1.2 and 2 billion persons worldwide, making it one of the common parasites globally.

Although effective treatment is available, if the environment is heavily contaminated in highly endemic areas, reinfection may occur very rapidly.

15.1.4.4.2 Information on transmission of *Ascaris lumbricoides* via fresh produce

Ascaris lends itself to transmission by the food-borne route as the eggs are excreted in large numbers (estimated to be in the hundreds of thousands of eggs per day per female) (O'Lorcain and Holland, 2000) and are not immediately infectious, thereby excluding direct hand-to-mouth infection. The eggs can contaminate fresh produce either directly from soil or by irrigation with contaminated water supplies, and probably the hands and clothing of agricultural workers or other people in contact with contaminated soil. The practice of using human faeces as fertilizer in subsistence farming presents a significant risk for contamination of crops.

Furthermore, *Ascaris* eggs are sticky, thick-shelled, and highly persistent in the environment, surviving for several years. They can also survive low temperatures, including freezing to some extent, desiccation, and exposure to some chemicals, but not cooking.

Only one small (12 cases) outbreak of food-borne ascariasis is reported in the literature (Räisänen *et al.*, 1985), but this is probably due to difficulties in associating infection with food-borne transmission due to the long period between infection and diagnosis (the period between infection and adults in the intestine takes around 6 weeks or more), and the lack of possibility of excluding other infection routes, especially in endemic settings. The outbreak reported occurred in Finland (where ascariasis is rare) and was considered to be associated with imported vegetables, possibly leeks (Räisänen

et al., 1985). Despite the lack of outbreaks of food-borne ascariasis, various surveys of fresh produce have identified contamination with *Ascaris* eggs (Eraky *et al.*, 2014; Kłapeć and Borecka, 2012; Uga *et al.*, 2009; Al-Binali *et al.*, 2006), indicating the potential for transmission. Although some studies identify consumption of raw produce as a risk factor for acquiring ascariasis (Knopp *et al.*, 2010), other studies place greater weight on factors such as disposal of excreta and educational attainment. Use of human excreta as a fertilizer for crops has also been identified as an important risk factor for infection, indirectly implicating food-borne infection via produce (Pham-Duc *et al.*, 2013).

15.2 Detection of selected parasites in fresh produce

Although legislated, regular monitoring of fresh produce for parasite contamination seems unlikely to be instigated, a robust, reproducible, efficient, and, preferably, user-friendly and cheap method for detecting parasite contamination in fresh produce would be a useful tool for many reasons.

Not only can microbiological evidence of contamination be used to support epidemiological data during outbreak investigations, but analysis of fresh produce for parasite contamination could provide data on the extent and concentration of contamination. Such data could be used as input for risk assessment, and for investigating how different parameters along the field to fork continuum may impact on the probability and extent of parasite contamination. Furthermore, a good detection method is essential for studies on possible decontamination procedures.

Although methods are available for some parasites, very few of these methods are standardized or validated by interlaboratory testing, and therefore different labs tend to use different methods. This hampers comparison of results obtained in different studies and extrapolation

of reported results to provide information for risk assessment or for input into HACCP schemes is difficult.

Due to the transmission stages of different parasites being dissimilar to each other in many respects, including size, morphology, and structure, there is no single method that can be used for all parasites. Furthermore, of the methods available for different parasites, some have been subjected to greater development and testing than others. However, in general, most methods consist of a series of steps in which the parasite stage itself is eluted from the produce surface, concentrated, and, if possible, separated from other debris, before detection either of the stage itself by visualising it using microscopy or by amplifying its DNA by PCR and detecting that.

15.2.1 Methods used

15.2.1.1 *Cryptosporidium* spp.

As there has been extensive development and testing of methods for detection of *Cryptosporidium* oocysts in water, methods for detection in fresh produce are relatively well developed and available methods are based on those commonly used for water samples (US EPA Method 1623, ISO Method 15553 (Anonymous, 2006) or regional standard methods, e.g., the UK “SCA Blue book method” (Anonymous, 1990)).

For all these methods, methods for concentration of particles from large volumes of water (usually filtration) are followed by a purification step using immunomagnetic separation (IMS) in which oocysts are captured from the concentrate using paramagnetic beads coated with an antibody specific for the oocyst wall. Following oocyst release from the beads, detection is by microscopy using the immunofluorescent antibody test (IFAT), enhanced by dyes (DAPI) for highlighting nuclei.

The use of IMS followed by IFAT in analysis of fresh produce samples for *Cryptosporidium* oocysts was first introduced around 15 years ago (Robertson and Gjerde, 2000) and increased the sensitivity of detection from 1% (as previously reported by Bier, 1991, in which flotation

was used rather than IMS) to over 40%. It was noted that recovery efficiency was not identical from all types of fresh produce, and for mung-bean sprouts, in particular, recovery efficiency was reduced.

In the US, the *Bacteriological Analytical Manual* (BAM) describes a method for detecting and identifying *Cryptosporidium* oocysts from a range of food samples, including fresh produce (Orlandi *et al.*, 2004). In this method, following a gentle wash procedure in distilled water the parasites are separated by IMS before detection using IFAT for approximately 10% of the sample concentrate and separate molecular methods. The washing procedure seems not to have been optimized and no explanation is provided for the absence of a buffer or detergent that may assist in oocyst removal from the matrix. No recovery efficiencies for the method are provided.

Further investigations by Cook *et al.* (2006a) compared various elution methods and resulted in a method that was used in an interlaboratory validation trial (Cook *et al.*, 2006b). Despite some methodological difficulties, and although the results from participating laboratories were around 50% that reported from the developing laboratory (30% compared with 60%), and some labs reported negative samples as positive (Robertson, 2014b), this method has become the basis of an ISO Method (ISO/DIS 18744) and has been used in various investigations of fresh produce for *Cryptosporidium* (e.g., Rzezutka *et al.*, 2010; Amorós *et al.*, 2010). Results demonstrate variable recovery efficiency, but nevertheless widespread, low-level contamination of fresh produce with *Cryptosporidium* oocysts seems to be relatively usual.

The procedures used in this method are summarized in Table 15.2.

Comparisons of molecular methods with IFAT for detection (Ripabelli *et al.*, 2004; Keserue *et al.*, 2012) have generally shown that IFAT seems to be more sensitive, although other studies have provided more positive results from molecular detection (Shields *et al.*, 2013).

Table 15.2 Summary of procedures in analysis of fresh produce for contamination with *Cryptosporidium* oocysts using the ISO method currently under development; recovery efficiencies of around 15–60% were reported (depending on the analytical lab, study, and matrix) and the theoretical limit of detection is 1 oocyst per sample analyzed (25 g or more depending on the product).

Step	Procedure	Details of procedure
1	Elution	A glycine buffer is used as elution solution. For berry fruit, the fruit are immersed in the buffer and shaken; for leafy green vegetables a stomacher is used
2	Concentration	The elution solution is collected and concentrated by centrifugation
3	Isolation or purification	An immunomagnetic separation (IMS) procedure is used to isolate oocysts from other particles in the eluate concentrate
4	Detection	Immunofluorescent antibody testing (IFAT) is used for detection, with morphology enhanced by use of DAPI, and putative oocysts also inspected by light microscopy with Normarski optics. Potential oocysts may be scored as confirmed or putative depending on the characteristics observed
5	Downstream testing	Molecular methods may be used downstream for determining species and genotype as necessary

In an alternative approach, a recent “proof of concept” study investigated the feasibility of using adhesive tape to remove *Cryptosporidium* oocysts from the surfaces of fresh produce for subsequent detection either by IFAT or PCR (Fayer *et al.*, 2013). This was successful down to levels of 10 oocysts applied per area of fresh produce surface. At lower levels of contamination, IFAT was more sensitive than PCR. Recovery was also dependent on the surface of the produce analysed. Peaches were problematic to work with due to their hair-like projections (trichomes) on the skin (exocarp) of peaches providing a physical barrier preventing the adhesive tape from reaching all the oocysts, particularly those located at the base of the trichomes (Fayer *et al.*, 2013).

15.2.1.2 *Cyclospora cayetanensis*

Despite outbreaks of cyclosporiasis associated with fresh produce being relatively frequent and affecting large numbers of people, an effective analytical method is lacking. Of those studies conducted, very few provide recovery efficiency data. Nevertheless, the process can be basically divided into three or four distinct steps (Shields and Ortega, 2012; Table 15.3). As with *Cryptosporidium*, the first step removes the oocysts from the produce surface by a wash

procedure (Shields *et al.*, 2012). As the produce most commonly investigated for contamination is soft fruit, predominantly raspberries, the washing technique is gentle to avoid maceration of the fruit that could result in difficulties in subsequent stages. The second step is concentration of the oocysts from debris. This step can be a simple centrifugation to remove excess water/liquid, while a flotation or magnetic bead separation may be employed if there is excess debris. Due to the lack of appropriate monoclonal antibodies, IMS, as used for *Cryptosporidium*, is not an option for *Cyclospora*. However, magnetic beads coated with a lectin (WGA) that binds to *Cyclospora* oocyst walls can be used to concentrate *Cyclospora* oocysts (Robertson *et al.*, 2000). Although using WGA beads did not increase the recovery rate, the resultant pellets were much cleaner and easier to examine.

The third step is organism identification, typically by microscopy or molecular methods. Due to the lack of appropriate monoclonal antibodies IFAT is not an option. However, the presence of protein–tyrosine cross-links in the oocyst wall (Belli *et al.*, 2006) means that oocysts autofluoresce under ultraviolet (UV) light, appearing blue when viewed at 365 nm (Ortega *et al.*, 1994). Nevertheless, detection is usually

Table 15.3 Summary of procedures in the analysis of fresh produce for contamination with *Cyclospora* (largely adapted from Shields and Ortega, 2012).

Step	Procedure	Details of procedure
1	Elution	Different elution solutions have been tried; inclusion of anionic detergent is reported to improve recovery
2	Concentration	Concentration is largely based on centrifugation, but lectin-coated beads may be used for a very dirty eluate
3	DNA isolation	Harsh methods (use of glass beads or freeze–thawing) is often used to open the oocysts before DNA isolation, usually using a commercial kit
4	Detection	Different PCR protocols (both conventional and real-time) are available and most commonly used, but microscopy is also an option, especially utilizing the autofluorescent properties of the oocysts

conducted by PCR for which several primer sets are published (Shields and Ortega, 2012) and for which detection limits of 40 oocysts per 100 g of raspberries or less have been reported (Steele *et al.*, 2003).

15.2.1.3 *Taenia solium*

Despite cysticercosis having been ranked as being of greatest importance globally among food-borne parasitoses (FAO/WHO, 2014), investigations on methods for detection of *T. solium* eggs as contaminants on fresh produce are lacking.

Although the eggs are small by helminth standards (30–40 μm in diameter), they are large compared with cysts and oocysts of most protozoa (around 10 times the size of *Cryptosporidium* oocysts), and therefore detection by microscopy is relatively easy. However, as most taeniid eggs are morphologically indistinguishable from each other, detection of these eggs only indicates contamination with transmission stages from a taeniid worm – not necessarily *T. solium* and not necessarily of public health significance. Thus, in the absence of any other criteria, molecular methods are necessary to determine the species of egg. Molecular methods for identification of *T. solium* eggs have been published, including PCR and LAMP (e.g., Nkouawa *et al.*, 2009; Yamasaki *et al.*, 2004). However, exploration of methods for eluting and concentrating *T. solium* eggs from fresh produce are lacking, and whether a molecular test is more

appropriate for initial detection or should be used downstream of a microscopy-based test has not been explored. In the absence of an assay method, surveillance studies of fresh produce for contamination with *T. solium* eggs are also lacking.

15.2.1.4 *Ascaris lumbricoides*

Ascaris eggs as contaminants of fresh produce may be fertilized or non-fertilized, and these differ morphologically, but both are relatively large in size (around 50–75 μm in length) and can be readily detected by microscopy. Thus, surveys of fresh produce for *Ascaris* eggs have tended to rely upon a basic technique, with microscopy of concentrated eluate sediment, sometimes with flotation or filtration included (e.g., Eraky *et al.*, 2014; Kłapeć and Borecka, 2012; Ogbolu *et al.*, 2009; Uga *et al.*, 2009; Vuong *et al.*, 2007; Al-Binali *et al.*, 2006; Robertson and Gjerde, 2001).

Nevertheless, some formal method of development has been published; an early interlaboratory study investigated the use of the Nacconol–ether centrifugation method for detecting *Ascaris* eggs on cabbage, but the efficiency was considered poor (around 20%). Poor reproducibility by individual laboratories resulted in this method not being recommended for quantitative estimates of nematode egg contamination of foods (Rude *et al.*, 1982). Further work using sonication and detergent solution for elution, followed by centrifugation, flotation for

dirty samples, and detection by microscopy reported a recovery efficiency from cabbage of around 10% (Bier *et al.*, 1991). This method continues to be used in surveys, demonstrating the lack of development in this technique (Shahnazi and Jafari-Sabe, 2010). A similar method, with elution sonication, concentration by centrifugation, and identification from a variety of fresh produce, resulted in a recovery efficiency of over 70% (Robertson and Gjerde, 2000). This higher recovery efficiency may be due to the more vigorous elution procedure, including two rounds in a rotating drum and sonication.

15.3 Effects of fresh produce treatments on selected parasites

The most effective control of parasites on fresh produce is by *prevention* of contamination at all points along the farm-to-fork continuum. This could probably be best achieved by all actors within the chain conducting an HACCP-based risk assessment, in which the most likely sources of contamination are identified, their severity, and the risk that they pose for consumer safety, and prevention or mitigation procedures (corrective actions) implemented (McClure *et al.*, 2012).

Although the most likely sources of contamination depend upon specific situations and conditions, obvious considerations are water quality, contact with animals, and hygiene in connection with human handlers.

While attempting to eliminate or minimize contamination is important, other barriers can assist in reducing the possibility of ingestion of infectious parasitic transmission stages with fresh produce. Two additional barriers are *removal* (implementation of procedures by which contaminating parasite transmission stages are removed) and *inactivation* (implementation of procedures by which parasite transmission stages contaminating of fresh produce are no longer capable of causing infection in a susceptible host). Some procedures (such as

washing in a disinfectant solution) attempt to combine both barriers.

It should be noted that a washing procedure might actually introduce, rather than remove, contamination (if the washwater is contaminated). Also, if a washing procedure is to be implemented in a food unit operation, the operator should consider how the used wash solution is going to be disposed of or treated and recirculated.

15.3.1 Methods for determining control (particularly removal or inactivation)

In order to determine whether control procedures are effective, some method of evaluating their efficacy must first be secured. For example, for evaluation of whether a particular washing procedure provides satisfactory removal of a parasite transmission stage, an experiment may be designed in which the pathogen is added to the specific produce at a known concentration, the procedure followed, and then the product investigated to determine the extent of removal of the contaminant. In designing such an experiment, various questions should be addressed, including:

- What is the uncertainty in the amount (concentration) of parasite added?
- Is it best to assess removal by looking for the pathogen in the wash solution (amount removed) or remaining on the product (amount that is not removed) – or both?
- How effective and efficient are the methods for determining the amount of pathogen in the wash solution or on the product? What is the uncertainty and how can this be incorporated into the results?
- Given a known uncertainty, how many replicate experiments need to be conducted in order to provide meaningful results?
- How long should the contact time be between the parasite and the fresh produce before the removal procedure is started? Will the contact time impact on the results obtained?
- Should the actual organism in question be used for the experiments or is a surrogate sufficient?

- Are there any other factors that should be considered: e.g. temperature of operation, size of product portion, intactness of product portion, effects of other contaminants or particles such as soil, sand, biofilms, etc.

Similarly, investigations on whether a particular procedure can be used to inactivate parasite transmission stages on fresh produce raise several questions. Some of these questions are similar to those raised for investigating removal procedures. However, in addition, it is important to have a method for determining whether a specific parasite transmission stage is viable and potentially infective or whether it has been inactivated and is incapable of causing infection.

Approaches to assessing parasite transmission stage viability have been a topic of investigation and debate for many years. Methods investigated (varying for different parasites) include: assessment of morphology, assessment of progress in embryonation, inclusion or exclusion of specific “vital” dyes, fluorescent *in situ* hybridization (FISH), infection of cell cultures, use of genes as viability markers, sometimes in combination with cell culture (CC-PCR), and infection of animal models (bioassay).

For some parasites there has been a considerable number of studies directed towards developing viability assays for the transmission stages (e.g., *Cryptosporidium*), whereas for others (e.g., *Taenia* eggs and *Cyclospora* oocysts) studies are relatively scarce. For *Ascaris*, studies have been mostly directed towards methods of ascertaining die-off of *Ascaris* eggs in slurry or faeces, rather than methods at inactivating *Ascaris* eggs post-embryonation on contaminated fresh produce.

15.3.2 Effects of different processes

Provided that there are satisfactory methods for quantifying parasite transmission stages and determining their viability (or infectivity), then investigations can be conducted to determine the effects of different post-harvest procedures on their removal or inactivation on fresh produce. Removal procedures are basically washing,

whilst inactivation procedures include both physical approaches (temperature, pressure, irradiation, etc.) and chemical approaches, including various liquid and gaseous sanitization regimes.

It is important to bear in mind that the methods employed should not only be effective in removal and/or inactivation, but also not be deleterious to the produce. While some fresh produce is relatively hardy and can withstand quite vigorous treatment without any significant loss in quality, taste, or appearance, other fresh produce, such as berries and other soft fruit, will be spoiled if subjected to prolonged or harsh treatments.

15.3.2.1 Wash processes

Washing procedures in the fresh produce industry are designed to remove dirt, pesticide residues, and microorganisms responsible for quality loss, as well as to pre-cool cut produce and remove cell exudates that may support microbial growth (Gil *et al.*, 2009). Washing usually consists of spraying or deluging fresh produce with water, possible mechanical treatment of the surface by brushes or spray washers, followed by rinsing with potable water, which may include a sanitizer treatment. The water that is used for washing and sanitizing purposes must also be clean, so that it does not become a vehicle for contamination. The quality of washwater, especially if the water is recycled and not treated prior to reuse, is a concern of the fresh produce industry regarding washing system efficiency (Parish *et al.*, 2003).

Although hardy items of fresh produce can be brush-washed, with oscillating brushes to scrub surfaces for the physical removal of soil and microorganisms (Parish *et al.*, 2003), this is obviously not appropriate for fresh produce such as lettuce and other leafy vegetables, and soft fruit such as raspberries or strawberries. The addition of particles to the water to increase abrasion is also usually not practical, due to the need for additional wastewater treatment and particle recovery. However, introducing air into the liquid provides additional cleaning forces

and modern aeration “jacuzzi” washers are considered to reduce bacterial loads on vegetables by between 1 and 2 log units (Gil *et al.*, 2009). Although the larger size of parasites compared with bacteria may increase the shear forces, and thus the ease of removal of adherent parasites during washing, the transmission stages of many parasites are “sticky”, and thus they may actually be more difficult to remove than bacteria. In addition, characteristics of the surfaces of the fresh produce (cracks, crevices, hydrophobic tendency, texture, etc.) may affect the ability of the washing procedure to remove adherent parasites.

Considerable attention has been directed towards efficient removal of bacteria from fresh produce by washing procedures, including process engineering variables such as spray qualities including optimal droplet spectrum, droplet velocity, angle of droplet impingement, number and orientation of nozzles, spray rate, resident time of produce in the sprayer, etc. The comparative research conducted on removal of different parasite transmission stages (including the four parasites in focus in this chapter) by washing procedures is minimal.

15.3.2.1.1 *Cryptosporidium* spp.

A study on removal of *Cryptosporidium parvum* oocysts from the surface of apples (a fruit of particular relevance due to several documented food-borne outbreaks of cryptosporidiosis associated with apple cider) found that none of the washing methods tested completely succeeded in removing oocysts from apple peel (Macarisin *et al.*, 2010b). The most efficient removal (37.5%) was achieved by rigorous manual washing in water with a detergent and agitation in an orbital shaker with tris-sodium dodecyl sulphate buffer (Macarisin *et al.*, 2010b). Furthermore, scanning electron microscopy revealed that some oocysts were attached in deep natural crevices in the apple exocarp, and some oocysts were closely associated with an amorphous substance with which they might have been attached to the apple surface (Macarisin *et al.*, 2010b). Another

study demonstrated strong adherence of *Cryptosporidium* oocysts to spinach leaves, and also internalization of oocysts through the leaf stomata, such that removal by washing was totally ineffective (Macarisin *et al.*, 2010a). In addition, Armon *et al.* (2002) reports that removal of *Cryptosporidium* oocysts from courgette (zucchini) surfaces is difficult, possibly due to the hairs (trichomes) on the vegetable surface.

15.3.2.1.2 *Cyclospora cayetanensis*

Cyclospora oocysts are also notoriously sticky and difficult to remove from surfaces of fresh produce (Shields and Ortega, 2012) and have been visualized attached to the surface of vegetables after washing (Ortega *et al.*, 1997). It has been reported that the “stickiness” of *Cyclospora* oocysts is stronger than that of *Cryptosporidium* oocysts, but the adhesins responsible for this capability for strong attachment to produce have not been identified (Ortega and Sanchez, 2010).

An experiment in which *Eimeria acervulina* (a coccidian parasite of chickens) was used as a model for *Cyclospora* on raspberries demonstrated that washing was generally inadequate to remove them (Lee and Lee, 2001). It should be noted that the hairs on raspberries add another difficulty for adequate washing of this particular fruit.

15.3.2.1.3 *Taenia solium*

No studies have been published on adherence of *Taenia solium* eggs to fresh produce or their removal by washing procedures. However, it should be noted that the eggs of *T. solium* are also considered sticky (OIE, 2014).

15.3.2.1.4 *Ascaris lumbricoides*

Ascaris eggs have long been recognized to adhere to surfaces of, for example, vegetables (Rudolfs *et al.*, 1951; Gaspard *et al.*, 1994; Jimenez, 2007) due to the presence of a sticky external layer (Quilès *et al.*, 2006) with some hydrophobic properties (Capizzi and Schwartzbrod, 2001). Nevertheless, experiments on the removal of *Ascaris* eggs from

fresh produce by different washing procedures appear to be lacking.

15.3.2.2 Physical treatments

A number of physical treatments can be used for inactivating pathogens on fresh produce. Not all physical treatments are equally suitable for all types of fresh produce and, depending on the type of produce, may alter or deteriorate the fundamental qualities of the produce. Some of the different physical processes that may be used in the fresh produce industry are briefly described below, along with information on how this treatment may affect the survival of selected parasite transmission stages.

15.3.2.2.1 Chilling and freezing

Chilling and freezing are commonly used for preservation in the fresh produce industry. Although these procedures may inactivate contaminant pathogens, the primary aim of chilling and freezing is to extend the shelf life by keeping the produce fresh and edible for a prolonged period. Freezing is considered to be the most satisfactory method for preserving quality during long storage periods; extreme cold retards microorganism growth and delays chemical changes that affect produce quality or cause food to spoil.

Chilling or cooling does not enable very prolonged storage, but it does extend the shelf life of fresh produce by reducing the rate of physiological change (respiration, ethylene production, enzymatic processes and water loss) and by retarding microorganism growth.

There are various approaches in the fresh produce industry to chilling (room cooling, forced air cooling, hydrocooling, ice cooling, top icing, vacuum cooling), and for freezing, which is usually down to -18°C or lower, approaches include air-blast freezers, tunnel freezers, belt freezers, fluid-bed freezers, contact freezers, immersion freezers, cryogenic freezers (produce exposed to an atmosphere below -60°C), liquid nitrogen freezers (produce exposed to liquid nitrogen spray, with a boiling temperature of -196°C) and liquid carbon

dioxide freezers (produce exposed to liquid carbon dioxide at a temperature of -70°C).

As parasite transmission stages do not proliferate on fresh produce, the question is not whether these lower temperatures affect proliferation but whether they have an impact on survival. For refrigerated fresh produce, the temperature for consideration is usually close to 0°C for temperate and subtropical produce, whereas tropical produce is often stored at temperatures above 10°C . For frozen produce, the temperatures to which the parasite transmission stages are exposed are likely to be around -18°C and lower.

In general, all parasite transmission stages survive refrigeration. However, for those that require a sporulation or embryonation period before they become infective (e.g., *Cyclospora* oocysts or *Ascaris* eggs), this development may not occur or may be delayed at refrigeration temperatures. The viability of transmission stages of most parasites is reduced by freezing, but some may be able to survive. The effects of refrigeration and freezing on parasite transmission stage survival for the four selected parasites of this chapter are summarized in Tables 15.4 and 15.5.

15.3.2.2.2 Heat treatment, including blanching and pasteurization

Although low temperatures, rather than high, are usually considered regarding preservation of fresh produce, some heat treatments are relevant. Heat shock treatment (45°C for 105 min) or low-temperature blanching (95°C for 3 min) may be used to prevent browning by inactivating polyphenol oxidases. Blanching may also be used prior to freezing or drying, especially of vegetables, while pasteurization may be used in preparing fruit juices. Heating is also used in canning and bottling of fresh produce.

In general, heating of any parasite transmission stage to 100°C kills them within seconds, while 75°C or more for less than a minute or 55°C for around 5 min also results in inactivation. For some parasites the cut-off temperature is lower; almost all parasite transmission stages

Table 15.4 Effect of refrigeration on the survival of transmission stages of selected parasites.

Parasite	Refrigeration	
	Effect	References
<i>Cryptosporidium</i> spp.	<i>Cryptosporidium</i> oocysts survive best in cool, damp conditions	Robertson, 2014b
<i>Cyclospora cayetanensis</i>	Although sporulation may be retarded at refrigeration temperatures, around 50% survive refrigeration and are able to sporulate subsequently	Sathyanarayanan and Ortega, 2006; Ortega and Sanchez, 2010; Shields and Ortega, 2012.
<i>Taenia solium</i>	Formal experiments on refrigeration of <i>T. solium</i> eggs are lacking, but prolonged survival of taeniid eggs at low temperatures is well recognized; <i>Taenia pisiformis</i> eggs showed survival at 4 °C for 300 days	Coman, 1975
<i>Ascaris lumbricoides</i>	Although embryonation may be delayed or abrogated at refrigeration temperatures, most unembryonated eggs that are refrigerated (5 °C) for 60 days are able to embryonate subsequently	Kim <i>et al.</i> , 2012

Table 15.5 Effect of freezing on the survival of transmission stages of selected parasites.

Parasite	Freezing	
	Effect	References
<i>Cryptosporidium</i> spp.	Freeze–thaw cycles decrease oocyst survival, but some studies indicate that a few oocysts survive freezing, even to around –20 °C. Blast freezing of green peppers inoculated with oocysts resulted in a reduction in viability by about 20%	Robertson <i>et al.</i> , 1992; Fayer and Nerad, 1996; Robertson and Gjerde, 2006; Duhain <i>et al.</i> , 2012
<i>Cyclospora cayetanensis</i>	Unlikely to sporulate, or sporulate extremely slowly, when frozen. A small proportion (<1%) can sporulate after exposure to –20 °C, but not –70 °C. An outbreak of food-borne cyclosporiasis was associated with the raspberry filling of a wedding cake in which the food had been frozen prior to consumption, indicating that sporulated oocysts survive freezing	Ho <i>et al.</i> , 2002; Sathyanarayanan and Ortega, 2006; Ortega and Sanchez, 2010; Shields and Ortega, 2012
<i>Taenia solium</i>	Experiments on survival of <i>T. solium</i> eggs following freezing are lacking. A conservative approach is to consider that <i>T. solium</i> eggs have similar survival properties to other taeniid eggs, such as those of <i>Echinococcus</i> , which can survive freezing to below –30 °C	
<i>Ascaris lumbricoides</i>	<i>Ascaris</i> eggs are considered very resistant to low temperatures. Early studies investigated survival of <i>A. lumbricoides</i> eggs for up to 40 days at a minimum of approximately –25 °C, and viability (embryonation) and infectivity in a guinea pig model were demonstrated. However, a study that exposed <i>Ascaris</i> eggs to –80 °C reported a 3 log inactivation after 3 hours	Cram, 1924; Bowman <i>et al.</i> , 2009

are adversely affected by temperatures above 45 °C. Pasteurization (high-temperature, short-time, or flash pasteurization; 72 °C for 15 seconds) should inactivate all parasite transmission stages. In line with these generalities, flash pasteurization is effective against *Cryptosporidium* oocysts in apple cider (Deng and Cliver, 2001).

Although heating is a good method for inactivating parasite transmission stages, it should be noted that microwave ovens may produce inconsistent results, perhaps due to hot and cold spots within them. In studies on *Cyclospora* and *Cryptosporidium* (Ortega and Liao, 2006), differences were found between microwaves and the temperatures reached. *Cryptosporidium* oocysts could be inactivated in microwave ovens following cooking for 20 seconds at 80 °C, while 45 seconds was needed to prevent unsporulated *Cyclospora* oocysts being capable of subsequent sporulation. In another study, both microwave heating and blanching inactivated most (>93%) *Cryptosporidium* oocysts on green peppers (Duhain *et al.*, 2012). It should be noted that microwave irradiation, as well as heat, could play a role here.

15.3.2.2.3 Drying

Fresh produce contains around 95% water, which is sufficient to support enzyme activity and microorganism growth, both of which result in a short shelf life. Both enzymatic activity and microorganism growth are hampered by reducing water content to about 10–15%. Either solar heat or artificial heat can be used to dry fresh produce; solar drying can be cheaper and may be the obvious approach in tropical countries, but is difficult to control. Unless a purpose-built cabinet is used such drying may expose the produce to contamination from the environment, particularly dust and insects. Although dried fruit production used to be almost entirely solar, a large proportion now use dehydration tunnels, where time and temperature can be accurately regulated and the resultant product has a pre-defined water activity (a_w). Fruit may also be freeze-dried, in which the fresh fruit is frozen and then

dried under vacuum, such that the water evaporates from the fruit while they are frozen. Desiccation and drying tends to have a deleterious effect on most parasite transmission stages, despite many having a trehalose synthetic pathway (Yu *et al.*, 2010; Elbein *et al.*, 2003; Behm, 1997); trehalose is known to function as a stress protectant against desiccation (and freezing). Desiccation has been shown to be lethal to *Cryptosporidium* oocysts (Robertson *et al.*, 1992; Deng and Cliver, 1999; Dawson *et al.*, 2004), taeniid eggs generally (Laws, 1968), and *Ascaris* eggs (Magri *et al.*, 2013; Wharton, 1979). However, most of these investigations have not been conducted with respect to survival of the parasites on fresh produce being subjected to drying procedures, and for some parasites (*Cyclospora* oocysts, *Taenia solium* eggs) data on the effects of desiccation on survival are entirely lacking. It should be noted that although desiccation may ultimately inactivate parasite transmission stages, this may not happen immediately and survival of transmission stages may be prolonged in crevices of fresh produce that are not completely dried out.

15.3.2.2.4 Irradiation

Various irradiation technologies are used in the fresh produce industry. These include ionizing radiation (e.g., gamma irradiation using cobalt-60 or caesium-137 as a radioactive source, X-ray, and electron beam processing) and non-ionizing radiation (e.g., UV irradiation at 254 nm). The former, particularly gamma irradiation, have been more traditionally used in the fresh produce industry, particularly with respect to insect control and inhibition of ripening/sprouting, but also have potential in controlling microbial pathogens. In general, only low doses (<1 to 3 joules per gram (kGy)) of gamma irradiation are useful for fresh produce, otherwise sensory qualities (flavour, texture, etc.) are adversely affected and/or accelerated senescence occurs due to irreparable damage to the product. Regulations for using ionizing radiation for fresh produce varies between countries; some countries

require that produce treated with ionized radiation is labeled as such.

With respect to non-ionizing irradiation (UV at 254 nm), this only inactivates those pathogens that come into contact with the UV beam. This might be satisfactory for inactivation in water or for smooth surfaces, but for products with uneven surfaces, crevices, etc., the inactivation curve will have a “tail” due to microorganisms that are shielded from the UV (Gardner and Shama, 2000; Turtoi, 2013). Thus, although UV treatment of produce may reduce the microbial load, it may be difficult to guarantee complete inactivation of all contaminating pathogens.

Various studies have investigated the effects of different types of irradiation (ionizing and

non-ionizing) for inactivating different parasite transmission stages. The results for the selected parasites of this chapter are outlined below and summarized in Tables 15.6 and 15.7.

A study on *Cryptosporidium parvum* oocysts demonstrated that in order to eliminate infectivity in mice completely, a dose of 50 kGy was necessary (Yu and Park, 2003). Although there is currently no internationally recommended upper limit regarding radiation of food (WHO, 1999), most food irradiation that intends to inactivate microbial pathogens does not exceed 10 kGy. It is suggested that *Cryptosporidium* may have unusual free radical scavenging or repair systems, and experiments should be conducted using irradiation in combination with other methods, such as radiosensitizers,

Table 15.6 Summary of effects of ionizing irradiation on the survival of transmission stages of selected parasites.

Parasite	Ionizing irradiation (e.g., gamma irradiation)	
	Effect	References
<i>Cryptosporidium</i> spp.	50 kGy necessary to eliminate infectivity in mice	Yu and Park, 2003
<i>Cyclospora cayetanensis</i>	Studies lacking for <i>Cyclospora</i> , but doses of 0.4 to 0.8 kGy effective at inactivating <i>Toxoplasma</i> oocysts	Dubey <i>et al.</i> , 1998
Taeniid species (<i>Taenia solium</i>)	Studies lacking for <i>Taenia solium</i> eggs, but exposure of <i>Echinococcus</i> eggs to doses of 0.4 kGy prevented development	Veit <i>et al.</i> , 1995;
<i>Ascaris lumbricoides</i>	10 kGy affects development within the eggs and of around 2 kGy abrogates infectivity	Chyu <i>et al.</i> , 1970; Lee <i>et al.</i> , 1970

Table 15.7 Summary of the effects of non-ionizing irradiation on survival of transmission stages of selected parasites.

Parasite	Non-ionizing irradiation (germicidal ultraviolet radiation)	
	Effect	References
<i>Cryptosporidium</i> spp.	Susceptible at fluences of 25 or 15 mJ/cm ² of pulsed UV	Craik <i>et al.</i> , 2001; Lee <i>et al.</i> , 2008; Le Goff <i>et al.</i> , 2014
<i>Cyclospora cayetanensis</i>	<i>Eimeria acervulina</i> susceptible at 261 mW exposure/cm ²	Kniel <i>et al.</i> , 2007
Taeniid species (<i>Taenia solium</i>)	Susceptible at 2430–2880 mJ/cm ²	Konno <i>et al.</i> , 1997; Lagapa <i>et al.</i> , 2001
<i>Ascaris lumbricoides</i>	Susceptible at 400 mJ/cm ² (>2 log inactivation)	Brownell and Nelson, 2006

in order to reduce the lethal radiation dose (Yu and Park, 2003).

A poster presentation of work on use of pulsed UV light to inactivate *Cryptosporidium* oocysts on raspberries (Le Goff *et al.*, 2014) noted a reduction in infectious load, but infection in mice was not entirely abrogated. A minimum dose of 15 mJ/cm² of pulsed UV light has previously been shown to inactivate *C. parvum* oocysts (Lee *et al.*, 2008), and 3 log inactivation at 25 mJ/cm² for UV light in general (Craik *et al.*, 2001).

Experiments on UV irradiation and the coccidian parasite of chickens, *Eimeria acervulina* (a model for *Cyclospora*), found that this treatment was more effective at inactivating parasites contaminating basil leaves than those on raspberries (Kniel *et al.*, 2007), presumably due to the flatter surface of basil that would reduce the shading effect. Although infectivity was reduced on the raspberries, it was not completely abrogated. There is currently a lack of experiments on gamma irradiation or other ionizing radiation on infectivity of *Cyclospora* oocysts, although the use of ionizing radiation to treat foods, particularly with respect to *Cyclospora* on raspberries, has been argued for by Osterholm (1999), who also advocated that urgent additional research be conducted and petitioned FDA for approval for higher levels of irradiation to be used on produce (Osterholm, 1999). The effects of caesium-137 irradiation on both unsporulated and sporulated *Toxoplasma gondii* oocysts have been investigated (Dubey *et al.*, 1998), and the results were considered to be of relevance for other protozoan parasites also, including *Cyclospora* and *Cryptosporidium*. Unsporulated oocysts irradiated at between 0.4 and 0.8 kGy sporulated, but were not infective to mice, whilst sporulated oocysts irradiated at 0.4 kGy and above were able to excyst, and although the sporozoites were infective they could not establish a viable infection in mice (Dubey *et al.*, 1998). Furthermore, raspberries inoculated with sporulated *T. gondii* oocysts and irradiated at 0.4 kGy did not cause

infection in mice. The authors suggest that irradiation at 0.5 kGy is effective at inactivating coccidian oocysts on fruits and vegetables, including those of *Toxoplasma*, *Cyclospora*, and *Cryptosporidium*.

The effects of irradiation on helminths have also been tested. UV at 254 nm inactivates taeniid eggs (Konno *et al.*, 1997; Lagapa *et al.*, 2001), but, as with the protozoan parasites, use of this technology on fresh produce may be ineffective if the eggs are protected from the UV dose due to the topography of the produce.

Irradiation with a cobalt-60 source on *Ascaris* eggs demonstrated inactivation at around 10 kGy in terms of development proceeding within the eggs (Chyu *et al.*, 1970) and around 2 kGy in terms of infectivity to mice (Lee *et al.*, 1970). However, *Ascaris* eggs seem particularly resistant to UV radiation (Brownell and Nelson, 2006), with typical fluences (less than 100 mJ/cm²) resulting in inactivation of less than 1 log for intact eggs. Decortication of eggs prior to UV exposure increased susceptibility considerably (with a 1.8 log reduction achieved at a fluence of 50 mJ/cm²). The authors suggest that the outer layers of the egg must protect against UV irradiation, perhaps due to absorption by the proteins (Brownell and Nelson, 2006). Again, for use on fresh produce, surface topography might shelter the eggs from the full UV dose administered.

15.3.2.2.5 Ultrasound

Ultrasound is a relatively new technology in the food industry. Sound waves at frequencies too high to be detected by the human ear (>20 kHz) are used for removing dirt from surfaces. It has been proposed that it may also be useful for inactivation (by cavitation) and removal of contaminant microorganisms on fresh produce (De São José *et al.*, 2014). Experiments on application of ultrasound have generally been conducted in combination with the use of a chemical sanitizer; this is of relevance as ultrasound technologies are generally applied to objects in suspension, and ultrasound may make bacterial pathogens more susceptible to chemical sanitizers (Seymour *et al.*, 2002).

Combining sonication with heat and/or pressure has also been suggested as potentially promising (Rastogi, 2011). Although various studies have investigated the use of ultrasound for inactivating bacteria, including on fresh produce (e.g. Birmpa *et al.*, 2013), inactivating parasite transmission stages using ultrasound has been the subject of few studies. Some investigations have demonstrated ultrasound for inactivating *Cryptosporidium* oocysts in water (Ashokkumar *et al.*, 2003; Oyane *et al.*, 2005; Olvera *et al.*, 2008). The effects of ultrasound on survival of the transmission stages of *Cyclospora*, *Taenia*, and *Ascaris* have not been investigated.

15.3.2.2.6 High pressure

In high-pressure processing (HPP), also known as high hydrostatic pressure (HHP) or ultra-high pressure (UHP) processing, fresh produce is subject to pressures between 100 MPa and above with the purpose of inactivating some enzymes, spoilage organisms, and microbial pathogens, while the nutritional and sensory characteristics of the fresh product are retained (Oey *et al.*, 2008). This procedure is particularly applicable to fruit juices and fruit and vegetable purees, but can also be used for fresh produce such as strawberries. The equipment and setup for HPP is extremely capital-intensive, but it is gaining interest as a technology within the food industry (Rastogi *et al.*, 2007).

The effectiveness of HPP treatment at inactivating microorganisms depends primarily on the pressure applied and the holding time. The resistance of microorganisms is highly variable, depending both on the organism and the food matrix (Rendules *et al.*, 2011). As HPP usually has a higher destructive effect on organisms with a greater degree of organization and structural complexity, eukaryotes (including parasites) are generally considered less resistant to HPP than bacteria (Rendules *et al.*, 2011). Although research has concentrated mainly on the effect of HPP on the spores and vegetative cells of different pathogenic bacteria, there is some research on the effects of high-pressure

processing on parasite transmission stages. Studies generally demonstrate a reduction in viability at pressures between 300 and 600 MPa for protozoan oocysts and helminth eggs, while lower doses decreased viability or delayed development (see Table 15.8).

15.3.2.2.7 Other physical treatments

Other physical treatments that have been explored for inactivation of pathogens on fresh produce include high-intensity electric field pulses and radio frequency (Gil *et al.*, 2009). Studies on the effects of these technologies on parasite transmission stages on fresh produce are currently lacking.

15.3.2.3 Chemical treatments and sanitizers

Chemical treatments, either in combination with washing or alone, are widely used in the fresh produce industry to reduce the load of pathogenic organisms; washing with disinfectants is the main method of reducing microbial load on fresh produce. Various compounds, known as sanitizers, are available. Chlorine-based compounds are the most widely applied, in particular as sodium hypochlorite solution or hypochlorous acid (HOCl), with solution pH maintained between 6.5 and 7.5 to optimize effectiveness. Free chlorine (commonly used at concentrations of 50–200 ppm for food, and usually 100 ppm for fresh produce, with a contact time of 1–2 minutes) is more effective as a disinfectant than chlorine dioxide under controlled conditions. However, chlorine dioxide is more effective when the washwater has a high organic load or there is biofilm growth, can work over a broad pH range, and its main by-product, chlorite, is not considered carcinogenic.

Although the fresh produce industry has a long history of using chlorination, and it is generally effective against bacterial pathogens and relatively cheap (about 5 to 10 times cheaper than chlorine dioxide), there is, nevertheless, a trend towards eliminating chlorine from the fresh produce disinfection process.

Table 15.8 Summary of effects of high-pressure processing (HPP) on survival of transmission stages of selected parasites.

Parasite	Experiments on high-pressure processing		
	Experimental details	Effect	References
<i>Cryptosporidium</i> spp.	<i>C. parvum</i> oocysts suspended in orange juice and apple juice; one pressure (550 MPa) was used for different time periods (30–120 seconds). Survival was assessed by <i>in vitro</i> excystation and cell culture	>3 log reduction in viability after 30 s; infectivity completely abrogated after 60 s; 550 MPa concluded to be effective	Slifko <i>et al.</i> , 2000
<i>Cyclospora cayetanensis</i>	<i>Eimeria acervulina</i> used as a surrogate for <i>Cyclospora</i> oocysts; excystation and chicken infectivity were used for survival assessment after treatment on raspberries (one treatment level)	<i>E. acervulina</i> oocyst infectivity abrogated (exposed birds were asymptomatic and did not shed oocysts) on raspberries/basil after HPP at 550 MPa	Kniel <i>et al.</i> , 2007
Taeniid species (<i>Taenia solium</i>)	<i>Hymenolepis diminuta</i> eggs used as surrogates for tapeworms, including <i>Taenia</i> spp.; exposure to 100–600 MPa HPP for 1 min. Survival assessed by <i>in vitro</i> hatching and vital dye exclusion	Egg hatching abrogated by exposure to 300–600 MPa for 1 min. Treatment at 400 MPa and above caused oncosphere rupture	Merwad <i>et al.</i> , 2011
<i>Ascaris lumbricoides</i>	Unembryonated <i>A. suum</i> eggs subjected to 138–552 MPa for 10–60 s and survival assessed by embryonation	No embryonation in eggs exposed to 241 MPa or more for 60 s or to 276 MPa for 10–30 s; delayed embryonation after lower exposures	Rosypal <i>et al.</i> , 2007; Rosypal <i>et al.</i> , 2011

This is because of concerns about the risks associated with formation of potentially carcinogenic halogenated disinfection by-products, such as trihalomethanes and haloacetic acids (Gil *et al.*, 2009). Furthermore, the wastewater generated has a high level of biological oxygen demand. These negative aspects have resulted in some countries banning the use of chlorine for washing organic produce and a drive towards finding alternative sanitizers. There is also concern that chlorination is ineffective for some pathogens, including parasite transmission stages.

Other sanitizers proposed for use in the fresh produce industry include compounds with known disinfectant properties, such as alternative halogens (bromine, iodine), chlorine dioxide, trisodium phosphate, quaternary ammonium compounds (QACs) (not permitted for organic produce), hydrogen peroxide,

organic acids, and ozone. More novel methods are also coming into focus, such as electrolyzed oxidizing water (EOW) (also known as electrolyzed water, electro-activated water, or electrochemically activated water solution, produced by electrolysis of tap water containing dissolved salt) and atmospheric pressure cold plasma (APCP) (also known as non-thermal plasma). Knowledge on these newer technologies is relatively limited.

Extensive overviews of the properties and modes of action of various of these sanitizers can be found in a range of documents (e.g., WHO, 1998; Arbor, 2009; Gil *et al.*, 2009; Ölmez and Kretzschmar, 2009; Wang *et al.*, 2012; Misra *et al.*, 2014; Mahajan *et al.*, 2014). The effects of the various sanitizer options on pathogenic bacteria on fresh produce have been explored in depth; however, it has been commented that most experiments have been carried out in

unrealistic conditions and the results cannot be compared because of differing experimental conditions and that some studies are useless for practical application because of extreme doses and excessive washing times (Gil *et al.*, 2009).

Fewer investigations have been conducted on elimination of parasite transmission stages by different sanitizers, but these studies also suffer from the same problems to those mentioned for bacteria. For example, contact times of around an hour may be used in experiments, whilst in the fresh produce industry contact time is a matter of minutes for most produce, while for more delicate produce such as soft fruit, contact times may be a quick spray or a brief (10 second) immersion. Nevertheless, the results reported can provide useful clues regarding the efficacy of different sanitizers at eliminating or reducing the populations of potentially infective parasites from the surfaces of fresh produce. It should be noted that the use of sequential inactivation treatments might optimize existing treatments through synergistic effects (Erickson and Ortega, 2006).

15.3.2.3.1 *Cryptosporidium* spp.

Many experiments on the effects of disinfectants on survival of *Cryptosporidium* oocysts are based on the potential for water-borne transmission and approaches to treatment of drinking water. This is not the same as washwater where organic load is frequently very high and contact times may also differ. For example, experiments on survival of *C. parvum* oocysts following exposure to chlorine dioxide, monochloramine, and chlorine (Korich *et al.*, 1990) demonstrated that 1.3 ppm of chlorine dioxide yielded 90% inactivation after 1 h, while 80 ppm of chlorine and 80 ppm of monochloramine required approximately 90 min. Electroporation during exposure to chlorine or chloramine did not affect oocyst viability, as assessed by *in vitro* excystation (Haas and Aturaliye, 1999). Another study demonstrated that 0.4 mg of chlorine dioxide per litre significantly reduced *C. parvum* infectivity within 15 min of contact, although some oocysts remained viable (Peeters

et al., 1989). Thus, with contact times used in the fresh product industry, these chemicals are unlikely to be effective at inactivating contaminant *Cryptosporidium* oocysts (Robertson, 2014b). This is supported by the results of some challenge experiments in which strips of green pepper inoculated with *C. parvum* oocysts were exposed to chlorination (100 and 200 ppm) for 40 s (Duhain *et al.*, 2012). Vital dye inclusion demonstrated that these regimes had a negligible effect on oocyst viability. Experiments on *Cryptosporidium* oocysts on basil leaves and lettuce have demonstrated that exposure to gaseous chlorine dioxide (for 20 min at concentrations of 4.1 mg/l) reduced cell culture infectivity by over 2 log (Ortega *et al.*, 2008). However, the dose (*Ct* value) used is high due to the prolonged contact time.

Sodium dichloroisocyanurate is a cleansing agent and disinfectant, acting as a source of slow release of chlorine in low concentrations at a relatively constant rate, and has been suggested for use in emergency water treatment and also for decontaminating fresh produce. Investigations into its efficacy have not been extensive, but a study from Egypt indicated a negative effect on *Cryptosporidium* oocyst viability (El-Zawawy *et al.*, 2010), but it was more effective against other protozoa such as *Entamoeba* and *Giardia*.

Ozone has been demonstrated to inactivate *Cryptosporidium* oocysts, with a level of 2.27 mg per litre being effective within 8 min (Peeters *et al.*, 1989) and over 90% reduction in infectivity achieved by treating oocysts with 1 ppm of ozone (1 mg/l) for 5 min (Korich *et al.*, 1990). Although ozone has been touted as an effective, clean, alternative technology for use as a sanitizer in the fresh produce industry (Rosenblum *et al.*, 2012; Horvitz and Cantalejo, 2014; Glowacz *et al.*, 2015), investigations on ozonation and inactivation of *Cryptosporidium* oocysts on fresh produce are limited. Furthermore, an outbreak of cryptosporidiosis was associated with consumption of apple cider that had been treated with ozone, suggesting that ozonation is insufficient for inactivating oocysts in this type of product

(Blackburn *et al.*, 2006). Investigations of other chemicals for inactivating *Cryptosporidium* oocysts in apple cider have suggested that hydrogen peroxide may be a useful alternative, as addition of 0.025% hydrogen peroxide to each of 3 juices investigated (apple cider, orange juice, and grape juice) resulted in a >5 log reduction of *C. parvum* infectivity as determined with a most-probable-number-based cell culture infectivity assay (Kniel *et al.*, 2003). It is suggested that hydrogen peroxide may reduce infectivity of *Cryptosporidium* oocysts by oxidation of oocyst wall proteins or lipids, chelating of cations necessary for infection, or hydroxyl radical-induced DNA damage to sporozoites (Kniel *et al.*, 2004). Hydrogen peroxide has also been shown to have an adverse effect on *Cryptosporidium* oocyst viability and infectivity in another study (e.g., Castro-Hermida *et al.*, 2006), although fresh produce or juices were not included. Another study in which oocysts were exposed to hydrogen peroxide (10 mg/l) reports modest inactivation, as assessed by *in vitro* excystation, which was synergistically enhanced by electroporation during exposure (Haas and Aturaliye, 1999).

The effect of three organic acids (malic, citric, and tartaric) on oocyst viability in juices, including apple cider, has been investigated (Kniel *et al.*, 2003). Concentrations of between and 1 and 5% were explored and the maximum reduction in infectivity of cell culture achieved was 88% (Kniel *et al.*, 2003). Levulinic acid, another organic acid derived from degradation of cellulose, together with sodium dodecyl sulphate (SDS), has been investigated for its effect on *C. parvum* oocysts (3% levulinic acid–2% SDS and 2% levulinic acid–1% SDS) with exposure for 30, 60, and 120 min (Ortega *et al.*, 2011). However, viability using a cell culture system was not affected.

Peracetic acid, also known as peroxyacetic acid (PAA), is another organic acid that has been considered for use as a sanitizer for fresh produce (Alvaro *et al.*, 2009). There are few experiments on the effects of this compound on *Cryptosporidium* oocyst survival and none on

fresh produce; however, some studies indicate that exposure to PAA reduces oocyst viability (Holton *et al.*, 1994; Briancesco *et al.*, 2005).

Studies on the effects of QACs and trisodium phosphate on *Cryptosporidium* oocyst viability are largely lacking, but a zeolite filter media treated with a QAC resulted in a reduction in *Cryptosporidium* oocyst viability of 1.19 log (Abbaszadegan *et al.*, 2006).

Exposure of *Cryptosporidium* oocysts to potassium permanganate (4 mg/l) resulted in some reduction in oocyst viability (as assessed by *in vitro* excystation) and this was synergistically enhanced by electroporation during exposure (Haas and Aturaliye, 1999).

Regarding the novel methodologies currently coming into the fresh produce industry, investigations are even more scant. A study on the use of electrolyzed oxidizing water (EOW) using a specific device designed for continuous flow water treatment produced encouraging results (Huffman *et al.*, 2000), but does not seem to have been followed up, and investigations on inactivation of *Cryptosporidium* oocysts for the fresh produce industry using EOW are entirely lacking. Similarly, there have been few published investigations on the effects of APCP technologies on *Cryptosporidium*. However, a study on the use of pulsed-plasma gas-discharge (PPGD) on the viability of *C. parvum* oocysts demonstrated a 4 log reduction following 32 min exposure (Hayes *et al.*, 2013). It should be noted that in the same experiment, analysis of PPGD-treated water revealed that electrode metals were at around 30 times the accepted values for EU drinking water.

15.3.2.3.2 *Cyclospora cayetanensis*

In comparison with *Cryptosporidium*, experiments directly investigating the effect of different sanitizers on *Cyclospora* are very few (Shields and Ortega, 2012). This could be due to the fact that the majority of outbreaks of cyclosporiasis have been associated with raspberries, which, as for other soft fruit, are not usually subject to sanitizing washes. However, recent outbreaks of cyclosporiasis in the US implicated salad (2013)

as a probable infection vehicle for some cases (Robertson *et al.*, 2014) and also cilantro (2014), and these may encourage further studies on the effects of sanitizers on *Cyclospora* oocysts. Experiments in which *Cyclospora* oocysts on basil leaves and lettuce were exposed to gaseous chlorine dioxide (for 20 min at concentrations of 4.1 mg/l) demonstrated that some oocysts were still able to sporulate (Ortega *et al.*, 2008), with no difference in sporulation between control and experimental oocysts on basil, but a significant reduction in sporulation for oocysts on lettuce. A greater effect was seen on *Cryptosporidium* oocysts and *Encephalitozoon intestinalis* spores exposed to the same treatment regime on both lettuce and basil leaves. A limited effect is also reported for *Cyclospora* oocysts exposed to sodium dichloroisocyanurate (El-Zawawy *et al.*, 2010), which was more effective against other protozoa such as *Entamoeba* and *Giardia*. Other studies have indicated that *Cyclospora* oocysts are able to sporulate following exposure for 15 min to hydrogen peroxide (0.5%), PAA-based sanitizers (40 µg/l), and QACs (*N*-alkyl dimethyl benzyl ammonium chloride) (400 ppm) (Ortega, 2009). These results suggest that *Cyclospora* oocyst may be more resistant to sanitizers than *Cryptosporidium* oocysts, but it should be noted that viability in *Cyclospora*, as evinced by sporulation, may not reflect infectivity. Furthermore, sporulated oocysts may have different sensitivities to sanitizers than non-sporulated oocysts.

15.3.2.3.3 *Taenia solium*

Investigations on survival of *Taenia solium* eggs following exposure to sanitizers used in the fresh produce industry are lacking, but it is reasonable to suppose that the majority would be ineffective, based on studies of laboratory disinfection protocols for *Echinococcus* eggs, from which *T. solium* eggs are morphologically indistinguishable. Research from almost 50 years ago on the effect of a range of compounds on the eggs of *Taenia hydatigena* and *Taenia ovis* demonstrated that most of them had a negligible impact (Mackie and Parnell, 1967); however, susceptibility to 1%

sodium hypochlorite was noted, but this concentration is about 100 times greater than that used in the fresh produce industry.

15.3.2.3.4 *Ascaris lumbricoides*

The thick (3 to 4 µm) four-layer wall of *Ascaris* eggs, consisting of an inner lipoprotein layer (made largely of lipid-containing ascarosides that are responsible for the impermeability), a thicker chitin/protein layer, a lipoprotein vitelline layer, and an outer acid mucopolysaccharide/protein uterine layer, means that it is almost impermeable to chemicals (Brownell and Nelson, 2006). However, the effects of a range of chemical disinfectants on the viability of *Ascaris* eggs have been explored, including hypochlorite, iodine compounds, acids, bases, oxidants, reductants, protein-disrupting agents, surface-active agents, and QACs (Krishnaswami and Post, 1968; Massara *et al.*, 2003; Brownell and Nelson, 2006; Labare *et al.*, 2013). In general, embryonation of *Ascaris* eggs was not affected, with the only disinfectant that abrogated viability being chlorophene, which is not an appropriate sanitizer for use in the fresh produce industry.

Application of ozone to *Ascaris* eggs in suspension at concentrations of between 3.5 and 4.7 mg/l, pH 5.9 inactivated 90% of eggs after 1 h, with a 2 log inactivation occurred at a *Ct* value of around 4.7 mg/min/l (Orta de Velásquez *et al.*, 2004), but only a 0.13 log inactivation of *Ascaris* eggs was achieved by exposure to approximately 5.8 mg/l of dissolved ozone dose for 30 min in a continuous diffusion reactor (Mun *et al.*, 2009). Although this sounds promising, in the fresh produce industry ozone tends to be used at concentrations of 1–5 ppm (mg/l), but for contact times of 5 minutes and less (Rosenblum *et al.*, 2012).

15.4 Conclusion

Knowledge on the occurrence, survival, and potential for removal or inactivation of parasite transmission stages on fresh produce is limited, but some general comments can be made.

At least 14 different parasites have the potential for transmission to humans via consumption of fresh produce contaminated with their transmission stages. The wide range in size, structure, lifecycle, etc., of these parasites mean it is impossible to consider all parasites together.

In this chapter, four different parasites were selected, based upon their perceived importance (proven food-borne transmission, sometimes with outbreaks, high prevalence either globally or in specific regions, potentially very severe pathology) and as representatives of different parasite groups. However, it should be noted that extrapolation from one parasite to another should be done with extreme caution, even if there are similarities in lifecycle or morphology. Information on these different parasites and their transmission via contaminated produce varies considerably. The global distribution also varies widely and for some parasites it is difficult to determine the contribution of fresh produce as a vehicle of infection to transmission.

Data on the extent to which washing procedures may be effective at removing parasite transmission stages to an acceptable level is lacking for most parasites. However, for many parasites the transmission stages are considered to have adhesive properties that may limit their removal by washing procedures. For delicate produce, such as berries, vigorous washing is not possible, and washing procedures are unlikely to remove any more than a very low proportion of contaminant parasites.

Physical and chemical processes (sanitizers) in the fresh produce chain may impact on survival of parasite transmission stages to some extent, but, in general, parasite transmission stages are very robust. Some parasite transmission stages are known to survive prolonged freezing, whilst others are resistant to chemical sanitizers, at least at the regimes used in the fresh produce industry. Although some procedures, such as high pressure, appear to be very promising, further research is necessary in order to verify initial results and to develop

reliable methods for inactivating or removing parasite transmission stages in the fresh produce industry.

References

- Abbaszadegan, M., Monteiro, P., Ouwers, R.N., Ryu, H., and Alum, A. 2006. Removal and inactivation of *Cryptosporidium* and microbial indicators by a quaternary ammonium chloride (QAC)-treated zeolite in pilot filters. *Journal of Environmental Science Health: A Toxic Hazard Substance in Environmental Engineering*, **41**, 1201–1210.
- Alarcón de Noya, B., Díaz-Bello, Z., Colmenares, C., Ruiz-Guevara, R., *et al.* 2010. Large urban outbreak of orally acquired acute Chagas disease at a school in Caracas, Venezuela. *Journal of Infectious Diseases*, **201**, 1308–1315.
- Al-Binali, A.M., Bello, C.S., El-Shewy, K., and Abdulla, S.E. 2006. The prevalence of parasites in commonly used leafy vegetables in South Western, Saudi Arabia. *Saudi Medical Journal*, **27**, 613–616.
- Alvaro, J.E., Moreno, S., Dianez, F., Santos, M., Carrasco, G., and Urrestarazu, M. 2009. Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *Journal of Food Engineering*, **95**, 11–15.
- Amorós, I., Alonso, J.L., and Cuesta, G. 2010. *Cryptosporidium* oocysts and *Giardia* cysts on salad products irrigated with contaminated water. *Journal of Food Protection*, **73**, 1138–1140.
- Anonymous. 1990. Methods for the examination of waters and associated materials. In: *Isolation and Identification of Giardia Cysts, Cryptosporidium Oocysts and Free Living Pathogenic Amoebae in Water, etc.* 1989. Department of Environment, Standing Committee of Analysts, HMSO Publication, London.
- Anonymous. 2006. Water quality – Isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts from water. ISO 15553:2006.
- Anonymous 2013. Outbreak of cryptosporidiosis in England and Scotland, May 2012. Health

- Protection Report 7 (12). Available at: http://www.hpa.org.uk/hpr/news/default_0413_aa_crptsprdm.htm.
- Arbor, A. 2009. Benefits and risks of the use of chlorine-containing disinfectants in food production and food processing: report of a joint FAO/WHO expert meeting, 2008. © FAO and WHO 2009. ISBN 978 92 4 159894 1. Available at: <http://www.fao.org/docrep/012/i1357e/i1357e.pdf>.
- Armon, R., Gold, D., Brodsky, M., and Oron, G. 2002. Surface and subsurface irrigation with effluents of different qualities and presence of *Cryptosporidium* oocysts in soil and on crops. *Water Science and Technology*, **46**, 115–122.
- Ashokkumar, M., Vu, T., Grieser, F., Weerawardena, A., Anderson, N., Pilkington, N., and Dixon, D.R. 2003. Ultrasonic treatment of *Cryptosporidium* oocysts. *Water Science and Technology*, **47**, 173–177.
- Behm, C.A. 1997. The role of trehalose in the physiology of nematodes. *International Journal of Parasitology*, **27**, 215–229.
- Belli, S.I., Smith, N.C., and Ferguson, D.J. 2006. The coccidian oocyst: a tough nut to crack! *Trends in Parasitology*, **22**, 416–423.
- Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., and Frankel, G. 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology*, **12**, 2385–2397.
- Bier, J.W. 1991. Isolation of parasites on fruits and vegetables. *Southeast Asian Journal of Tropical Medicine and Public Health*, **22** (Suppl.), 144–145.
- Birmpa, A., Sfika, V., and Vantarakis, A. 2013. Ultraviolet light and ultrasound as non-thermal treatments for the inactivation of microorganisms in fresh ready-to-eat foods. *International Journal of Food Microbiology*, **167**, 96–102.
- Blackburn, B.G., Mazurek, J.M., Hlavsa, M., Park, J., et al. 2006. Cryptosporidiosis associated with ozonated apple cider. *Emerging Infectious Diseases*, **12**, 684–686.
- Bowman, D.D., Chun, K., and Liotta, J.L. 2009. Effects of freezing on the viability of the eggs of *Ascaris suum*. In: *Proceedings of the Water Environment Federation, Disinfection*, Orlando, Florida, pp. 54–58(5).
- Briancesco, R., Veschetti, E., Ottaviani, M., and Bonadonna, L. 2005. Peracetic acid and sodium hypochlorite effectiveness in reducing resistant stages of microorganisms. *Central European Journal of Public Health*, **13**, 159–162.
- Brownell, S.A. and Nelson, K.L. 2006. Inactivation of single-celled *Ascaris suum* eggs by low-pressure UV radiation. *Applied and Environmental Microbiology*, **72**, 2178–2184.
- Capizzi, S. and Schwartzbrod, J. 2001. Surface properties of *Ascaris suum* eggs: hydrophobic potential and Lewis acid–base interactions. *Colloids Surface B Biointerfaces*, **22**, 99–105.
- Castro-Hermida, J.A., Pors, I., Méndez-Hermida, F., Ares-Mazás, E., and Chartier, C. 2006. Evaluation of two commercial disinfectants on the viability and infectivity of *Cryptosporidium parvum* oocysts. *Veterinary Journal*, **171**, 340–345.
- Chaidez, C., Soto, M., Gortares, P., and Mena, K. 2005. Occurrence of *Cryptosporidium* and *Giardia* in irrigation water and its impact on the fresh produce industry. *International Journal of Environmental Health Research*, **15**, 339–345.
- Chyu, I., Lee, W.H., Woo, C.K., and Lee, K.B. 1970. Effects of irradiation with cobalt 60 to the development of *Ascaris* eggs in stool. *Kisaengchunghak Chapchi*, **8**, 1–4.
- Collier, S.A., Smith, S., Lowe, A., Hawkins, P., et al. 2011. Cryptosporidiosis outbreak at a summer camp – North Carolina. *Morbidity. Mortality Weekly (MMWR)*, **60**, 918–922.
- Coman, B.J. 1975. The survival of *Taenia pisiformis* eggs under laboratory conditions and in the field environment. *Australian Veterinary Journal*, **51**, 560–565.
- Cook, N., Paton, C.A., Wilkinson, N., Nichols, R.A., Barker, K., and Smith, H.V. 2006a. Towards standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries. Part 1: development and optimization of methods. *International Journal of Food Microbiology*, **109**, 215–221.
- Cook, N., Paton, C.A., Wilkinson, N., Nichols, R.A., Barker, K., and Smith, H.V. 2006b. Towards

- standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries. Part 2: validation. *International Journal of Food Microbiology*, **109**, 222–228.
- Cowie, R.H. 2013. Pathways for transmission of angiostrongyliasis and the risk of disease associated with them. *Hawaii Journal of Medicine and Public Health*, **72** (6 Suppl. 2), 70–74.
- Craik, S.A., Weldon, D., Finch, G.R., Bolton, J.R., and Belosevic, M. 2001. Inactivation of *Cryptosporidium parvum* oocysts using medium- and low-pressure ultraviolet radiation. *Water Research*, **35**, 1387–1398.
- Cram, E. 1924. The influence of low temperatures and of disinfectants on the eggs of *Ascaris lumbricoides*. *Journal of Agricultural Research*, **27**, 167–175.
- Current, W.L. and Garcia, L.S. 1991. Cryptosporidiosis. *Clinical Microbiology Review*, **4**, 325–358.
- Dawson, D.J., Samuel, C.M., Scrannage, V., and Atherton, C.J. 2004. Survival of *Cryptosporidium* species in environments relevant to foods and beverages. *Journal of Applied Microbiology*, **96**, 1222–1229.
- De Leon, W.U., Monzon, R.B., Aganon, A.A., Arceo, R.E., Ignacio, E.J., and Santos, G. 1992. Parasitic contamination of selected vegetables sold in Metropolitan Manila, Philippines. *Southeast Asian Journal of Tropical Medicine and Public Health*, **23**, 162–164.
- Deng, M.Q. and Cliver, D.O. 1999. *Cryptosporidium parvum* studies with dairy products. *International Journal of Food Microbiology*, **46**, 113–121.
- Deng, M.Q. and Cliver, D.O. 2001. Inactivation of *Cryptosporidium parvum* oocysts in cider by flash pasteurization. *Journal of Food Protection*, **64**, 523–527.
- De São José, J.F.B., José de Andrade, N., Mota Ramos, A., Dantas Vanetti, M.C., Stringheta, P.C., and Paes Chaves, J.B. 2014. Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control*, **25**, 36–50.
- Dubey, J.P., Thayer, D.W., Speer, C.A., and Shen, S.K. 1998. Effect of gamma irradiation on unsporulated and sporulated *Toxoplasma gondii* oocysts. *International Journal of Parasitology*, **28**, 369–375.
- Duhain, G.L., Minnaar, A., and Buys, E.M. 2012. Effect of chlorine, blanching, freezing, and microwave heating on *Cryptosporidium parvum* viability inoculated on green peppers. *Journal of Food Protection*, **75**, 936–941.
- Ekman, C.C., Chiossi, M.F., Meireles, L.R., Andrade Júnior, H.F., Figueiredo, W.M., Marciano, M.A., and Luna, E.J. 2012. Case-control study of an outbreak of acute toxoplasmosis in an industrial plant in the state of São Paulo, Brazil. *Review of Institute of Medicine in Tropical Sao Paulo*, **54**, 239–244.
- Elbein, A.D., Pan, Y.T., Pastuszak, I., and Carroll, D. 2003. New insights on trehalose: a multifunctional molecule. *Glycobiology*, **13**, 17R–27R.
- El-Zawawy, L.A., El-Said, D., Ali, S.M., and Fathy, F.M. 2010. Disinfection efficacy of sodium dichloroisocyanurate (NaDCC) against common food-borne intestinal protozoa. *Journal of Egypt Society Parasitology*, **40**, 165–185.
- Ensink, J.H., Mahmood, T., and Dalsgaard, A. 2007. Wastewater-irrigated vegetables: market handling versus irrigation water quality. *Tropical Medicine and International Health*, **12** (Suppl. 2), 2–7.
- Eraky, M.A., Rashed, S.M., Nasr, M.-S., El-Hamshary, A.M., and Salah El-Ghannam, A. 2014. Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. *Journal of Parasitology Research*, **2014**, 613960.
- Erickson, M.C. and Ortega, Y.R. 2006. Inactivation of protozoan parasites in food, water, and environmental systems. *Journal of Food Protection*, **69**, 2786–2808.
- FAO/WHO (Food and Agriculture Organization of the United Nations and World Health Organization). 2014. Multicriteria-based ranking for risk management of foodborne parasites. Report of a Joint FAO/WHO Expert Meeting, 3–7 September 2012, FAO Headquarters, Rome, Italy. ISBN 978 92 4 156470 0 (WHO) (NLM classification: WA 701). ISBN 978-92-5-108199-0 (print) (FAO). E-ISBN 978-92-5-108200-3 (PDF) (FAO). ISSN 1726-5274. Available at: <http://www.fao.org/3/a-i3649e.pdf>.

- Fayer, R. 2008. Biology. In: *Cryptosporidium and Cryptosporidiosis*, 2nd edn (eds Fayer, R. and Xiao, L.). CRC Press and IWA Publishing, Boca Raton, FL, USA.
- Fayer, R. and Nerad, T. 1996. Effects of low temperatures on viability of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, **62**, 1431–1433.
- Fayer, R., Santin, M., Macarisin, D., and Bauchan, G. 2013. Adhesive-tape recovery combined with molecular and microscopic testing for the detection of *Cryptosporidium* oocysts on experimentally contaminated fresh produce and a food preparation surface. *Parasitology Research*, **112**, 1567–1574.
- Fetene, T., Worku, N., Huruy, K., and Kebede, N. 2011. *Cryptosporidium* recovered from *Musca domestica*, *Musca sorbens* and mango juice accessed by synanthropic flies in Bahirdar, Ethiopia. *Zoonoses Public Health*, **58**, 69–75.
- Gardner, D.W. and Shama, G. 2000. Modeling UV-induced inactivation of microorganisms on surfaces. *Journal of Food Protection*, **63**, 63–70.
- Gaspard, P.G., Wiart, J., and Schwartzbrod, J. 1994. Experimental study of the helminth eggs adhesion (*Ascaris suum*): analysis of the environmental implications. *Revue des Sciences de L'Eau*, **7**, 367–376.
- Gibbs, R.A., Nanyonjo, R., Pingault, N.M., Combs, B.G., Mazzucchelli, T., Armstrong, P., Tarling, G., and Dowse, G.K. 2013. An outbreak of *Cyclospora* infection on a cruise ship. *Epidemiological Infection*, **141**, 508–516.
- Gil, M.I., Selma, M.V., López-Gálvez, F., and Allende, A. 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. *International Journal of Food Microbiology*, **134**, 37–45.
- Glowacz, M., Colgan, R., and Rees, D. 2015. The use of ozone to extend the shelf-life and maintain quality of fresh produce. *Journal of Science and Food Agriculture*, **95**, 662–671. doi: 10.1002/jsfa.6776.
- Goodgame, R.W., Genta, R.M., White, A.C., and Chappell, C.L. 1993. Intensity of infection in AIDS-associated cryptosporidiosis. *Journal of Infectious Diseases*, **167**, 704–709.
- Graczyk, T.K., Grimes, B.H., Knight, R., Szostakowska, B., et al. 2004. Mechanical transmission of *Cryptosporidium parvum* oocysts by flies. *Wiad Parazytology*, **50**, 243–247.
- Greig, J.D., Todd, E.C., Bartleson, C.A., and Michaels, B.S. 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 1. Description of the problem, methods, and agents involved. *Journal of Food Protection*, **70**, 1752–1761.
- Haas, C.N. and Aturaliye, D. 1999. Semi-quantitative characterization of electroporation-assisted disinfection processes for inactivation of *Giardia* and *Cryptosporidium*. *Journal of Applied Microbiology*, **86**, 899–905.
- Hawk, M.W., Shahlaie, K., Kim, K.D., and Theis, J.H. 2005. Neurocysticercosis: a review. *Surgical Neurology*, **63**, 123–132.
- Hayes, J., Kirf, D., Garvey, M., and Rowan, N. 2013. Disinfection and toxicological assessments of pulsed UV and pulsed-plasma gas-discharge treated-water containing the waterborne protozoan enteroparasite *Cryptosporidium parvum*. *Journal of Microbiology Methods*, **94**, 325–337.
- Ho, A.Y., Lopez, A.S., Eberhart, M.G., Levenson, R., et al. 2002. Outbreak of cyclosporiasis associated with imported raspberries, Philadelphia, Pennsylvania. *Emerging Infectious Diseases*, **8**, 783–788.
- Holton, J., Nye, P., and McDonald, V. 1994. Efficacy of selected disinfectants against mycobacteria and cryptosporidia. *Journal of Hospital Infections*, **27**, 105–115.
- Horvitz, S. and Cantalejo, M.J. 2014. Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Review of Food Science and Nutrition*, **54**, 312–339.
- Huffman, D.E., Coulliette, A., Rose, J.B., Cardenas, R., Molof, A.H., Om, J., and Mignone, E.J. 2000. Inactivation of *Cryptosporidium parvum* using International Water Technologies' pH Balancer. *Proceedings of the Water Environment Federation, WEFTEC 2000: Session 71 through Session 79*, pp. 332–340(9).
- Jimenez, B. 2007. Helminth ova removal from wastewater for agriculture and aquaculture reuse. *Water Science and Technology*, **55**, 485–493.

- Kern, P., Ammon, A., Kron, M., Sinn, G., Sander, S., Petersen, L.R., Gaus, W., and Kern, P. 2004. Risk factors for alveolar echinococcosis in humans. *Emerging Infectious Diseases*, **10**, 2088–2093.
- Keserue, H.A., Fuchslin, H.P., Wittwer, M., Nguyen-Viet, H., *et al.* 2012. Comparison of rapid methods for detection of *Giardia* spp. and *Cryptosporidium* spp. (oo)cysts using transportable instrumentation in a field deployment. *Environmental Science and Technology*, **46**, 8952–8959.
- Kim, J.S., Oh, D.S., Ahn, K.S., and Shin, S.S. 2012. Effects of kimchi extract and temperature on embryostasis of *Ascaris suum* eggs. *Korean Journal of Parasitology*, **50**, 83–87.
- Kłapeć, T. and Borecka, A. 2012. Contamination of vegetables, fruits and soil with geohelminths eggs on organic farms in Poland. *Annals of Agricultural and Environmental Medicine*, **19**, 421–425.
- Kniel, K.E., Sumner, S.S., Lindsay, D.S., Hackney, C.R., Pierson, M.D., Zajac, A.M., Golden, D.A., and Fayer, R. 2003. Effect of organic acids and hydrogen peroxide on *Cryptosporidium parvum* viability in fruit juices. *Journal of Food Protection*, **66**, 1650–1657.
- Kniel, K.E., Sumner, S.S., Pierson, M.D., Zajac, A.M., Hackney, C.R., Fayer, R., and Lindsay, D.S. 2004. Effect of hydrogen peroxide and other protease inhibitors on *Cryptosporidium parvum* excystation and *in vitro* development. *Journal of Parasitology*, **90**, 885–888.
- Kniel, K.E., Shearer, A.E., Cascarino, J.L., Wilkins, G.C., and Jenkins, M.C. 2007. High hydrostatic pressure and UV light treatment of produce contaminated with *Eimeria acervulina* as a *Cyclospora cayetanensis* surrogate. *Journal of Food Protection*, **70**, 2837–2842.
- Knopp, S., Mohammed, K.A., Stothard, J.R., Khamis, I.S., Rollinson, D., Marti, H., and Utzinger, J. 2010. Patterns and risk factors of helminthiasis and anemia in a rural and a peri-urban community in Zanzibar, in the context of helminth control programs. *PLoS Neglect in Tropical Diseases*, **4**, e681.
- Konno, K., Oku, Y., Sakai, H., and Kamiya, M. 1997. Effect of ultraviolet radiation on the infectivity of *Taenia taeniaeformis* eggs. *Japan Journal of Veterinary Research*, **45**, 75–79.
- Korich, D.G., Mead, J.R., Madore, M.S., Sinclair, N.A., and Sterling, C.R. 1990. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Applied and Environmental Microbiology*, **56**, 1423–1428.
- Koruk, I., Simsek, Z., Tekin Koruk, S., Doni, N., and Gürses, G. 2010. Intestinal parasites, nutritional status and psychomotor development delay in migratory farm worker's children. *Child Care Health Development*, **36**, 888–894.
- Kozak, G.K., MacDonald, D., Landry, L., and Farber, J.M. 2013. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. *Journal of Food Protection*, **76**, 173–183.
- Krishnaswami, S.K. and Post, F.J. 1968. Effect of chlorine on *Ascaris* (Nematoda) eggs. *Health Laboratory Science*, **5**, 225–232.
- Labare, M.P., Soohoo, H., Kim, D., Tsoi, K.Y., Liotta, J.L., and Bowman, D.D. 2013. Ineffectiveness of a quaternary ammonium salt and povidone-iodine for the inactivation of *Ascaris suum* eggs. *American Journal of Infection Control*, **41**, 360–361.
- Laberge, I. and Griffiths, M.W. 1996. Prevalence, detection and control of *Cryptosporidium* in food. *International Journal of Food Microbiology*, **32**, 1–26.
- Lagapa, J.T., Konno, K., Oku, Y., Nonaka, N., and Kamiya, M. 2001. Inhibitory effect of different UV lamps on the infectivity of taeniid eggs. *Parasitology Research*, **87**, 593–597.
- Laws, G.F. 1968. Physical factors influencing survival of taeniid eggs. *Experimental Parasitology*, **22**, 227–239.
- Lee, M.B. and Lee, E.H. 2001. Coccidial contamination of raspberries: mock contamination with *Eimeria acervulina* as a model for decontamination treatment studies. *Journal of Food Protection*, **64**, 1854–1857.
- Lee, O.R., Yun, B.H., and Choi, W.Y. 1970. The experiments on the infectivity to mice of the *Ascaris* eggs irradiated with Cobalt (60). *Kisaengchunghak Chapchi*, **8**, 90–95.

- Lee, S.U., Joung, M., Yang, D.J., Park, S.H., Huh, S., Park, W.Y., and Yu, J.R. 2008. Pulsed-UV light inactivation of *Cryptosporidium parvum*. *Parasitology Research*, **102**, 1293–1299.
- Le Goff, L., Hubert, B., Favenec, L., Villena, I., Agoulon, A., and Gargala, G. 2014. Pilot-scale evaluation of an innovative pulsed UV light technology's efficacy: irradiation of raspberries experimentally contaminated reduces *Cryptosporidium parvum* infectivity in immunocompetent suckling mice. In: *5th International Giardia and Cryptosporidium Conference, Poster Presentation. Abstract Book*. Uppsala, Sweden, May 2014.
- Lonigro, A., Pollice, A., Spinelli, R., Berrilli, F., Di Cave, D., D'Orazi, C., Cavallo, P., and Brandonisio, O. 2006. *Giardia* cysts and *Cryptosporidium* oocysts in membrane-filtered municipal wastewater used for irrigation. *Applied and Environmental Microbiology*, **72**, 7916–7918.
- Lynch, M.F., Tauxe, R.V., and Hedberg, C.W. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiology and Infections*, **137**, 307–315.
- Macarasin, D., Bauchan, G., and Fayer, R. 2010a. *Spinacia oleracea* L. leaf stomata harboring *Cryptosporidium parvum* oocysts: a potential threat to food safety. *Applied and Environmental Microbiology*, **76**, 555–559.
- Macarasin, D., Santín, M., Bauchan, G., and Fayer, R. 2010b. Infectivity of *Cryptosporidium parvum* oocysts after storage of experimentally contaminated apples. *Journal of Food Protection*, **73**, 1824–1829.
- Mackie, A. and Parnell, I.W. 1967. Some observations on taeniid oviducts: the effects of some organic compounds and pesticides on activity and hatching. *Journal of Helminthology*, **41**, 167–210.
- Magri, M.E., Philippi, L.S., and Vinnerås, B. 2013. Inactivation of pathogens in feces by desiccation and urea treatment for application in urine-diverting dry toilets. *Applied and Environmental Microbiology*, **79**, 2156–2163.
- Mahajan, P.V., Caleb, O.J., Singh, Z., Watkins, C.B., and Geyer, M. 2014. Postharvest treatments of fresh produce. *Philosophical Trans. A Mathematics, Physics and Engineering Science*, **372**, 20130309.
- Massara, C.L., Costa, H.M., De Souza, D.W., Souza, M.S., and Carvalho Odo, S. 1991. Viability of *Ascaris lumbricoides* eggs eliminated after anti-helminthic therapy. *Memórias do Instituto Oswaldo Cruz*, **86**, 233–237.
- Massara, C.L., Ferreira, R.S., de Andrade, L.D., Guerra, H.L., and Carvalho Odo, S. 2003. Effects of detergents and disinfectants on the development of *Ascaris lumbricoides* eggs. *Cad. Saude Publica*, **19**, 335–340.
- McClure, P., Ronnie, N., and Waskar, M. 2012. HACCP, risk assessment and risk communication, Chapter 9. In: *Foodborne Protozoan Parasites* (eds Robertson, L.J. and Smith, H.V.), pp. 239–266. Nova Biomedical, Hauppauge, NY, USA.
- Merwad, A.M., Mitchell, S.M., Zajac, A.M., Flick, G.J., and Lindsay, D.S. 2011. Effects of high pressure processing on hatching of eggs of the zoonotic rat tapeworm *Hymenolepis diminuta*. *Veterinary Parasitology*, **176**, 185–188.
- Mintz, E.D., Hudson-Wragg, M., Mshar, P., Cartter, M.L., and Hadler, J.L. 1993. Foodborne giardiasis in a corporate office setting. *Journal of Infectious Diseases*, **167**, 250–253.
- Misra, N.N., Keener, K.M., Bourke, P., Mosnier, J.P., and Cullen, P.J. 2014. In-package atmospheric pressure cold plasma treatment of cherry tomatoes. *Journal of Bioscience and Bioengineering*, **118**, 177–182.
- Mossallam, S.F. 2010. Detection of some intestinal protozoa in commercial fresh juices. *Journal of Egypt Society of Parasitology*, **40**, 135–149.
- Mun, S., Cho, S.H., Kim, T.S., Oh, B.T., and Yoon, J. 2009. Inactivation of *Ascaris* eggs in soil by microwave treatment compared to UV and ozone treatment. *Chemosphere*, **77**, 285–290.
- Nkouawa, A., Sako, Y., Nakao, M., Nakaya, K., and Ito, A. 2009. Loop-mediated isothermal amplification method for differentiation and rapid detection of *Taenia* species. *Journal of Clinical Microbiology*, **47**, 168–174.

- Nydam, D.V., Wade, S.E., Schaaf, S.L., and Mohammed, H.O. 2001. Number of *Cryptosporidium parvum* oocysts or *Giardia* spp. cysts shed by dairy calves after natural infection. *American Journal of Veterinary Research*, **62**, 1612–1615.
- Oey, I., Lille, M., Van Loey, A., and Hendrickx, M. 2008. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in Food Science and Technology*, **19**, 320–328.
- Ogbolu, D.O., Alli, O.A., Ogunleye, V.F., Olusoga-Ogbolu, F.F., and Olaosun, I. 2009. The presence of intestinal parasites in selected vegetables from open markets in south western Nigeria. *African Journal of Medical Science*, **38**, 319–324.
- OIE. 2014. Cysticercosis, Chapter 2.9.5. In: *OIE Terrestrial Manual*. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.09.05_CYSTICERCOSIS.pdf.
- Ölmez, H. and Kretzschmar, U. 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT – Food Science and Technology*, **42**, 686–693.
- O’Lorcain, P. and Holland, C.V. 2000. The public health importance of *Ascaris lumbricoides*. *Parasitology*, **121**, S51–S71.
- Olvera, M., Eguía, A., Rodríguez, O., Chong, E., Pillai, S.D., and Ilangoan, K. 2008. Inactivation of *Cryptosporidium parvum* oocysts in water using ultrasonic treatment. *Bioresource Technology*, **99**, 2046–2049.
- Orlandi, P.A., Frazar, C., Carter, L., and Chu, D.-M.T. 2004. Detection of *Cyclospora* and *Cryptosporidium* from fresh produce: isolation and identification by polymerase chain reaction (PCR) and microscopic analysis, Chapter 19A. In: *Bacteriological Analytical Manual*, USDA/FSIS Microbiological Laboratory Guidebook, 3rd edn. Available at: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm073638.htm>.
- Orta de Velásquez, T., Rojas-Valencia, N., Martínez, J.L., and Monje-Ramírez, I. 2004. Destruction of helminth (*Ascaris suum*) eggs by ozone. *Ozone Science and Engineering Journal*, **26**, 359–366.
- Ortega, Y.R. 2009. Environmental factors influencing the survival of *Cyclospora cayetanensis*. In: *Giardia and Cryptosporidium – From Molecules to Disease* (eds Ortega-Pierres, G., Cacciò, S., Fayer, R., Mank, T., Smith, H.V., and Thompson, R.C.A.). CABI, Oxfordshire, UK. ISBN-13: 978-1845933913.
- Ortega, Y.R. and Liao, J. 2006. Microwave inactivation of *Cyclospora cayetanensis* sporulation and viability of *Cryptosporidium parvum* oocysts. *Journal of Food Protection*, **69**, 1957–1960.
- Ortega, Y.R. and Sanchez, R. 2010. Update on *Cyclospora cayetanensis*, a food-borne and water-borne parasite. *Clinical Microbiology Review*, **23**, 218–234.
- Ortega, Y.R., Gilman, R.H., Sterling, C.R. 1994. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. *Journal of Parasitology*, **80**, 625–629.
- Ortega, Y.R., Roxas, C.R., Gilman, R.H., Miller, N.J., Cabrera, L., Taquiri, C., and Sterling, C.R. 1997. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *American Journal of Tropical Medicine and Hygiene*, **57**, 683–686.
- Ortega, Y.R., Mann, A., Torres, M.P., and Cama, V. 2008. Efficacy of gaseous chlorine dioxide as a sanitizer against *Cryptosporidium parvum*, *Cyclospora cayetanensis*, and *Encephalitozoon intestinalis* on produce. *Journal of Food Protection*, **71**, 2410–2414.
- Ortega, Y.R., Torres, M.P., and Tatum, J.M. 2011. Efficacy of levulinic acid-sodium dodecyl sulfate against *Encephalitozoon intestinalis*, *Escherichia coli* O157:H7, and *Cryptosporidium parvum*. *Journal of Food Protection*, **74**, 140–144.
- Osterholm, M.T. 1999. Lessons learned again: cyclosporiasis and raspberries. *Annals of International Medicine*, **130**, 233–234.
- Oyane, I., Furuta, M., Stavarache, C.E., Hashiba, K., Mukai, S., Nakanishi, J.M., Kimata, I., and Maeda, Y. 2005. Inactivation of *Cryptosporidium parvum* by ultrasonic irradiation. *Environmental Science and Technology*, **39**, 7294–7298.

- Pal, D.K., Carpio, A., and Sander, J.W. 2000. Neurocysticercosis and epilepsy in developing countries. *Journal of Neurology, Neurosurgery and Psychiatry*, **68**, 137–143.
- Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garret, E.H., Farber, J.N., and Busta, F.F. 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce, Chapter V. In: *Comprehensive Reviews in Food Science and Food Safety* (ed. Kroger, M). FDA, Chicago. Available at: <http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm091363.htm>.
- Peeters, J.E., Mazás, E.A., Masschelein, W.J., Villacorta Martiez de Maturana, I., and Debacker, E. 1989. Effect of disinfection of drinking water with ozone or chlorine dioxide on survival of *Cryptosporidium parvum* oocysts. *Applied and Environmental Microbiology*, **55**, 1519–1522.
- Pereira, K.S., Barbosa, R.L., Passos, L.A.C., Sousa de Aguiar, F., Rogez, H., de Noya, B., and Gonzalez, O.N. 2012. *Trypanosoma cruzi*, Chapter 7, pp. 189–216. In: *Foodborne Protozoan Parasites* (eds Robertson, L.J. and Smith, H.V.). Nova Biomedical, Hauppauge, NY, USA.
- Pham-Duc, P., Nguyen-Viet, H., Hattendorf, J., Zinsstag, J., Phung-Dac, C., Zurbrugg, C., and Odermatt, P. 2013. *Ascaris lumbricoides* and *Trichuris trichiura* infections associated with wastewater and human excreta use in agriculture in Vietnam. *Parasitology International*, **62**, 172–180.
- Porter, J.D., Gaffney, C., Heymann, D., and Parkin, W. 1990. Foodborne outbreak of *Giardia lamblia*. *American Journal of Public Health*, **80**, 1259–1260.
- Prasad, K.N., Verma, A., Srivastava, S., Gupta, R.K., Pandey, C.M., and Paliwal, V.K. 2011. An epidemiological study of asymptomatic neurocysticercosis in a pig farming community in northern India. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **105**, 531–536.
- Psarros, T.G., Zouros, A., and Coimbra, C. 2003. Neurocysticercosis: a neurosurgical perspective. *South Medical Journal*, **96**, 1019–1022.
- Quilès, F., Balandier, J.Y., and Capizzi-Banas, S. 2006 *In situ* characterisation of a microorganism surface by Raman microspectroscopy: the shell of *Ascaris* eggs. *Analysis of Bioanalytical Chemistry*, **386**, 249–255.
- Quiroz, E.S., Bern, C., MacArthur, J.R., Xiao, L., et al. 2000. An outbreak of cryptosporidiosis linked to a foodhandler. *Journal of Infectious Diseases*, **181**, 695–700.
- Räisänen, S., Ruuskanen, L., and Nyman, S. 1985. Epidemic ascariasis – evidence of transmission by imported vegetables. *Scandinavian Journal of Primary Health Care*, **3**, 189–191.
- Ranjbar-Bahadori, Sh., Mostoophi, A., and Shemshadi, B. 2013. Study on *Cryptosporidium* contamination in vegetable farms around Tehran. *Tropical Biomedicine*, **30**, 193–198.
- Rastogi, N.K. 2011. Opportunities and challenges in application of ultrasound in food processing. *Critical Review of Food Science and Nutrition*, **51**, 705–722.
- Rastogi, N.K., Raghavarao, K.S., Balasubramaniam, V.M., Niranjana, K., and Knorr, D. 2007. Opportunities and challenges in high pressure processing of foods. *Critical Review of Food Science and Nutrition*, **47**, 69–112.
- Rendueles, E., Omer, M.K., Alyseike, O., Alonso-Calleja, C., Capita, R., and Prieto, M. 2011. Microbiological food safety assessment of high hydrostatic pressure processing: a review. *LWT – Food Science and Technology*, **44**, 1251–1260.
- Ríos, J.F., Arboleda, M., Montoya, A.N., and Alarcón, E.P. 2011. Probable outbreak of oral transmission of Chagas disease in Turbo, Antioquia. *Biomedica*, **31**, 185–195.
- Ripabelli, G., Leone, A., Sammarco, M.L., Fanelli, I., Grasso, G.M., and McLauchlin, J. 2004. Detection of *Cryptosporidium parvum* oocysts in experimentally contaminated lettuce using filtration, immunomagnetic separation, light microscopy, and PCR. *Foodborne Pathogen Diseases*, **1**, 216–222.
- Robertson, L.J. 2014a. Protozoan parasites: a plethora of potentially foodborne pathogens, Chapter 13. In: *Food Associated Pathogens* (eds Tham, W. and Danielsson-Tham, M.-L.), pp. 169–216. CRC Press, Taylor & Francis. ISBN: 978-1466584983.
- Robertson, L.J. 2014b. *Cryptosporidium* as a Foodborne Pathogen. Springer Briefs in Food,

- Health and Nutrition, Springer, New York, Heidelberg, Dordrecht, London. ISBN: 978-1461493778.
- Robertson, L.J. and Chalmers, R.M. 2013. Foodborne cryptosporidiosis: is there really more in Nordic countries? *Trends in Parasitology*, **29**, 3–9.
- Robertson, L.J. and Gjerde, B. 2000. Isolation and enumeration of *Giardia* cysts, *Cryptosporidium* oocysts, and *Ascaris* eggs from fruits and vegetables. *Journal of Food Protection*, **63**, 775–778.
- Robertson, L.J. and Gjerde, B. 2001. Occurrence of parasites on fruits and vegetables in Norway. *Journal of Food Protection*, **64**, 1793–1798.
- Robertson, L.J. and Gjerde, B.K. 2006. Fate of *Cryptosporidium* oocysts and *Giardia* cysts in the Norwegian aquatic environment over winter. *Microbiology and Ecology*, **52**, 597–602.
- Robertson, L.J. and Gjerde, B. 2007. *Cryptosporidium* oocysts: challenging adversaries? *Trends in Parasitology*, **23**, 344–347.
- Robertson, L.J., Campbell, A.T., and Smith, H.V. 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. *Applied and Environmental Microbiology*, **58**, 3494–3500.
- Robertson, L.J., Gjerde, B., and Campbell, A.T. 2000. Isolation of *Cyclospora* oocysts from fruits and vegetables using lectin-coated paramagnetic beads. *Journal of Food Protection*, **63**, 1410–1414.
- Robertson, L.J., Johannessen, G.S., Gjerde, B.K., and Loncarevic, S. 2002. Microbiological analysis of seed sprouts in Norway. *International Journal of Food Microbiology*, **75**, 119–126.
- Robertson, L.J., van der Giessen, J.W., Batz, M.B., Kojima, M., and Cahill, S. 2013. Have foodborne parasites finally become a global concern? *Trends in Parasitology*, **29**, 101–103.
- Robertson, L.J., Sprong, H., Ortega, Y.R., van der Giessen, J.W., and Fayer, R. 2014. Impacts of globalisation on foodborne parasites. *Trends in Parasitology*, **30**, 37–52.
- Robinson, G. and Chalmers, R.M. 2010. The European rabbit (*Oryctolagus cuniculus*), a source of zoonotic cryptosporidiosis. *Zoonoses and Public Health*, **57**, e1–13.
- Rojas, L., Vazquez, A., Domenech, I., and Robertson, L.J. 2010. Fascioliasis: can Cuba conquer this emerging parasitosis? *Trends in Parasitology*, **26**, 26–34.
- Rosenblum, J., Ge, C., Bohrerova, Z., Yousef, A., and Lee, J. 2012. Ozonation as a clean technology for fresh produce industry and environment: sanitizer efficiency and wastewater quality. *Journal of Applied Microbiology*, **113**, 837–845.
- Rosypal, A.C., Bowman, D.D., Holliman, D., Flick, G.J., and Lindsay, D.S. 2007. Effects of high hydrostatic pressure on embryonation of *Ascaris suum* eggs. *Veterinary Parasitology*, **145**, 86–89.
- Rosypal, A.C., Zajac, A.M., Flick, G.J., Bowman, D.D., and Lindsay, D.S. 2011. High pressure processing treatment prevents embryonation of eggs of *Trichuris vulpis* and *Ascaris suum* and induces delay in development of eggs. *Veterinary Parasitology*, **181**, 350–353.
- Rude, R.A., Bier, J.W., Jackson, G.J., and McClure, F.D. 1982. Recovery of eggs of two parasitic nematodes, *Ascaris* sp. and *Trichuris* sp.: interlaboratory study. *Journal of Association Of Analytical Chemistry*, **65**, 79–81.
- Rudolfs, W., Falk, L.L., and Ragotzkie, R.A. 1951. Contamination of vegetables grown in polluted soil. III. Field studies on *Ascaris* eggs. *Sewage and Industrial Waste*, **23**, 656–660.
- Rzezutka, A., Nichols, R.A., Connelly, L., Kaupke, A., Kozyra, I., Cook, N., Birrell, S., and Smith, H.V. 2010. *Cryptosporidium* oocysts on fresh produce from areas of high livestock production in Poland. *International Journal of Food Microbiology*, **139**, 96–101. doi: 10.1016/j.ijfoodmicro.2010.01.027.
- Sathyanarayanan, L. and Ortega, Y. 2006. Effects of temperature and different food matrices on *Cyclospora cayetanensis* oocyst sporulation. *Journal of Parasitology*, **92**, 218–222.
- Schantz, P.M. 2002. Overview of global distribution and transmission. In: *Taenia solium Cysticercosis: From Basic to Clinical Science* (eds

- Singh, G. and Prabhakar, S.). CAB International, Wallingford, UK.
- Schantz, P.M., Moore, A.C., Muñoz, J.L., Hartman, B.J., *et al.* 1992 Neurocysticercosis in an Orthodox Jewish community in New York City. *New England Journal of Medicine*, **327**, 692–695.
- Seymour, I.J., Burfoot, D., Smith, R.L., Cox, L.A., and Lockwood, A. 2002. Ultrasound decontamination of minimally processed fruits and vegetables. *International Journal of Food Science Technology*, **37**, 547–557.
- Shahnazi, M. and Jafari-Sabet, M. 2010. Prevalence of parasitic contamination of raw vegetables in villages of Qazvin Province, Iran. *Foodborne Pathogen Diseases*, **7**, 1025–1030.
- Shields, J.M. and Ortega, Y. 2012. *Cyclospora cay- etanensis*, Chapter 3. In: *Foodborne Protozoan Parasites* (eds Robertson, L.J. and Smith, H.V.), pp. 65–103. Nova Biomedical, Hauppauge, NY, USA.
- Shields, J.M., Lee, M.M., and Murphy, H.R. 2012. Use of a common laboratory glassware detergent improves recovery of *Cryptosporidium par- vum* and *Cyclospora cayetanensis* from lettuce, herbs and raspberries. *International Journal of Food Microbiology*, **153**, 123–128.
- Shields, J.M., Joo, J., Kim, R., and Murphy, H.R. 2013. Assessment of three commercial DNA extraction kits and a laboratory-developed method for detecting *Cryptosporidium* and *Cyclospora* in raspberry wash, basil wash and pesto. *Journal of Microbiology Methods*, **92**, 51–58.
- Sivapalasingam, S., Friedman, C.R., Cohen, L., and Tauxe, R.V. 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *Journal of Food Protection*, **67**, 2342–2353.
- Slifko, T.R., Raghubeer, E., and Rose, J.B. 2000. Effect of high hydrostatic pressure on *Cryptosporidium parvum* infectivity. *Journal of Food Protection*, **63**, 1262–1267.
- Sorvillo, F.J., DeGiorgio, C., and Waterman, S.H. 2007. Deaths from cysticercosis, United States. *Emerging Infectious Diseases*, **13**, 230–235.
- Srikanth, R. and Naik, D. 2004. Prevalence of giar- diasis due to wastewater reuse for agriculture in the suburbs of Asmara City, Eritrea. *International Journal of Environmental Health Research*, **14**, 43–52.
- Steele, M., Unger, S., and Odumeru, J. 2003. Sensitivity of PCR detection of *Cyclospora cay- etanensis* in raspberries, basil, and mesclun lettuce. *Journal of Microbiology Methods*, **54**, 277–280.
- Theel, E. and Pritt, B.S. 2012. *Balantidium coli* and *Entamoeba histolytica*, Chapter 1. In: *Foodborne Protozoan Parasites* (eds Robertson, L.J. and Smith, H.V.), pp. 5–31. Nova Biomedical, Hauppauge, NY, USA.
- Turtoi, M. 2013. Ultraviolet light treatment of fresh fruits and vegetables surface: a review. *Journal of Agroalimentary Processes and Technologies*, **19**, 325–337.
- Ueno, H., Arandia, R., Morales, G., and Medina, G. 1975. Fascioliasis of livestock and snail host for *Fasciola* in the Altiplano region of Bolivia. *National Institute of Animal Health Q (Tokyo)*, **15**, 61–67.
- Uga, S., Matsuo, J., Kono, E., Kimura, K., Inoue, M., Rai, S.K., and Ono, K. 2000. Prevalence of *Cryptosporidium parvum* infection and pattern of oocyst shedding in calves in Japan. *Veterinary Parasitology*, **94**, 27–32.
- Uga, S., Hoa, N.T., Noda, S., Moji, K., Cong, L., Aoki, Y., Rai, S.K., and Fujimaki, Y. 2009. Parasite egg contamination of vegetables from a sub- urban market in Hanoi, Vietnam. *Nepal Medical College Journal*, **11**, 75–78.
- Veit, P., Bilger, B., Schad, V., Schäfer, J., Frank, W., Lucius, R. 1995. Influence of environmental factors on the infectivity of *Echinococcus multi- locularis* eggs. *Parasitology*, **110** (Pt. 1), 79–86.
- Vuong, T.A., Nguyen, T.T., Klank, L.T., Phung, D.C., Dalsgaard, A. 2007. Faecal and protozoan parasite contamination of water spinach (*Ipomoea aquatica*) cultivated in urban waste- water in Phnom Penh, Cambodia. *Tropical Medicine and International Health*, **12**, 73–81.
- Wang, R.X., Nian, W.F., Wu, H.Y., Feng, H.Q., *et al.* 2012. Atmospheric-pressure cold plasma

- treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation. *European Physical Journal D*, **66**, 276
- Wharton, D.A. 1979. *Ascaris* sp.: water loss during desiccation of embryonating eggs. *Experimental Parasitology*, **48**, 398–406.
- WHO. 1998. World Health Organization. Food Safety Team, Food and Agriculture Organization of the United Nations. Surface decontamination of fruits and vegetables eaten raw: a review prepared by Larry R. Beuchat. World Health Organization, Geneva. Available at: http://www.who.int/foodsafety/publications/fs_management/en/surface_decon.pdf.
- WHO. 1999. World Health Organization: Joint FAO/IAEA/WHO Study Group on High-Dose Irradiation (Wholesomeness of Food Irradiated with Doses above 10 kGy). (WHO Technical Report Series: 890). ISBN: 92 4 120890 2. Available at: http://www.who.int/foodsafety/publications/fs_management/en/irrad.pdf.
- Yamasaki, H., Allan, J.C., Sato, M.O., Nakao, M., *et al.* 2004. DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *Journal of Clinical Microbiology*, **42**, 548–553.
- Yu, J.R. and Park, W.Y. 2003. The effect of gamma-irradiation on the viability of *Cryptosporidium parvum*. *Journal of Parasitology*, **89**, 639–642.
- Yu, Y., Zhang, H., and Zhu, G. 2010. Plant-type trehalose synthetic pathway in *Cryptosporidium* and some other apicomplexans. *PLoS One*, **5**, e12593.

CHAPTER 16

Impact of food unit operations on probiotic microorganisms

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16.1 Introduction

“Probiotics” (Greek: for life) are defined as “live microorganisms which when administered in adequate amount confer a health benefit on the host” (FAO/WHO, 2002). Probiotic foods contain live microorganisms that are believed to actively enhance health by improving the balance of microflora in the gut. From time immemorial, probiotics have been consumed in food products such as yogurt. Even though there was awareness about the health beneficial effects of these food products, it was only in the 1900s that the scientific community started to investigate further to understand the reasons for those benefits.

Various genera of bacteria (and yeast) such as *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, and *Enterococcus* are commonly used as probiotics. However, *Lb. acidophilus*, *Bifidobacterium* spp., and *Lb. casei* are considered the main species. *Lactobacillus* and *Bifidobacterium* species are found as a part of the gut microflora and are regarded as safe for the production of dairy products.

The health benefits associated with probiotic bacteria are well documented. Some of the most commonly studied and potentially the most health beneficial probiotic bacteria are *Lb. rhamnosus* GG (ATCC 53103), *Lb. acidophilus* NCFB 1478, *Lb. casei* Shirota, *Lb. johnsonii* La1, *B. animalis* Bb12, and *Lb. reuteri*. Further to

their conventional use in yogurt and other fermented food products, these bacteria have been included in drinks and are also made available as supplements in the form of capsules and freeze-dried preparations (Capela *et al.*, 2006). Various products across the world, including yogurt, buttermilk, sour cream, powdered milk, and desserts, are produced by addition of *Bifidobacterium* species and *Lb. acidophilus*. Japan alone produces more than 53 varieties of milk products containing probiotic bacteria. Though the European market is rich in probiotics, it is dominated mainly by yogurt.

16.1.1 Selection criteria for probiotics

The scientific community has provided enough evidence regarding the health benefits conferred by probiotics on human health, by improving the intestinal microflora through defence against the disease-causing pathogens. The most important criteria for classifying microorganisms as probiotics is the ability of the bacteria to survive and be metabolically active in the gastrointestinal tract. The stability of the microorganisms in the gut environment is dependent on the resistance of bacteria to the low pH and the ability to adhere to the epithelial mucosa. It is also critical that the cell viability and the desirable health beneficial characteristics of the probiotic are maintained during the product production and

Table 16.1 Challenges with probiotics and probiotic products.

Physiological trait	Products
Acid tolerance	Yogurt, cheese, spreads, dip
Oxygen tolerance	Yogurt
Heat and cold tolerance	Dried products (spray dried or freeze dried)
Osmotic tolerance	Cheese, chocolate

throughout the shelf life of the product (Gilliland, 2003). The resistance of probiotics to a food processing environment includes its tolerance to acidic environment, oxidative stress, and thermo tolerance. Some of the desired characteristics of probiotic bacteria required in specific food products are listed in Table 16.1. Vasijevic and Shah (2008) have comprehensively reviewed the literature on probiotics, their properties, and health benefits. The processes to classify bacteria as probiotic require exhaustive screening for ensuring that the health benefits are conferred to the host. Since it is technically difficult to test each strain that can colonize the digestive tract, the colonization potential is tested *in vitro* by studying the capacity of the strains to adhere to gastrointestinal mucosa and their capacity to grow in intestinal extracts.

16.2 Probiotic products

Yogurt is one of the most popular dairy products around the world. With more innovations in the product formulations, its popularity has increased in the recent times. Yogurt, traditionally prepared from the milk of cow, buffalo, sheep, and goat, has a semi-solid consistency and is prepared from heat-treated yogurt mix, with the help of a starter culture. The yogurt mix is often fortified with non-fat dry milk or protein concentrate. The most common organisms used as the starter culture for yogurt preparation are *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus*. Often,

other bacteria, such as *Lb. acidophilus*, *Lb. casei*, *Lb. rhamnosus* GG, *Lb. johnsonii* LA1, and *Bifidobacterium* spp., are also found in yogurt. A variety of products are available on the market including plain yogurt, drinking yogurt, dried yogurt, frozen yogurt, fruit flavored and whipped yogurt (Shah, 2003).

The increasing popularity of yogurt is mainly due to: (1) increasing awareness about its health benefits and (2) the innovations and variety in the product production. Improved gastrointestinal health, enhanced immunity of the host, amelioration of the symptoms associated with lactose malabsorption, prevention of cancer, and reduction in diarrhea caused by antibiotic treatment and rotavirus are some of the health benefits conferred to the host upon consumption of yogurt. With the increasing awareness about the health benefits conferred by yogurt consumption, there are increasing innovative products available on the market. Probiotic yogurt has multiple health benefits including antimicrobial properties, improved lactose metabolism, antimutagenic properties, anticarcinogenic properties, reduction in serum cholesterol, antidiarrhoeal properties, immune system stimulation, improvement in inflammatory bowel disease, and suppression of *Helicobacter pylori* infection (Shah, 2006). Probiotic cheese, cheese spreads, and dips are some other well-established dairy probiotic products on the market. Among the other probiotic products, probiotic chocolates, ice creams, milk powder, fermented milk beverages, mayonnaise, and frozen desserts have also gained popularity in recent years.

16.3 Probiotics and environmental stress: cellular mechanisms and resistance

Probiotic bacteria encounter various stresses depending on the product and its processing. Fermented dairy products like yogurt expose the bacteria to oxygen toxicity and acid stress. The probiotic bacteria in cheese undergo osmotic stress owing to varying sodium chloride levels

and stress due to low moisture. Osmotic stress is also encountered due to high sugar in probiotic chocolates. Probiotics present in cheese spreads and dips encounter a survival challenge due to low pH. On the other hand, dried products like milk powder subject the bacteria to extreme temperatures during spray drying and freeze drying. Studies on responses of bacteria to combat stress have provided the opportunity to genetically manipulate the probiotic bacteria and improve their resistance to stress during food processing.

16.3.1 Acid tolerance: fermented products

One of the major challenges associated with the viability of probiotic bacteria is the survival of the organism in the gastrointestinal (GI) environment. A continuous flux exists in the GI environment depending on the food and varies from pH 1.0 to 5.0 (Marteau *et al.*, 1997). Gastric juices, being extremely acidic, pose a challenge for survival of probiotic bacteria in the gut. Bacteria also encounter low pH in fermented probiotic products like yogurt, cheese, spreads, and dips. Acid stress injury is caused by

accumulation of protons within the cell, leading to a reduced intracellular pH and an altered trans-membrane pH and proton motive force, which are essential in regulating transport across membranes. Acid stress damages not only the cell membrane but also affects DNA and proteins within the cell. Although the extent of acid tolerance differs with different species and strains, *Bifidobacterium* in general are extremely sensitive to low pH. Bacteria respond to acid stress by up-regulation of chaperones like GroEL, GroES, DnaK, ClpE, and GrpE (Frees *et al.*, 2003) and production of novel shock proteins (Tables 16.2 and 16.3). Alterations in membrane fatty acids, particularly monounsaturated fatty acids, occur in bacteria as a result of high acid in the growth medium. Cyclopropane fatty acid (CFA) plays a significant role in cell responses to acid stress (Chang and Cronan, 1999; Budin-Verneuil *et al.*, 2005). Reduced survival of bacteria was observed on deletion of CFA synthase activity (Klaenhammer *et al.*, 2005) whereas external addition of CFA in growth medium increased the acid resistance of the bacteria (Chang and Cronan, 1999). Certain

Table 16.2 Studies identifying genes and/or proteins involved in an acid stress response.

Bacteria	Gene and/or protein involved	Reference
<i>Lb. acidophilus</i>	LBA1272 (CFA synthase) LBA1524HK (histidine kinase)	Klaenhammer <i>et al.</i> , 2005 Azcarate-Peril <i>et al.</i> , 2005
<i>Lc. lactis</i>	cfa GroES, GroEL, DnaK mleS, ldh, citE, citF	Budin-Verneuil <i>et al.</i> , 2005 Frees <i>et al.</i> , 2003 Xie <i>et al.</i> , 2004

Table 16.3 Some genes and proteins up-regulated as a response to heat and cold stress.

Bacteria	Stress conditions	Gene and/or protein up-regulated	Reference
<i>Lb. plantarum</i>	37°C, 50 min	hsp18.5, hsp19.3	Spano <i>et al.</i> , 2004, 2005
<i>B. breve</i>	43°C, 150 min	DnaK, GroEL, GroES	Ventura <i>et al.</i> , 2004, 2005a, 2005b
<i>Lb. rhamnosus</i>	50°C, 30 min	clpL1	Suokko <i>et al.</i> , 2005
<i>Lb. paracasei</i>	52°C, 40 min	GroEL	Desmond <i>et al.</i> , 2004
<i>Lb. plantarum</i>	8°C, 3 h	cspL	Derzelle <i>et al.</i> , 2002
<i>Lc. lactis</i>	10°C, 4 h	clpX	Skinner and Trempey, 2001

bacteria also utilize amino acids like glutamate, aspartate, and arginine for survival in acidic conditions, by decarboxylating the amino acid and exporting the alkaline product (Cotter and Hill, 2003). This helps the cell to release the alkaline product out of the cell in the environment and accumulate protons in the cell. Furthermore, studies have also revealed that addition of vitamins and antioxidant-rich extracts offer protection to probiotic bacteria under acidic conditions (Shah *et al.*, 2010). The ability to survive an acidic environment is considered one of the most important characteristics of a probiotic bacteria, since the exposure to gastric acid when ingested is certain and survivability of bacteria in this environment is important for it to be able to impart health benefits to the host.

16.3.2 Oxygen tolerance: fermented products

Probiotic bacteria encounter oxidative stress during fermentation, drying, and storage in various products like probiotic yogurt, kefir, probiotic spread, and non-dairy products like probiotic chocolates and cookies. The high levels of dissolved oxygen in yogurts adversely affect the viability of probiotic bacteria. Oxygen exposure damages the probiotic bacteria and the sensitivity of the probiotic bacteria to oxidative stress is strain-dependent. Probiotics have often been categorized on the basis of their oxygen tolerance from their quantitative index of their oxygen sensitivity (Talwalkar *et al.*, 2001). Oxygen toxicity affects the cells in various ways (Shin and Park, 1997; Ahn *et al.*, 2001; Talwalkar and Kailasapathy, 2003). Bacterial cells elongate, developing a rough surface and extended lag phase. Cellular fatty acid profile changes on exposure to oxygen. Furthermore, changes at the metabolic and biochemical level occur due to oxygen sensitivity of the cells. Decreased lactate production and increased NADH oxidase and NADH peroxidase activities are observed in probiotic bacteria subjected to oxygen stress. These enzymes have a significant role in oxygen tolerance of certain strains of *Lb. acidophilus* and

Bifidobacterium spp. and strains with high aerotolerance have shown high activities of these enzymes. Also, certain probiotics such as *Lb. acidophilus* and *Bifidobacterium* spp. possess reducing activity and the ability to scavenge reactive oxygen species and to chelate metal ions (Archibald and Fridovich, 1981; Kot *et al.*, 1994; Lin and Yen, 1999; Kim *et al.*, 2001). Stress proteins released by the bacteria when subjected to oxidative stress have been studied in *E. coli* (Greenberg and Dimple, 1989) and up to 80 stress proteins were induced including heat shock proteins, superoxide dismutase, and a DNA repair protein and up-regulation of heat shock genes up to more than four times was observed.

Exposing the probiotic bacteria to gradually increasing levels of stress can lead to an adaptive response of the cell. Oxidative stress has also been shown to induce an adaptive response in the bacteria. An oxygen-tolerant mutant of *B. longum* was developed in culture broth and in yogurt as well. In a study by Talwalkar and Kailasapathy (2004), oxygen tolerance of several strains of *Lb. acidophilus* and *Bifidobacterium* spp. was developed by exposure to varying levels of dissolved oxygen in yogurt. Results demonstrated successful adaptation of cells to resist high levels of oxygen after storage. Adaptation to oxidative stress thus possesses the potential to develop and use oxygen-tolerant probiotic bacteria in a food matrix.

16.3.3 Heat and cold tolerance: dried products

Probiotic bacteria experience extremes of temperature during production of dried probiotic products involving spray drying or freeze-drying. Heat stress leads to cell death by damaging the cell membrane of the bacteria. Membrane fatty acids are the primary targets, followed by aggregation of cellular proteins and damage of ribosomes and RNA (Somero, 1995; Teixeira *et al.*, 1997). Heat tolerance varies between strains and species, but in general the common probiotics, *Lactobacillus* and *Bifidobacterium*, are sensitive to temperatures of more than 50 °C

(Gardiner *et al.*, 2000; Simpson *et al.*, 2005). The most common cellular stress response is uptake and accumulation of metabolites, particularly glycine and betaine. However, when subjected to heat stress, genes involved in the uptake of glycine and betaine are also affected. Genes related to nucleotide salvage and methionine biosynthesis pathways are affected and increased expression of glycolytic enzymes is observed in cells exposed to heat stress. Synthesis of heat shock proteins is also one of the cell's defense mechanisms. Two-dimensional gel electrophoresis and microarray analysis have revealed that heat shock proteins form a significant part of the heat shock responses of lactic acid bacteria. The key heat shock proteins are chaperones like GroES/ GroEL, DnaK, and DnaJ and proteases including HtrA, FtsH, and Clp. The significance of heat shock proteins as a defense mechanism for survival of bacteria has been demonstrated by knockout, complementation, and overexpression studies. Heat shock proteins form oligomeric structures of up to 24 subunits and high temperature leads to altered surface hydrophobicity of the chaperone structure (Shearstone and Baneyx, 1999; Lentze *et al.*, 2003). Increased expression of these smaller heat shock proteins is observed on an increase in environmental heat stress. Expression of small heat shock proteins increased to a 300-fold increase in *E. coli* was observed when subjected to 50°C for 7 minutes (Richmond *et al.*, 1999). Heat shock proteins also contribute to maintaining thermal stability in lactic acid bacteria (El Demerdash *et al.*, 2003).

Cold stress is also encountered by probiotic bacteria during processing and storage of products at low temperatures. A reduction in temperature reduces the membrane fluidity of the cell. DNA supercoiling and the rate of strand breakage are affected by low temperature, thereby causing an alteration in the process of replication, transcription, and translation. Significant effects on the bacteria's enzyme activity, ribosomal functions, and protein folding are observed when subjected to cold stress (Graumann and Marahiel, 1999; Bayles

et al., 2000; van de Guchte *et al.*, 2002). Increased sensitivity to bile stress and salt stress are also related to cold stress following membrane damage by low temperatures. Freezing of water around the cell leads to concentration of solutes and gases in the matrix, which in turn exerts osmotic pressure on the cells (Fernandez Murga *et al.*, 1998; Murga *et al.*, 2001). In order to maintain the membrane fluidity at low temperatures, alterations in membrane fatty acids are observed, mainly the unsaturated fatty acids. In response to the cold stress, cold shock proteins are produced in the cell that target mRNA stabilization (Phadtare and Inouye, 2001). Cold shock proteins are expressed in most of the lactic acid bacteria and are proteins mainly comprising of aromatic and basic amino acids at the surface binding to nucleic acids (Schindelin *et al.*, 1993). Cold tolerance, like heat, is also strain and species dependent and must be observed for the bacteria being used before its application to the product involving low-temperature storage.

16.3.4 Osmotic tolerance: cheese, probiotic chocolates

Probiotic bacteria experience varying levels of osmotic stress during production and storage of products. Products like cheese expose the bacteria to a high sodium chloride stress whereas a high sugar stress is encountered in products like probiotic chocolates. Cell death due to high osmotic stress is caused by loss of water and excess accumulation of osmolytes in the cell. Water activity (a_w) plays a significant role in survival of bacteria in food products. For instance, the water activity of milk (a_w 0.97) is higher than brined cheeses (a_w 0.85–0.95), thus making the bacteria easier to grow and survive in milk. Furthermore, food products like chocolate (a_w 0.4–0.5), biscuits (a_w 0.20), and powdered milk (a_w 0.11) have even lower water activity, subjecting the bacteria to enhanced stress. Cells respond to reduced water activity in the product by osmoregulation, which results in increased internal osmotic pressure and restores turgor pressure. In particular, the sodium and

potassium ions interfere with the cell transport system and maintenance of turgor pressure inside the cell is a challenge for the bacteria. Transport of osmolytes plays an important role in osmotic tolerance of the cell. Some other bacteria have genes that can synthesize glycine and betaine in the cell (Kapfhammer *et al.*, 2005) and help in the protective mechanism under osmotic stress. Protective effects of disaccharides like trehalose on the bacteria when subjected to osmotic stress have been demonstrated in *Lb. acidophilus*. The presence of di- and tri-peptides has shown to increase the growth rate of the *Lb. casei* up to threefold in the presence of sodium chloride. Increased activities of cell envelope proteinase PrtP and peptidase PepX have also been observed in the presence of NaCl (Piuri *et al.*, 2003). Furthermore, addition of oxygen scavengers like ascorbic acid has a protective effect on probiotics in yogurt during storage (Dave and Shah, 1997). Various techniques have been applied to monitor the effect of osmotic stress on probiotic bacteria. Fourier transform infrared (FTIR) spectroscopy was used to study the effect of salt stress on probiotic bacteria in a recent study. The spectral changes observed by FTIR reveal the changes in the membrane structure of the probiotics as a result of exposure to sodium chloride (Gandhi and Shah, 2014). Advancements in the application of FTIR to determine the effects of osmotic stress to the bacteria have helped in determining a critical sodium chloride concentration in products (Gandhi *et al.*, 2014). Flow cytometric analysis of bacteria subjected to osmotic stress has enabled a study of the degree of injury sustained by the cell membrane. This has an added advantage over the traditional plate count method, where the partly damaged cells may recover when provided with a fresh medium (agar plates) to grow. Two-dimensional gel electrophoresis has revealed increased expression of GroEL and DnaK in *Lc. lactis* (Kilstrup *et al.*, 1997) and *Lb. rhamnosus* (Prasad *et al.*, 2003) following osmotic stress. Enhancing the resistance of bacteria to osmotic stress still remains a challenge for the food industry.

However, with the application of new technologies and advances in research, the future looks promising.

16.4 Enhancing stress resistance of probiotics

Pre-adaptation of bacteria to various environmental stress conditions has been employed as a technique to enhance the stress resistance of the bacteria. Environmental adaptation of bacteria to adverse conditions increases the stress resistance of bacteria (Desmond *et al.*, 2001). Genetic engineering has been conventionally used to enhance the stress resistance of an organism. Genomic and proteomic studies have identified specific mechanisms involved in cellular responses to stress. The knowledge of these mechanisms has been used to enhance the stress tolerance of probiotic bacteria. Since genetic engineering is specific to bacteria and the stress it is subjected to, more widely applicable fast and effective methods, like microencapsulation, incorporation of cryoprotectants, and prebiotics, have been applied to enhance the resistance of bacteria (Capela *et al.*, 2006). Microencapsulation, a generic technique, has been used to protect the probiotic bacteria from environmental stresses.

16.4.1 Microencapsulation

Microencapsulation is defined as the technology for packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Shahidi and Han, 1993). A microcapsule is a semipermeable thin and strong membrane that offers protection to the contents, in this case probiotic bacteria, from environmental conditions that pose a threat to their viability. Microencapsulation of bacteria not only enhances the viability but also facilitates cell handling and enables a controlled dosage of cells. The protective effects of microencapsulation on probiotic bacteria during

various processing and storage conditions have been reviewed by Dianawati *et al.* (2016).

For microencapsulation of probiotics several coating materials have been used, including polysaccharides from seaweeds, plants, and bacteria, and animal proteins, like alginate, starch and its derivatives, chitosan, gum Arabic, gellan, xanthan, milk, gelatin, etc. A comparative study on the protective effects of various encapsulating materials on probiotic bacteria has been carried out by Ding and Shah (2009). Physical entrapment of the cell in the encapsulating matrix prevents direct exposure of the cell to the environment. However, being semi-permeable in nature, it allows the inflow of nutrients, thereby ensuring the viability of the microencapsulated bacteria. There are different microencapsulation techniques like emulsion, extrusion, adhesion, and spray drying. The major difference between these techniques is in the size of the microencapsulated bacteria. The emulsion method using alginate beads results in the formation of beads of 20 μm to 2 mm, whereas by extrusion the bead size is around

2 to 4 mm. The approximate size of the starch granules that are the base for adhesion of probiotic bacteria has been found to be 50 μm . The size of the microcapsule using spray drying produces capsules of 5 to 80 μm . For microencapsulation, bacteria are cultured in their optimum growth media and conditions and then freeze dried before encapsulating. The size of the microcapsule plays a very significant role in the sensory properties of the food product. Microencapsulated probiotic bacteria have been incorporated in a variety of food matrices owing to the protective effects of microencapsulation (Rokka and Rantamaki, 2010). Further, microencapsulated probiotics have also been used in cereal-based products, sausages, and juices. Some of the products containing encapsulated probiotics are listed in Table 16.4.

A major challenge in the use of microencapsulated probiotic bacteria is their survival during the process of microencapsulation and stability in the food matrix, while maintaining their probiotic properties. The process of microencapsulation subjects the bacteria to

Table 16.4 Products containing microencapsulated probiotic bacteria.

Product	Coating material	Bacteria	References
Cheese	Alginate and hi-maize starch	<i>B. infantis</i> , <i>B. lactis</i> , <i>Lb. acidophilus</i>	Godward and Kailasapathy, 2003; Kailasapathy and Masondole, 2005
Yogurt	Alginate, whey, k-carrageenan	<i>B. longum</i> , <i>B. breve</i> , <i>Lb. acidophilus</i> , <i>Lb. casei</i> , <i>Lb. rhamnosus</i>	Adhikari <i>et al.</i> , 2000; Picot and Lacroix, 2004; Capela <i>et al.</i> , 2006
Milk	Alginate and chitosan	<i>Lb. bulgaricus</i>	Lee <i>et al.</i> , 2004
Ice cream	Alginate and hi-maize starch	<i>Lb. casei</i> and <i>B. lactis</i>	Homayouni <i>et al.</i> , 2008
Mayonnaise	Alginate	<i>B. bifidum</i>	Khalil and Mansour, 1998
Cereals	Wheat dextrin and polydextrose	Bifidobacterium spp., <i>Lb. rhamnosus</i>	O'Riordan <i>et al.</i> , 2001; Saarela <i>et al.</i> , 2006
Sausage	Alginate	<i>Lb. reuteri</i> , <i>B. longum</i>	Muthukumarasamy and Holley, 2007
Juice	Alginate	<i>Lb. rhamnosus</i> , <i>B. longum</i> , <i>Lb. salivarius</i> , <i>Lb. plantarum</i> , <i>Lb. acidophilus</i> , <i>Lb. paracasei</i> , <i>B. lactis</i> type Bi-04, <i>B. lactis</i> type Bi-07	Ding and Shah, 2008

extreme temperatures and osmotic stress. The coating material plays a protective role for the bacteria and thus should be carefully selected. Survivability of bacteria during microencapsulation is better for cells grown in fresh culture as compared to freeze-dried cultures. The protective effect of calcium alginate and mannitol has been observed in *Bifidobacterium* during freeze drying (Dianawati *et al.*, 2012). Also, incorporation of Hi-maize starch during encapsulation with alginate results in an increased number of live encapsulated bacteria (Sultana *et al.*, 2000). Stability of microencapsulated probiotics has been verified in various food matrices. Microencapsulated *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *Lb. salivarius*, *Lb. plantarum*, *Lb. acidophilus*, and *Lb. paracasei* showed prolonged survival in fruit juices as compared to free bacteria (Ding and Shah, 2008). Encapsulation has also improved viability of probiotic bacteria in ice creams and mayonnaise (Khalil and Mansour, 1998; Homayouni *et al.*, 2008). Encapsulation with wheat dextrin and polydextrose improved the survival of *Lb. rhamnosus* in chocolate-coated breakfast cereals (Saarela *et al.*, 2006). Microencapsulation of *B. longum* enhanced its survival in fermented dry sausage, but also reduced its ability to inhibit *E. coli* O157:H7 (Muthukumarasamy and Holley, 2007). Improved survival of microencapsulated probiotic bacteria in fermented frozen dairy desserts has also been shown (Shah and Ravula, 2000).

Another major challenge associated with the use of microencapsulated probiotics in food is the effect on its sensory attributes. The bead size of the microencapsulated probiotic influences the texture of the product. A particle size less than 10 μm has been shown to have no effect on the mouth-feel properties of most of the food products. The size of the bead is also influenced by the conditions during spray drying and can be regulated to a certain extent. Furthermore, the shape of the microcapsule also contributes to the flow properties, which is important for industrial processes. Starch and sodium alginate used for encapsulation of probiotics have been shown to alter the mouth-feel of the product. The use of

exopolysaccharides from probiotics and starch as a filler polymer has been shown to enhance the stability of yogurt gel (Kailasapathy, 2006) and increased water retention in feta cheese (Kailasapathy and Masondole, 2005). The material for coating, the technique used for microencapsulation, and the product being produced, all contribute to the sensory quality of the product and need to be optimized to obtain the desired sensory properties of the product. This becomes a major challenge for the food industry, particularly with respect to the technique and cost when developing products on an industrial scale. Research on microencapsulation has taken a great leap forward in recent years, yet there is need for more research to be able to better develop products containing microencapsulated probiotic bacteria.

16.5 Conclusion

Probiotics have gained popularity due to their ability to promote gut health. However, the viability of probiotics is of the utmost importance for them to be able to confer health benefits upon ingestion. In this regard, resistance of probiotic bacteria to various environmental factors is crucial to monitor and to enhance the stress tolerance of probiotic bacteria. There have been novel approaches to maintain the viability of probiotics during processing and storage of food products. Genetic manipulation, microencapsulation, and developing adaptive stress tolerance have been used to protect the probiotic bacteria from environmental stress. Innovation in the use of these techniques may contribute to increase the efficacy and viability of probiotics in the food matrix and also in the gut environment.

References

- Adhikari, K., Mustapha, A., Grun, I.U., and Fernando, L. 2000. Viability of microencapsulated bifidobacteria in set yogurt during refrigerated storage. *Journal of Dairy Science*, **83**, 1946–1951.

- Ahn, J.B., Hwang, H.J., and Park, J.H. 2001. Physiological responses of oxygen-tolerant anaerobic *Bifidobacterium longum* under oxygen. *Journal of Microbiology and Biotechnology*, **11**, 443–451.
- Archibald, F.S. and Fridovich, I. 1981. Manganese, superoxide dismutase, and oxygen tolerance in some lactic acid bacteria. *Journal of Bacteriology*, **146**, 928–936.
- Azcarate-Peril, M.A., McAuliffe, O., Altermann, E., Lick, S., Russell, W.M., and Klaenhammer, T.R. 2005. Microarray analysis of a two-component regulatory system involved in acid resistance and proteolytic activity in *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, **71**, 5794–5804.
- Bayles, D.O., Tunick, M.H., Foglia, T.A., and Miller, A.J. 2000. Cold shock and its effect on ribosomes and thermal tolerance in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, **66**, 4351–4355.
- Budin-Verneuil, A., Pichereau, V., Auffray, Y., Ehrlich, D.S., and Maguin, E. 2005. Proteomic characterization of the acid tolerance response in *Lactococcus lactis* MG1363. *Proteomics*, **5**, 4794–4807.
- Capela, P., Hay, T.K.C., and Shah, N.P. 2006. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International*, **39**, 203–211.
- Chang, Y.Y. and Cronan, J.E. 1999. Membrane cyclopropane fatty acid content is a major factor in acid resistance of *Escherichia coli*. *Molecular Microbiology*, **33**, 249–259.
- Cotter, P.D. and Hill, C. 2003. Surviving the acid test: responses of Gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, **67**, 429–453.
- Dave, R.I. and Shah, N.P. 1997. Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. *International Dairy Journal*, **7**, 435–443.
- Derzelle, S., Hallet, B., Ferain, T., Delcour, J., and Hols, P. 2002. Cold shock induction of the cspL gene in *Lactobacillus plantarum* involves transcriptional regulation. *Journal of Bacteriology*, **184**, 5518–5523.
- Desmond, C., Stanton, C., Fitzgerald, G.F., Collins, K., and Ross, R.P. 2001. Environmental adaptation of probiotic lactobacilli towards improvement of performance during spray drying. *International Dairy Journal*, **11**, 801–808.
- Desmond, C., Fitzgerald, G.F., Stanton, C., and Ross, R.P. 2004. Improved stress tolerance of GroESL-overproducing *Lactococcus lactis* and probiotic *Lactobacillus paracasei* NFBC 338. *Applied and Environmental Microbiology*, **70**, 5929–5936.
- Dianawati, D., Mishra, V., and Shah, N.P. 2012. Role of calcium alginate and mannitol in protecting *Bifidobacterium*. *Applied and Environmental Microbiology*, **78**, 6914–6921.
- Dianawati, D., Mishra, V., and Shah, N.P. 2016. Survival of microencapsulated probiotic bacteria after processing and during storage: a review. *Critical Reviews in Food Science and Nutrition*. doi: 10.1080/10408398.2013.798779.
- Ding, W. and Shah, N.P. 2008. Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *International Food Research Journal*, **15**, 219–232.
- Ding, W. and Shah, N. 2009. Effect of various encapsulating materials on the stability of probiotic bacteria. *Journal of Food Science*, **74**, M100–M107.
- El Demerdash, H.A., Heller, K.J., and Geis, A. 2003. Application of the shsp gene, encoding a small heat shock protein, as a food-grade selection marker for lactic acid bacteria. *Applied and Environmental Microbiology*, **69**, 4408–4412.
- FAO/WHO. 2002. Guidelines for the evaluation of probiotics in food. *Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food*.
- Fernandez Murga, M.a.L., De Ruiz Holgado, A.d.P., and De Valdez, G.F. 1998. Survival rate and enzyme activities of *Lactobacillus acidophilus* following frozen storage. *Cryobiology*, **36**, 315–319.
- Frees, D., Vogensen, F.K., and Ingmer, H. 2003. Identification of proteins induced at low pH in *Lactococcus lactis*. *International Journal of Food Microbiology*, **87**, 293–300.
- Gandhi, A. and Shah, N.P. 2014. Effects of salt concentration and pH on structural and

- functional properties of *Lactobacillus acidophilus*: FT-IR spectroscopic analysis. *International Journal of Food Microbiology*, **173**, 41–47.
- Gandhi, A., Cui, Y., Zhou, M., and Shah, N.P. 2014. Effect of KCl substitution on bacterial viability of *Escherichia coli* (ATCC 25922) and selected probiotics. *Journal of Dairy Science*, **97**, 5939–5951.
- Gardiner, G.E., O'Sullivan, E., Kelly, J., Auty, M.A., Fitzgerald, G.F., Collins, J.K., Ross, R.P., and Stanton, C. 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. *Applied and Environmental Microbiology*, **66**, 2605–2612.
- Gilliland, S. 2003. Probiotics. In: *Encyclopedia of Food Sciences and Nutrition*, vol. 8. Academic Press, London.
- Godward, G. and Kailasapathy, K. 2003. Viability and survival of free and encapsulated probiotic bacteria in Cheddar cheese. *Milchwissenschaft-Milk Science International*, **58**, 624–627.
- Graumann, P.L. and Marahiel, M.A. 1999. Cold shock response in *Bacillus subtilis*. *Journal of Molecular Microbiology and Biotechnology*, **1**, 203–209.
- Greenberg, J.T. and Demple, B. 1989. A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. *Journal of Bacteriology*, **171**, 3933–3939.
- Homayouni, A., Azizi, A., Ehsani, M.R., Yarmand, M.S., and Razavi, S.H. 2008. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry*, **111**, 50–55.
- Kailasapathy, K. 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT – Food Science and Technology*, **39**, 1221–1227.
- Kailasapathy, K. and Masondole, L. 2005. Survival of free and microencapsulated *Lactobacillus acidophilus* and *Bifidobacterium lactis* and their effect on texture of feta cheese. *Australian Journal of Dairy Technology*, **60**, 252–258.
- Kapfhammer, D., Karatan, E., Pflughoeft, K.J., and Watnick, P.I. 2005. Role for glycine betaine transport in *Vibrio cholerae* osmoadaptation and biofilm formation within microbial communities. *Applied and Environmental Microbiology*, **71**, 3840–3847.
- Khalil, A.H. and Mansour, E.H. 1998. Alginate encapsulated bifidobacteria survival in mayonnaise. *Journal of Food Science*, **63**, 702–705.
- Kilstrup, M., Jacobsen, S., Hammer, K., and Vogensen, F.K. 1997. Induction of heat shock proteins DnaK, GroEL, and GroES by salt stress in *Lactococcus lactis*. *Applied and Environmental Microbiology*, **63**, 1826–1837.
- Kim, W.S., Perl, L., Park, J.H., Tandianus, J.E., and Dunn, N.W. 2001. Assessment of stress response of the probiotic *Lactobacillus acidophilus*. *Current Microbiology*, **43**, 346–350.
- Klaenhammer, T.R., Barrangou, R., Buck, B.L., Azcarate-Peril, M.A., and Altermann, E. 2005. Genomic features of lactic acid bacteria effecting bioprocessing and health. *FEMS Microbiology Reviews*, **29**, 393–409.
- Kot, E., Haloftis, G., and Bezkorovainy, A. 1994. Iron accumulation by *Bifidobacteria* at low Po₂ and in air – action of putative ferroxidase. *Journal of Agricultural and Food Chemistry*, **42**, 685–688.
- Lee, J.S., Cha, D.S., and Park, H.J. 2004. Survival of freeze-dried *Lactobacillus bulgaricus* KFERI 673 in chitosan-coated calcium alginate microparticles. *Journal of Agricultural and Food Chemistry*, **52**, 7300–7305.
- Lentze, N., Studer, S., and Narberhaus, F. 2003. Structural and functional defects caused by point mutations in the alpha-crystallin domain of a bacterial alpha-heat shock protein. *Journal of Molecular Biology*, **328**, 927–937.
- Lin, M.Y. and Yen, C.L. 1999. Antioxidative ability of lactic acid bacteria. *Journal of Agricultural and Food Chemistry*, **47**, 1460–1466.
- Marteau, P., Minekus, M., Havenaar, R., and Huis in't Veld, J.H. 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. *Journal of Dairy Science*, **80**, 1031–1037.
- Murga, M.a.L.F., de Valdez, G.F., and Disalvo, E.A. 2001. Effect of lipid composition on the stability of cellular membranes during freeze–thawing of

- Lactobacillus acidophilus* grown at different temperatures. *Archives of Biochemistry and Biophysics*, **388**, 179–184.
- Muthukumarasamy, P. and Holley, R.A. 2007. Survival of *Escherichia coli* O157:H7 in dry fermented sausages containing micro-encapsulated probiotic lactic acid bacteria. *Food Microbiology*, **24**, 82–88.
- O’Riordan, K., Andrews, D., Buckle, K., and Conway, P. 2001. Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology*, **91**, 1059–1066.
- Phadtare, S. and Inouye, M. 2001. Role of CspC and CspE in regulation of expression of RpoS and UspA, the stress response proteins in *Escherichia coli*. *Journal of Bacteriology*, **183**, 1205–1214.
- Picot, A. and Lacroix, C. 2004. Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal*, **14**, 505–515.
- Piuri, M., Sanchez-Rivas, C., and Ruzal, S.M. 2003. Adaptation to high salt in *Lactobacillus*: role of peptides and proteolytic enzymes. *Journal of Applied Microbiology*, **95**, 372–379.
- Prasad, J., McJarrow, P., and Gopal, P. 2003. Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. *Applied and Environmental Microbiology*, **69**, 917–925.
- Richmond, C.S., Glasner, J.D., Mau, R., Jin, H., and Blattner, F.R. 1999. Genome-wide expression profiling in *Escherichia coli* K-12. *Nucleic Acids Research*, **27**, 3821–3835.
- Rokka, S. and Rantamaki, P. 2010. Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research and Technology*, **231**, 1–12.
- Saarela, M., Virkajarvi, I., Nohynek, L., Vaari, A., and Matto, J. 2006. Fibres as carriers for *Lactobacillus rhamnosus* during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. *International Journal of Food Microbiology*, **112**, 171–178.
- Schindelin, H., Marahiel, M.A., and Heinemann, U. 1993. Universal nucleic acid-binding domain revealed by crystal-structure of the *Bacillus subtilis* major cold-shock protein. *Nature*, **364**, 164–168.
- Shah, N. 2003. Yogurt: the product and its manufacture. *Encyclopedia of Food Science and Nutrition*, **10**, 6252–6260.
- Shah, N.P. 2006. Health benefits of yogurt and fermented milks. In: *Manufacturing Yogurt and Fermented Milks* (eds Chandan, R.C., White, C., Kilara, A., and Hui, H.), pp. 327–340. Blackwell Publishing Professional, Iowa, USA.
- Shah, N.P. and Ravula, R.R. 2000. Micro-encapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. *Australian Journal of Dairy Technology*, **55**, 139–144.
- Shah, N.P., Ding, W.K., Fallourd, M.J., and Leyer, G. 2010. Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants. *Journal of Food Science*, **75**, M278–M282.
- Shahidi, F. and Han, X.Q. 1993. Encapsulation of food ingredients. *Critical Reviews in Food Science and Nutrition*, **33**, 501–547.
- Shearstone, J.R. and Baneyx, F. 1999. Biochemical characterization of the small heat shock protein IbpB from *Escherichia coli*. *Journal of Biology and Chemistry*, **274**, 9937–9945.
- Shin, S.Y. and Park, J.H. 1997. Activities of oxidative enzymes related with oxygen tolerance in *Bifidobacterium* sp. *Journal of Microbiology and Biotechnology*, **7**, 356–359.
- Simpson, P.J., Stanton, C., Fitzgerald, G.F., and Ross, R. P. 2005. Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. *Journal of Applied Microbiology*, **99**, 493–501.
- Skinner, M.M. and Trempey, J.E. 2001. Expression of clpX, an ATPase subunit of the Clp protease, is heat and cold shock inducible in *Lactococcus lactis*. *Journal of Dairy Science*, **84**, 1783–1785.
- Somero, G.N. 1995. Proteins and temperature. *Annual Review of Physiology*, **57**, 43–68.
- Spano, G., Capozzi, V., Vernile, A., and Massa, S. 2004. Cloning, molecular characterization and

- expression analysis of two small heat shock genes isolated from wine *Lactobacillus plantarum*. *Journal of Applied Microbiology*, **97**, 774–782.
- Spano, G., Beneduce, L., Perrotta, C., and Massa, S. 2005. Cloning and characterization of the hsp 18.55 gene, a new member of the small heat shock gene family isolated from wine *Lactobacillus plantarum*. *Research in Microbiology*, **156**, 219–224.
- Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P., and Kailasapathy, K. 2000. Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology*, **62**, 47–55.
- Suokko, A., Savijoki, K., Malinen, E., Palva, A., and Varmanen, P. 2005. Characterization of a mobile clpL gene from *Lactobacillus rhamnosus*. *Applied and Environmental Microbiology*, **71**, 2061–2069.
- Talwalkar, A. and Kailasapathy, K. 2003. Metabolic and biochemical responses of probiotic bacteria to oxygen. *Journal of Dairy Science*, **86**, 2537–2546.
- Talwalkar, A. and Kailasapathy, K. 2004. Oxidative stress adaptation of probiotic bacteria. *Milchwissenschaft*, **59**, 140–143.
- Talwalkar, A., Kailasapathy, K., Peiris, P., and Arumugaswamy, R. 2001. Application of RBGR – a simple way for screening of oxygen tolerance in probiotic bacteria. *International Journal of Food Microbiology*, **71**, 245–248.
- Teixeira, P., Castro, H., Mohacsi-Farkas, C., and Kirby, R. 1997. Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress. *Journal of Applied Microbiology*, **83**, 219–226.
- van de Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S.D., and Maguin, E. 2002. Stress responses in lactic acid bacteria. *Antonie Van Leeuwenhoek*, **82**, 187–216.
- Vasijevec, T. and Shah, N.P. 2008. Probiotics – from Metchnikoff to bioactives. *International Dairy Journal*, **18**, 714–728.
- Ventura, M., Canchaya, C., Zink, R., Fitzgerald, G.F., and van Sinderen, D. 2004. Characterization of the groEL and groES loci in *Bifidobacterium breve* UCC 2003: genetic, transcriptional, and phylogenetic analyses. *Applied and Environmental Microbiology*, **70**, 6197–6209.
- Ventura, M., Fitzgerald, G.F., and van Sinderen, D. 2005a. Genetic and transcriptional organization of the clpC locus in *Bifidobacterium breve* UCC 2003. *Applied and Environmental Microbiology*, **71**, 6282–6291.
- Ventura, M., Zink, R., Fitzgerald, G.F., and van Sinderen, D. 2005b. Gene structure and transcriptional organization of the dnaK operon of *Bifidobacterium breve* UCC 2003 and application of the operon in bifidobacterial tracing. *Applied and Environmental Microbiology*, **71**, 487–500.
- Xie, Y., Chou, L.S., Cutler, A., and Weimer, B. 2004. DNA macroarray profiling of *Lactococcus lactis* subsp. *lactis* IL1403 gene expression during environmental stresses. *Applied and Environmental Microbiology*, **70**, 6738–6747.

PART III

Microbial ecology of food products

CHAPTER 17

Microbial ecology of fresh vegetables

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The total consumption of fresh and processed vegetables has exceeded 123 kg per capita in the United States and 118 kg per capita in the European Union (<http://www.helgilibrary.com/indicators/index/vegetable-consumption-per-capita>) in 2009. Changes in lifestyles and consumption trends have prompted the sustained growth of fresh-cut or minimally processed vegetables that are fresh and ready-to-eat (RTE). In 2011, for example, total US fresh-cut produce sales through both food service and retail channels were estimated to surpass \$27 billion (Cook *et al.*, 2012).

1442 illnesses and two deaths (Mody *et al.*, 2011). In addition, contaminated pre-packaged baby spinach caused a devastating *E. coli* O157:H7 outbreak in 2006 in the US, involving 199 cases and three deaths (CDC, 2006). In the wake of these food-borne illness outbreaks, research has begun in earnest to define the complex but critical biological interactions among indigenous microorganisms, human pathogens, and fresh produce. In this chapter, an in-depth review of the microbial ecology of fresh and fresh-cut vegetables and their relationship to the major food-borne bacterial pathogens is presented.

17.1 Introduction

The incidence of food-borne illness outbreaks caused by contaminated fresh and fresh-cut vegetables has increased globally in recent years. In the US, an estimated 34% of all food-borne illnesses that led to hospitalization or death, from 1998 to 2008, were attributable to vegetables (Painter *et al.*, 2013). Among these, contaminated leafy vegetables were the leading causes (22%), followed by vine-stalk (7.9%), and other commodities. The pathogens most frequently linked to vegetable-related outbreaks include bacteria (*Salmonella*, *Escherichia coli*) and viruses (norovirus, hepatitis A) (Painter *et al.*, 2013). As an example, hot peppers were the source for a high profile salmonellosis outbreak in the US and Canada in 2008, leading to

17.2 Prevalence and diversity of microbial communities on fresh vegetables (post-harvest)

Vegetables are known to harbor a diverse and complex array of bacterial communities. Coliforms and fecal coliforms, like generic *E. coli*, are considered indicators of fecal contamination and their presence on food suggests that sanitary quality might be compromised. In 2009, The Consumer Union tested 16 different brands of salad greens ($n = 208$ bags) collected in the New York City Metro area and found about a third of the bags tested had more than 10,000 cfu/g of total coliforms and approximately 5% of the bags contained generic *E. coli* (Consumer Union, 2010). A Canadian-based group conducted a similar retail-level study

across five provinces involving imported herbs and pre-packaged leafy greens and spinach from five countries. The highest prevalence of coliforms were found in imported US leafy greens (mean of 21 samples (66.7% of total tested) – 3.3 log₁₀ cfu/g) followed by US herbs (mean of 12 samples (7% of total tested) – 2.6 log₁₀ cfu/g) (Allen *et al.*, 2013). Although not currently used in the US, hygienic standards for minimally processed vegetables exist in other countries. For example, the European Union guidelines are 100 cfu/g generic *E. coli* in RTE pre-cut fruits and vegetables. A recent field survey of both organically and conventionally grown lettuce grown in Spain found that 20% of samples exceeded this level (i.e., 35% of samples collected tested positive) (Oliveira *et al.*, 2010).

Pathogenic bacteria (e.g., *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*) can also be part of larger microbial communities on fresh produce (Shi *et al.*, 2009; Teplitski *et al.*, 2011). Field surveys on contamination of produce with pathogenic bacteria such as *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* found drastically different results depending on the methods, location, seasons, produce type, and criteria of the survey conducted. Some revealed little or no occurrence of contamination with these pathogenic bacteria (Consumer Union, 2010; Koseki *et al.*, 2011; Sant'Ana *et al.*, 2011; Allen *et al.*, 2013; Althaus *et al.*, 2012), while others showed widespread contamination. Salleh *et al.* (2003) examined 112 samples from four local salad vegetables in Selangor, Malaysia, and found 40 (35%) were contaminated with *Salmonella* with a total of 31 different *Salmonella* serovars being isolated from this study. In Spain, *Salmonella* spp. were detected in 0.7% of lettuce samples ($n=137$) (Sospedra *et al.*, 2013). In 2014, Wijnands *et al.* (2014) conducted a survey to estimate pathogen prevalence and contamination levels of raw produce as well as resulting minimally processed packaged salad as sold in the Netherlands. The overall prevalence for *L. monocytogenes*, *E. coli* O157, and *Salmonella* was 0.11, 0.11, and 0.38%, respectively, across 1800 samples of produce and 1900 samples of RTE mixed salads investi-

gated. Prevalence point estimates for *Salmonella* in specific produce ranged from 0.53% in iceberg lettuce to 5.1% in cucumber. In the Philippines (Vital *et al.*, 2014), 24.7% of retail fresh produce samples, including bell pepper, cabbage, carrot, lettuce, and tomato, were positive for *Salmonella* spp. It is noteworthy that all of these studies indicated that a wide range of vegetables can be contaminated by pathogenic bacteria and are potentially capable of serving as vehicles for human infection. However, manifestation of these capabilities can be greatly influenced by intrinsic and extrinsic ecological factors naturally present in produce or imposed at one or more points during pre-harvest and post-harvest process lines.

17.3 Post-harvest persistence, colonization, and survival on fresh vegetables

Numerous studies have examined the growth or die-off of pathogens under variable temperature conditions experienced during the processing, storage, and shipping of fresh-cut or minimally processed vegetables. Hard conclusions of growth rates and maximum population densities based upon these individual investigations are difficult due to variances in experimental design, test strains (including the use of stressed versus non-stressed bacterial cells), produce type, and distinct packaging materials. It is an accepted fact that native bacterial populations, including any pathogens that might be present, are in constant flux during post-harvest handling and storage. Moreover, variables such as storage temperature and time greatly impact microbial levels and product quality. The US Food and Drug Administration Food Code requires that packaged ready-to-eat fruits and leafy green vegetables be refrigerated at less than or equal to 5°C to minimize the growth of food-borne pathogens. Luo *et al.* (2009) noted increases in native microflora and *E. coli* O157:H7 after storage at ≥8°C within the labeled “Best If Used By” date of bagged baby spinach. In addition, *E. coli* has been noted to grow on

fresh-cut iceberg lettuce an additional 1 log cfu/g over a period of 6 hours at temperatures above 16°C (Rodríguez-Caturla *et al.*, 2012). Similarly, multiple studies have demonstrated *Salmonella*'s ability to persist on whole produce, internally and externally, across a broad range of temperatures from 4 to 25°C for 7 days up to 8 weeks (Liao *et al.*, 2010; Vandamm *et al.*, 2013; Shi *et al.*, 2007; Iturriaga *et al.*, 2007; Kroupitski *et al.*, 2009; Zhou *et al.*, 2014; Beuchat and Mann, 2008). Higher storage temperatures (above 15°C) and longer storage time typically allow *Salmonella* to grow on or within a produce commodity. An examination of growth kinetics (Table 17.1) of *Salmonella* Newport growth on beefsteak and Roma tomatoes shows no significant difference in growth between these two tomato types, and after a lag phase of approximately 6 h, the exponential growth rate reaches almost 0.3 log/h. Pre-storage of the tomatoes at 5°C did not alter the growth kinetics compared to pre-storage at 22°C (Table 17.2), suggesting that pre-storage temperature has little impact on the growth of *Salmonella* once it is held at a higher temperature for storage after slicing. Conversely, storage at lower temperatures (lower than 10°C) suppresses *Salmonella* growth, and with longer storage times, a

decrease in cell number may occur (Pao *et al.*, 2012; Vandamm *et al.*, 2013).

Luo and others (2010) looked at fresh-cut romaine and iceberg lettuce inoculated with *E. coli* O157:H7 and resealed in bags containing the original O₂ levels. At 5°C, *E. coli* O157:H7 populations decreased almost 2 log cfu/g by day 10 even though viable cells were still detected. McKellar *et al.* (2012) monitored and recorded the temperatures of 27 cases of packaged lettuce throughout various stages of storage and shipping from the processor to the retail shelf of three stores. The results indicate a nearly 1 log reduction in viable bacterial cells and the extent of die-off was proportional to the overall time spent in refrigeration. The authors suggested a reduced risk of illness to consumers when fresh-cut lettuce is stored at 5°C or below prior to consumption. *L. monocytogenes*, on the other hand, is psychrotropic and capable of growth at low temperatures due to a variety of intrinsic physiological attributes (Laksanalamai *et al.*, 2011).

Several studies examined the growth potential of *L. monocytogenes* in a variety of fresh produce (Ells and Truelstrup, 2006; Sant'Ana *et al.*, 2012, 2013; Skalina and Nikolajeva, 2010; Tian *et al.*, 2012). In all of these commodities,

Table 17.1 Growth kinetics of *S. Newport* in fresh-cut red ripe tomatoes stored at 22°C.

	pH	a _w	LDT (h)	EGR (log/h)
Beefsteak	4.25±0.03	0.994±0.001	6.40±0.90	0.299±0.010
Roma	4.18±0.09	0.994±0.002	5.77±0.49	0.298±0.014

Table 17.2 Effect of storage temperature before cutting on the growth kinetics of *S. Newport* inoculated in fresh-cut red ripe tomatoes stored at 22°C.

	Storage temperature			
	22°C		5°C	
	LDT (h)	EGR (log/h)	LDT (h)	EGR (log/h)
Beefsteak	2.28±0.95	0.232±0.029	3.81±0.91	0.244±0.036
Roma	2.46±0.73	0.251±0.006	3.34±0.44	0.260±0.005

L. monocytogenes grew 1–2 logs in 7–14 days at 5°C. Growth of 3–4 logs was noticed, however, when the temperature was increased to 10–15°C. In a detailed commodity-specific study of *L. monocytogenes*, the growth potentials of *L. monocytogenes* strains were examined on fresh-cut celery at different temperatures and incubation periods. A cocktail of three outbreak strains of *L. monocytogenes* including serotypes 1/2a, 1/2b, and 4b were used as an inoculum at $3\text{--}5 \times 10^3$ cfu per 5 g of celery. The inoculated samples were stored at 5°C for 30 days, 10°C for 12 days, and 25°C for 7 days. The growth pattern of all three serotypes was comparable at all temperatures; the growth rate was found to be much slower at 5°C followed by 10 and 25°C. The increase in counts at the end of the sampling periods were approximately 3–4 log cfu at 25°C, 2 log cfu at 10°C and 1 log cfu at 5°C per 5 g of celery (Sahu *et al.*, 2014; see Table 17.3). Maximum growth was found to be primarily a function of temperature and duration of incubation while the growth rate was dependent solely on the temperature of storage. These results clearly indicated that *L. monocytogenes* could survive and grow on a variety of fresh vegetables even at refrigerated temperatures. Zeng and others (2014) conducted a comprehensive large-scale study in the US placing temperature sensors in cross-country transport trucks, as well as in the storage rooms and display cases in nine supermarkets to determine temperature fluctuations that fresh-cut bagged

leafy greens might experience during a one to three day storage period in each location prior to purchase by the consumer. Temperature profiles were then reproduced with *E. coli* O157:H7 and *L. monocytogenes* artificially inoculated on to romaine lettuce. Retail storage showed the largest temperature fluctuations (i.e., range of 0.6 to 15.4°C) and, correspondingly, the levels of *E. coli* O157:H7 and *L. monocytogenes* showed the greatest increase, around 3.0 log cfu/g, over the three days of storage. Mean measured temperatures for transport and display in retail cases were rarely above 6°C. While *E. coli* O157:H7 growth was essentially static during this time, *L. monocytogenes* barely proliferated under transport conditions (≤ 0.6 log cfu/g) but increased up to 1.1 log cfu/g during three days of housing in display cases (Zeng *et al.*, 2014). This study illustrated the importance of maintaining an uninterrupted cold chain.

Temperature abuse has not only been noted to occur within the retail environment but can also be a problem within the consumer's home. Somewhat alarming was the notion made by James *et al.* (2008) who reviewed 20 published studies, conducted in parts of Europe, the US, and New Zealand from 1987 to 2006, and found only two studies reporting mean air temperatures of less than 5°C in domestic refrigerators. Most studies showed a great deal of concordance, citing temperatures in >50% of refrigerators above 5°C.

The pH of a produce commodity may also contribute to the ability of pathogens to grow on

Table 17.3 Growth of *Listeria monocytogenes* in artificially contaminated cut celery.

Strain	5°C		10°C		25°C	
	Maximum growth (days)	Doubling time (h)	Maximum growth (days)	Doubling time (h)	Maximum growth (days)	Doubling time (h)
LS806(4b)	2.6×10^4 (30 d)	87.6	1.2×10^5 (12 d)	25.3	8×10^6 (4 d)	4.6
LS814(1/2a)	2.7×10^4 (30 d)	72.8	1.7×10^5 (12 d)	25.9	2.6×10^7 (4 d)	3.8
LS810(1/2b)	4.3×10^4 (30 d)	74.1	2×10^5 (12 d)	23.9	8.2×10^6 (4 d)	4.4

it. For example, the optimal pH range for *Salmonella* is 6.5–7.5. However, the pH range in which salmonellae can survive and grow is much broader (i.e., 3.7–9.5). Tomatoes are considered to be relatively acidic with pH values ranging from 4.37 for round to 4.67 for grape tomatoes (Beuchat and Mann, 2008), but these values are not outside of the pH range to prevent the growth of *Salmonella*. As shown in Table 17.1, *S. Newport* was able to grow in both red round and Roma tomatoes with a pH of 4.2. Inspecting the growth kinetics of *S. Newport* in blended tomatoes with pH adjusted from 3.9 to 4.3 (Table 17.4), growth was observed at all pH levels, albeit at a lower rate and at the lowest pH. This low pH tolerance was also observed by Asplund and Nurmi (1991), where three different *Salmonella* serovars, Enteritidis, Infantis, and Typhimurium, were all able to grow on cut tomatoes with low pH values (3.99–4.37). The ability of *Salmonella* to grow to such high numbers in tomatoes may be due to the major acidulants within tomatoes, citric and malic acids, to which salmonellae may be more tolerant.

Packaging may affect the growth of pathogens on fresh-cut vegetables as well. Moisture enters bags of fresh-cut leafy greens as a residual from product washing. Vapor condensation could also lead to the accumulation of water inside the bags. Valentin-Bon *et al.* (2008) suggested that moisture (i.e., condensation) observed at the bottom of many bags may provide a better opportunity for microbial growth including that of any pathogens present. In a subsequent study, Kase *et al.* (2012) noted an

absence of moisture condensation in the bags, which may suggest industry advances in moisture control and bag design (e.g., anti-fog film, perforated versus non-perforated, etc.).

Differences in genetic make-up and efficiency in expression of stress-related pathways most likely dictate the ability of a particular serovar or strain to survive when exposed to various stressors. For example, *Salmonella* possesses the ability to survive in a desiccated state. The desiccation tolerance of several different serovars demonstrated a range of survival from 36 to 80%, depending on the serovar. Additionally, exposing salmonellae to desiccation conditions induced tolerance to several other stressors, including high salts, ethanol, bleach, high temperatures, and UV irradiation (Gruzdev *et al.*, 2011).

17.4 Routes of contamination during post-harvest handling of fresh and fresh-cut vegetables

During post-harvest processing, various routes of contamination with human pathogens may include contaminated water used for washing, chill tanks or sprays and shipping ice, processing equipment and transportation, infected workers, and cross-contamination from food preparation, display, and storage. Recently, Johnston *et al.* (2005) studied the quality of fresh produce at different stages from harvest throughout the packing shed in the southern United States. The group found that total aerobic plate count (APC) levels in cilantro increased from the field and throughout packing, with mean ranges of 5.7 log in the field to 6.7 log cfu/g in the samples obtained from boxes ready for distribution. Total coliforms increased significantly from harvest through packing, with a peak occurring mainly at the rinse step. All of these results suggested that microbiological contamination could either increase or originate during post-harvest processing. Several key areas have been identified as high risk for

Table 17.4 Growth kinetics of *S. Newport* on blended beefsteak and tomatoes as affected by pH incubated at 22 °C.

pH*	LDT (h)	EGR (log/h)
4.3	5.32 ± 0.87	0.189 ± 0.065
4.2	4.92 ± 3.47	0.105 ± 0.088
4.1	6.58 ± 3.11	0.167 ± 0.096
4.0	7.57 ± 2.73	0.146 ± 0.105
3.9	6.9 ± 2.10	0.123 ± 0.024

*pH adjusted with citric acid.

cross-contamination including water used to wash produce, kitchen surfaces, cutting surfaces, and worker hand hygiene, including the use of gloves (Doyle and Erickson, 2008; Todd *et al.*, 2010; Waitt *et al.*, 2013; Zhou *et al.*, 2014).

Water washes are often used immediately after harvest to remove field debris before subsequent processing steps. In the tomato industry, tomatoes are brought from fields to packing houses where they are placed in large dump tanks for washing before sorting and sizing. Contamination of this water can lead to internal and external contamination of the tomatoes. Internal contamination occurs when water and other foreign material, such as *Salmonella*, rush into the tomato via the stem scar due to differences in hydrostatic pressure, differences in temperature, or capillary action (Bartz, 1982; Smith *et al.*, 2006). Recent findings have demonstrated that even with a 10 °F positive temperature differential (i.e., washwater 10 °F warmer than incoming tomatoes) *Salmonella* was still able to internalize (Zhou *et al.*, 2014).

Common kitchen surfaces are another mechanism for transfer of pathogens to fresh produce. In one large study examining many common kitchen surfaces, such as ceramic, glass, plastic, and stainless steel, it was demonstrated that *Salmonella* can readily be transferred from contaminated surfaces to fresh produce, with higher transfer rates from wet surfaces (79–97% transfer) (Jensen *et al.*, 2013). The potential for fresh produce to contaminate the kitchen surface also exists, albeit at significantly lower rates of transfer. This directional tendency of pathogens to move from the kitchen surface to produce is attributed to the limited availability of nutrients and suitable attachment sites on abiotic surfaces. Additionally, microbial attachment to produce commodities is facilitated by the presence of complex carbohydrates (Jensen *et al.*, 2013). *S. Montevideo* was transferred from the surface of a tomato to the interior by cutting with a sterile knife (Lin and Wei, 1997). The amount transferred and the depth of transfer into the tomato pulp increased

in a dose-dependent manner. Additionally, when the same knife was used to cut subsequent tomatoes, transfer was noted well into the interior of the next tomato. A key example of cutting surface involvement leading to an outbreak situation happened in Queensland, Australia, where an outbreak of *S. Bovismorbificans* linked to cut lettuce leaves occurred. Investigators discovered the outbreak organisms on the surface of the cutting equipment used to process the lettuce and sited inadequate cleaning and sanitation of the cutting equipment leading to the contamination of lettuce products (Stafford *et al.*, 2002). *Listeria* has been isolated in several food processing environments. *Listeria*, specifically *L. monocytogenes*, has the ability to grow on different food contact surfaces where it is capable of establishing biofilms (Silva *et al.*, 2008). The organism has been isolated from various locations on food premises including drains, abattoirs, conveyor belts, freezers, smoke houses, slicing blades, packaging machines, floors and walls, footbaths, air ducts, and others (Moretro and Langsrud, 2004; Sofos, 2008). Biofilms can protect the embedded bacteria from antibacterial treatments such as sanitization, desiccation, UV radiation, concentrated disinfectants, etc., allowing the persistence of *L. monocytogenes* for long periods of time in the processing environment. These biofilm-coated surfaces become sources of frequent contamination when food products come into contact with them (Carpentier and Cerf, 2011; Hall-Stoodley *et al.*, 2004).

Good worker hygiene is critical to prevent the transfer of pathogens to fresh produce as well. The use of gloves as a barrier can be an effective means to reduce the risk of transfer. Unfortunately, glove use can lead to a false sense of security and unsafe practices are adopted because workers believe that gloves will prevent any cross-contamination (Todd *et al.*, 2010). Gloves were shown to aid in the transfer of *S. Enteritidis* to the edible portions of living lettuce during harvest (Waitt *et al.*, 2013). Moreover, high transfer rates were seen from the point of contamination to the subsequent three heads of

lettuce. Thus, infrequent glove changes can lead to the contamination of large numbers of produce items.

17.5 Microbial adaptation on produce commodity

It has become clear that natural selection plays a significant role in the adaptive change now observed among *Salmonella* strains associated with the food supply, produce notwithstanding. The recent application of whole-genome sequencing, with both fragment-based and long read technologies included, has yielded important clues into the specific genetic determinants that give rise to adapted phenotypes in *Salmonella*. Moreover, these data have provided substantial insight into the genetic mechanisms that underpin adaptive change among salmonellae now thriving in produce and other post-harvest processing niches including the mobilome (i.e., high-frequency horizontally transferrable elements such as phage, transposable elements, and other recombination hotspots across the genome) of *Salmonella*. The intersect of adaptive change and horizontal transfer is not insignificant as it has been postulated that those changes which are honed by selection are then transferred rapidly across the population by means of laterally enhanced regions of the genome (Allard *et al.*, 2012).

Adaptation among *Salmonella* strains may account for the novel genetic changes now emerging among certain produce vehicles. *S. Saintpaul*, for instance, recently associated

with one of the largest fresh produce contamination events ever documented in North America, appears to have acquired a number of nucleotide substitutions that distinguish it from other non-tomato/pepper associated Saintpauls. A recent study by Hayford *et al.* (2015) (Figure 17.1) documents a variety of non-synonymous single nucleotide changes across the genome when compared to other isolates from other produce- and non-produce-related sources. Interpretation of these nucleotide substitutions in the context of the amino acid PAM matrix pointed to several amino acid changes with functionally relevant differences in the host protein (Mount, 2008). That is, several non-synonymous changes were conserved biochemically and may play a role in the enhanced survival of *S. Saintpaul* strains in pepper and tomato and potentially in other members of the *Solanaceae*. Surprisingly, these changes have provided a substantial clue to potential areas of adaptation having occurred in genes responsible for propanediol utilization (*pduF*) and propanediol diffusion facilitator (*pudB*) genes. Interestingly, propanediol is a metabolite resulting from ripening or rotting of plant tissues (Bobik *et al.*, 1997; Brandl *et al.*, 2013; Goudeau *et al.*, 2013). Previous studies revealed that populations of mutants deficient in propanediol utilization were several logs lower than wild-type strains grown in cilantro (Goudeau *et al.*, 2013). In the light of *S. Saintpaul*'s documented association with at least Jalapeno and Serrano peppers, these changes may be of significance, representing key adaptive changes among produce-specific *Salmonella*. Moreover, these changes may signal

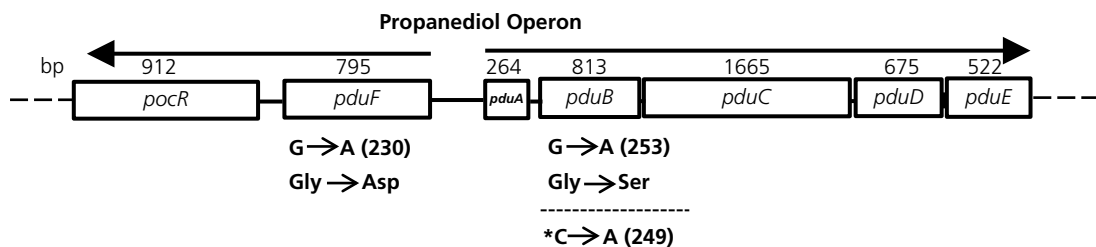


Figure 17.1 Genetic Map of *Salmonella* Saintpaul propanediol operon (see GenBank sequence gb|AOXY01000026.1). Regions of two propanediol genes (*pduF* and *pduB*) with location and positions of nonsynonymous and synonymous SNP mutations.

emerging alleles in produce niches such as Jalapeno peppers, yielding an adaptive metabolic premium allowing for survival of specific *S. Saintpaul* in harsh and relatively narrow produce settings.

Adaptive change among bacteria in the produce and produce-processing environment is likely to be further driven by intrinsic genetic factors known to enhance evolutionary change and the acquisition of adaptive change among enteric pathogens. Such evolution may be explained in part by the hypermutable phenotype (LeClerc *et al.*, 1996) caused by defects in the bacterial methyl-directed mismatch repair (MMR) system. Up to 73% of the MMR defects found in feral settings are due to lesions within the *mutS* gene, resulting in increased nucleotide substitution rates, enhanced DNA transposition, and, perhaps most importantly, a relaxation of the internal barriers that normally restrict homologous recombination following horizontal gene transfer (HGT) of foreign DNA (LeClerc and Cebula, 1997). The now incontrovertible connection between HGT and MMR gene evolution has led to the thesis that genetic exchange of *mutS* alleles could simultaneously quiet the mutator phenotype while rescuing adaptive changes from the population (LeClerc *et al.*, 1996; Brown *et al.*, 2001). Consistent with this hypothesis, the *mutS* gene is evolutionarily scrambled by HGT in subspecies I *S. enterica*, which has been documented in our laboratories (Brown *et al.*, 2002, 2003).

In *L. monocytogenes*, two cell–cell communication systems, *luxS* orthologous (Challan *et al.*, 2006) and virulence regulator, the *arg* system (Riedel *et al.*, 2009; Rieu *et al.*, 2007), have been reported to be involved in the regulation of biofilm formation. *luxS* mutants in *L. monocytogenes* strains are reported to form denser biofilms than parental strains (Sela *et al.*, 2006). Flagellum-mediated motility also plays an important role in biofilm formation in *L. monocytogenes* (Lemon *et al.*, 2007). Virulence genes like *prfA* and *inlA* are also reported to have important roles in biofilm development in *L. monocytogenes*. The *prfA* mutant is reported

to present a defective biofilm compared with the wild type (Lemon *et al.*, 2010), whereas truncated *InlA* showed enhanced biofilm formation compared with the full length one (Franciosa *et al.*, 2009). It is interesting to note that many proteins in *L. monocytogenes* have evolved to serve both for environmental adaptation such as biofilm formation and adaptation in human hosts leading to disease manifestation.

17.6 Effective post-harvest intervention technologies

The produce industry faces unique challenges for eliminating pathogen contamination when compared to other types of foods. In 2013, the US FDA Food Safety Modernization Act (FSMA) proposed rules for produce safety to set standards in identified routes of microbial contamination of produce, including: (1) agricultural water; (2) biological soil amendments of animal origin; (3) health and hygiene; (4) animals in the growing area; and (5) equipment, tools, and buildings (<http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm>). However, even with the proper plan and systems in place, it may still be possible for some microbial contamination to occur.

A better understanding of microbial ecosystems on the surface of raw vegetables would be extremely useful when developing interventions to minimize contamination, prevent the growth of pathogens, and kill or remove pathogens at various stages of pre- and post-harvest. The composition and abundance of microbial ecosystems unique to various types of produce (Leff and Fierer, 2013; Barak *et al.*, 2008) can be greatly influenced by changes in practice and field conditions prior to harvest and alterations in conditions of various stages after harvesting.

After weeks of sun and ambient temperature exposure, vegetables typically undergo a pre-cooling step soon after harvest to reduce the field heat. Pre-cooling is generally done through forced air cooling, hydrocooling (cold water

Table 17.5 Chemical and physical interventions on fresh and fresh-cut vegetables in post-harvest.

Disinfection technique	Dose/concentration	Investigated vegetable commodity	Targeted microorganism	Results	Reference
<i>Chemical-based disinfection</i>					
Chlorine	50–200 ppm	Carrots, lettuce, cabbage, bell peppers, artichokes, borages, cilantro, and coleslaw mix	Bacteria, virus	1–2 log reduction	Gonzalez et al. (2004), Akbas and Olmez (2007), Allwood et al. (2004), Foley et al. (2004), Hadjok et al. (2008), Sanz et al. (2002), Uyttendaele et al. (2004)
Chlorine dioxide	Aqueous: 100 ppm Gaseous: 1.2–4.1 mg/l	Cucumber, lettuce, carrot, tomato, onion, and cabbage	Bacteria, yeast, molds	1.5–5.8 log reduction	Singh et al. (2002), Chung et al. (2011), Sy et al. (2005)
Organic acids	0.5–1.0%	Iceberg lettuce, spinach	Bacteria	1–2 log reduction	Akbas and Olmez (2007), Neal et al. (2012)
Hydrogen peroxide	1–3%	Mushrooms, tomatoes, red bell peppers, lettuce, spinach, cucumbers, zucchini, and bell peppers	Bacteria	1–3 log reduction	Back et al. (2014), Moore et al. (2011), Kim et al. (2007), Sapers and Simmons (1998)
Peroxyacetic acid	80–100 ppm	Lettuce, spinach, celery, cabbage, and leek	Bacteria, yeast, molds, and virus	1–2 log reduction	Neal et al. (2012), Fraisse et al. (2011), Vandekinderen et al. (2009)
Calcium-based solutions	1.5%	Lettuce	Bacteria	1–2 log reduction	Martin-Diana et al. (2005)
Electrolyzed oxidizing water	10–50 ppm available chlorine	Lettuce, Chinese celery, bell pepper, Japanese radish, potato, carrots, spinach, cucumber, and kale	Bacteria, yeast, molds, virus, and fungus	Up to 2.9 log reduction	Buck et al. (2002), Hati et al. (2012), Issa-Zacharia et al. (2011), Izumi (1999), Tamaki et al. (2014), Mansur et al. (2014), Koide et al. (2011)
Ozone	Up to 10 ppm O ₃	Lettuce, potato, carrot, spinach, cucumber, tomato, baby leaf, brassica, cabbage, rocket leaf, pepper,	Bacteria, yeast, molds, and fungus	Up to 2.6 log reduction	Singh et al. (2002), Garcia et al. (2003), Beltran et al. (2005a, 2005b), Horvitz and Cantalejo (2014)
Plant extracts		Cilantro, parsley, spinach, carrot, and tomato	Bacteria	Up to 4 log reduction	Singh et al. (2002), Orue et al. (2013), Lu and Wu (2010), Mattson et al. (2011)
<i>Physical-based treatment</i>					
Thermal treatments	85–100 °C	Lettuce, green bell pepper	Bacteria, yeast, molds and parasite	1 log reduction	Rico et al. (2007), Duhaïn et al. (2012)
High-pressure processing	300–600 mPa	tomato	Bacteria	0.5–3.6 log reduction	Maitland et al. (2011)
Irradiation	≤1.0 kGy	Cilantro, lettuce	Bacteria, molds, yeast	Up to 6.7 log reduction	Foley et al. (2004), Goularte et al. (2004)
Ultraviolet light	1.18 kJ/m ² , 2.37 kJ/m ²	Lettuce	Bacteria, yeast, and molds	1–2 log reduction	Allende et al. (2006)

dunk or rinse), icing (direct or indirect contact with produce), vacuum cooling, or hydrovac cooling (i.e., water is sometimes sprayed on the produce prior to vacuum cooling). Vegetables in close contact with the ground usually undergo a washing step following harvest to remove dirt and other debris taken from the agricultural fields. Typically washwater contains a disinfectant which, if used correctly, can reduce the populations of both human pathogens and spoilage microorganisms. Care should be taken to ensure the presence of a sufficient amount of sanitizers in the washwater in order to prevent pathogen cross-contamination. Traditionally, chlorine in the form of a sodium hypochlorite solution or as a dry, powdered calcium hypochlorite is used in hydrocooling or washwater as a disinfectant. However, the reaction of chlorine with other organic compounds in perishable produce may lead to the formation of halogenated by-products in the presence of organic matter, giving rise to toxicity concerns. In some European countries, including Germany, the Netherlands, Switzerland, and Belgium, the use of chlorine in RTE products is prohibited (Rico *et al.*, 2007). Moreover, the efficacy of chlorine to reduce microbial pathogens on vegetables is often limited by pH (i.e., hypochlorous acid – the form with the most antimicrobial activity – is present at pH 6.5 to 7.5), temperature, exposure to light, levels of soil and organic matter, initial and residual free chlorine concentration, and length of exposure (Gonzalez *et al.*, 2004; Shen *et al.*, 2013). Several innovative approaches both chemically and physically have been explored for the decontamination of fresh or fresh-cut vegetables (Table 17.5). Effective washing and decontamination of fresh-cut vegetables is difficult to achieve due to the different types of vegetables, the inadequate efficacy of individual treatments alone (Table 17.5), the presence of biofilms on vegetables and on processing equipment (Jahid and Ha, 2012; Somers *et al.*, 1994), and internalization/infiltration of bacteria within produce (Zhuang *et al.*, 1995; Zheng *et al.*, 2013; Takeuchi and Frank, 2000). Therefore, a combination of

different disinfection methods (e.g., hurdle technology) (Rico *et al.*, 2007; Joshi *et al.*, 2013) is necessary to increase the efficacy of disinfectants against microbial population reduction. Novel biocontrol strategies using environmentally and ecologically friendly bacterial epiphytes, designed and now being evaluated with human health end points (i.e., prevention of salmonellosis and other produce-borne illnesses), may also play a significant role in the reduction of pathogen loads on fresh produce. Given the lack of effective technologies to eliminate pathogens from produce surfaces and the potential for pathogen cross-contamination during produce washing and post-harvest handling, preventing pathogen proliferation via temperature control is critical to mitigate food safety risks. Overall, technology advances spanning the detection, monitoring, and tracking of food-borne pathogens along with more highly effective preventive control and kill-step measures will be crucial to maintaining a safe fresh and fresh-cut produce supply for consumers in the US and around the world.

References

- Akbas, M.Y., and Olmez, H. 2007. Inactivation of *Escherichia coli* and *Listeria monocytogenes* on iceberg lettuce by dip wash treatments with organic acids. *Letters in Applied Microbiology*, **44**, 619–624.
- Allard, M.W., Luo, Y., Strain, E., Li, C., *et al.* 2012. High resolution clustering of *Salmonella enterica* serovar Montevideo strains using a next-generation sequencing approach. *BMC Genomics*, **13**, 32.
- Allen, K.J., Kovacevic, J., Cancarevic, A., Wood, J., Xu, J., Gill, B., Allen, J.K., and Mesak, L.R. 2013. Microbiological survey of imported produce available at retail across Canada. *International Journal of Food Microbiology*, **162**, 135–142.
- Allende, A., McEvoy, J.L., Luo, Y.G., Artes, F., and Wang, C.Y. 2006. Effectiveness of two-sided UV-C treatments in inhibiting natural microflora

- and extending the shelf-life of minimally processed "Red Oak Leaf" lettuce. *Food Microbiology*, **23**, 241–249.
- Allwood, P.B., Malik, Y.S., Hedberg, C.W., and Goyal, S.M. 2004. Effect of temperature and sanitizers on the survival of feline calicivirus, *Escherichia coli*, and F-specific coliphage MS2 on leafy salad vegetables. *Journal of Food Protection*, **67**, 1451–1456.
- Althaus, D., Hofer, E., Corti, S., Julmi, A., and Stephan, R. 2012. Bacteriological survey of ready-to-eat lettuce, fresh-cut fruit, and sprouts collected from the Swiss market. *Journal of Food Protection*, **75**, 1338–1341.
- Asplund, K. and Nurmi, E. 1991. The growth of salmonellae in tomatoes. *International Journal of Food Microbiology*, **13**, 177–182.
- Back, K.H., Ha, J.W., and Kang, D.H. 2014. Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. *Food Control*, **44**, 78–85.
- Barak, J.D., Liang, A., and Narm, K.-E. 2008. Differential attachment to and subsequent contamination of agricultural crops by *Salmonella enterica*. *Applied and Environmental Microbiology*, **74**, 5568–5570. doi: 10.1128/AEM.01077-08.
- Bartz, J.A. 1982. Infiltration of tomatoes immersed at different temperatures to different depths in suspensions of *Erwinia carotovora* subsp. *carotovora*. *Plant Diseases*, **66**, 302–305.
- Beltran, D., Selma, M.V., Tudela, J.A., Gil, M.I. 2005a. Effect of different sanitizers on microbial and sensory quality of fresh-cut potato strips stored under modified atmosphere or vacuum packaging. *Postharvest Biology and Technology*, **37**, 37–46.
- Beltran, D., Selma, M.V., Marin, A., Gil, M.I. 2005b. Ozonated water extends the shelf life of fresh-cut lettuce. *Journal of Agriculture and Food Chemistry*, **53**, 5654–5663.
- Beuchat, L.R. and Mann, D.A. 2008. Survival and growth of acid-adapted and unadapted *Salmonella* in and on raw tomatoes as affected by variety, stage of ripeness, and storage temperature. *Journal of Food Protection*, **71**, 1572–1579.
- Bobik, T.A., Xu, Y., Jeter, R.M., Otto, K.E., and Roth, J.R. 1997. Propanediol utilization genes (pdu) of *Salmonella typhimurium*: three genes for the propanediol dehydratase. *Journal of Bacteriology*, **179**, 6633–6639.
- Brandl, M.T., Cox, C.E., and Teplitski, M. 2013. *Salmonella* interactions with plants and their associated microbiota. *Phytopathology*, **103**, 316–325.
- Brown, E.W., LeClerc, J.E., Li, B., Payne, W.L., and Cebula, T.A. 2001. Phylogenetic evidence for horizontal transfer of mutS alleles among naturally occurring *Escherichia coli* strains. *Journal of Bacteriology*, **183**, 1631–1644.
- Brown, E.W., Kotewicz, M.L., and Cebula, T.A. 2002. Detection of recombination among *Salmonella enterica* strains using the incongruence length difference test. *Molecular Phylogenetic Evolution*, **24**, 102–120.
- Brown, E.W., Mammel, M.K., LeClerc, J.E., Cebula, T.A. 2003. Limited boundaries for extensive horizontal gene transfer among *Salmonella* pathogens. *PNAS*, **100**, 15676–15681.
- Buck, J.W., van Iersel, M.W., Oetting, R.D., Hung, Y.C. 2002. *In vitro* fungicidal activity of acidic electrolyzed oxidizing water. *Plant Diseases*, **86**, 278–281.
- Carpentier, B. and Cerf, P. 2011. Persistence of *Listeria monocytogenes* in food industry equipment and premises. *International Journal of Food Microbiology*, **145**, 1–8.
- Centers for Disease Control and Prevention (CDC). 2006. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach – United States, September 2006. *Morbidity and Mortality Weekly Report*, **55**, 1045–1046.
- Challan, B.S., et al. 2006. Assessment of the roles of LuxS, S-ribosyl homocysteine, and autoinducer 2 in cell attachment during biofilm formation by *Listeria monocytogenes* EGD-e. *Applied and Environmental Microbiology*, **72**, 2644–2650.
- Chung, C.-C., Huang, T.-C., Yu, C.-H., Shen, F.-Y., and Chen, H.-H. 2011. 2011 International Conference on Food Engineering and Biotechnology, Singapore, pp. 107–112.

- Consumer Union. 2010. Bacteria and bagged salads: better standards and enforcement needed. Available at: <http://www.consumersunion.org/pdf/BaggedSaladReport.pdf>.
- Cook, R. 2012. Trends in the marketing of fresh produce and fresh-cut products. University of California [Online]. Posted on September 18, 2012.
- Doyle, M.P. and Erickson, M.C. 2008. Summer meeting 2007 - the problems with fresh produce: an overview. *Journal of Applied Microbiology*, **105**, 317–330.
- Duhain, G.L.M.C., Minnaar, A., and Buys, E.M. 2012. Effect of chlorine, blanching, freezing, and microwave heating on *Cryptosporidium parvum* viability inoculated on green peppers. *Journal of Food Protection*, **75**, 936–941.
- Ells, T.C. and Truelstrup, H.L. 2006. Strain and growth temperature influence *Listeria* spp. attachment to intact and cut cabbage. *International Journal of Food Microbiology*, **111**, 34–42.
- Foley, D., Euper, M., Caporaso, F., and Prakash, A. 2004. Irradiation and chlorination effectively reduces *Escherichia coli* O157:H7 inoculated on cilantro (*Coriandrum sativum*) without negatively affecting quality. *Journal of Food Protection*, **67**, 2092–2098.
- Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., Vialette, M., Morin, T., and Perelle, S. 2011. Comparison of chlorine and peroxyacetic-based disinfectant to inactivate feline calicivirus, murine norovirus and hepatitis A virus on lettuce. *International Journal of Food Microbiology*, **151**, 98–104.
- Franciosa, G., et al. 2009. Expression of internalin A and biofilm formation among *Listeria monocytogenes* clinical isolates. *International Journal of Immunopathology and Pharmacology*, **22**, 183–193.
- Garcia, A., Mount, J.R., and Davidson, P.M. 2003. Ozone and chlorine treatment of minimally processed lettuce. *Journal of Food Science*, **68**, 2747–2751.
- Gonzalez, R.J., Luo, Y.G., Ruiz-Cruz, S., and McEvoy, J.L. 2004. Efficacy of sanitizers to inactivate *Escherichia coli* O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *Journal of Food Protection*, **67**, 2375–2380.
- Goudeau, D.M., Parker, C.T., Zhou, Y., Sela, S., Kroupitski, Y., and Brandl, M.T. 2013. The *Salmonella* transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. *Applied and Environmental Microbiology*, **79**, 250–262.
- Goularte, L., Martin, C.G., Morales-Aizpurua, I.C., Destro, M.T., et al. 2004. Combination of minimal processing and irradiation to improve the microbiological safety of lettuce (*Lactuca sativa*, L.). *Radiation and Physical Chemistry*, **71**, 157–161.
- Gruzdev, N., Pinto, R., and Sela, S. 2011. Effect of desiccation on tolerance of *Salmonella enterica* to multiple stresses. *Applied Environmental Microbiology*, **77**, 1667–1673.
- Hadjok, C., Mittal, G.S., and Warriner, K. 2008. Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *Journal of Applied Microbiology*, **104**, 1014–1024.
- Hall-Stoodley, L., Costerton, J.W., and Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious diseases. *National Review of Microbiology*, **2**, 95–108.
- Hati, S., Mandal, S., Minz, P.S., Vij, S., Khetra, Y., and Singh, B.P. 2012. Electrolyzed oxidized water (EOW): non-thermal approach for decontamination of food borne microorganisms in food industry. *Food and Nutrition Sciences*, **3**, 760–768.
- Hayford, A.E., Brown, E.W., Zhao, S., Mammel, M.K., et al. 2015. Genetic and resistance phenotypic subtyping of *Salmonella* Saintpaul isolates from various food sources and humans: Phylogenetic concordance in combinatory analyses. *Infection, Genetics and Evolution*, **36**, 92–107.
- Horvitz, S. and Cantalejo, M.J. 2014. Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, **54**, 312–339.
- Issa-Zacharia, A., Kamitani, Y., Miwa, N., Muhimbula, H., and Iwasaki, K. 2011. Application of slightly

- acidic electrolyzed water as a potential non-thermal food sanitizer for decontamination of fresh ready-to-eat vegetables and sprouts. *Food Control*, **22**, 601–607.
- Iturriaga, M.H., Tamplin, M.L., and Escartin, E.F. 2007. Colonization of tomatoes by *Salmonella* Montevideo is affected by relative humidity and storage temperature. *Journal of Food Protection*, **70**, 30–34.
- Izumi, H. 1999. Electrolyzed water as a disinfectant for fresh-cut vegetables. *Journal of Food Science*, **64**, 536–539.
- Jahid, I.K. and Ha, S.D. 2012. A review of microbial biofilms of produce: future challenge to food safety. *Food Science and Biotechnology*, **21**, 299–316.
- James, S.J., Evans, J., and James, C. 2008. A review of the performance of domestic refrigerators. *Journal of Food Engineering*, **87**, 2–10.
- Jensen, D.A., Friedrich, L.M., Harris, L.J., Danyluk, M.D., and Schaffner, D.W. 2013. Quantifying transfer rates of *Salmonella* and *Escherichia coli* O157:H7 between fresh-cut produce and common kitchen surfaces. *Journal of Food Protection*, **76**, 1530–1538.
- Johnston, L.M., Jaykus, L.A., Moll, D., Martinez, M.C., Anciso, J., Mora, B., and Moe, C.L. 2005. A field study of the microbiological quality of fresh produce. *Journal of Food Protection*, **68**, 1840–1847.
- Joshi, K., Mahendran, R., Alagusundaram, K., Norton, T., and Tiwari, B.K. 2013. Novel disinfectants for fresh produce. *Trends in Food and Science Technology*, **34**, 54–61.
- Kase, J.A., Borenstein, S., and Feng, P.C.H. 2012. Microbial quality of bagged baby spinach and romaine lettuce – effects of top vs. bottom sampling. *Journal of Food Protection*, **75**, 132–136.
- Kim, H.J., Fonseca, J.M., Kubota, C., Choi, J.H. 2007. Effect of hydrogen peroxide on quality of fresh-cut tomato. *Journal of Food Science*, **72**, S463–S467.
- Koide, S., Shitanda, D., Note, M., and Cao, W. 2011. Effects of mildly heated, slightly acidic electrolyzed water on the disinfection and physicochemical properties of sliced carrot. *Food Control*, **22**, 452–456.
- Koseki, S., Mizuno, Y., Kawasaki, S., and Yamamoto, K. 2011. A survey of iceberg lettuce for the presence of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Japan. *Journal of Food Protection*, **74**, 1543–1546.
- Kroupitski, Y., Pinto, R., Brandl, M.T., Belausov, E., and Sela, S. 2009. Interactions of *Salmonella enterica* with lettuce leaves. *Journal of Applied Microbiology*, **106**, 1876–1885.
- Laksanalamai, P., Burall, L., and Datta, A.R. 2011. *Adaptation Mechanisms of Psychrotolerant Bacterial Pathogens. Extremophiles Handbook* (ed. Horikoshi, K.). Springer.
- LeClerc, J.E. and Cebula, T.A. 1997. Hypermutability and homeologous recombination: ingredients for rapid evolution. *Bulletin of Institute of Pasteur*, **95**, 97–106.
- LeClerc, J.E., Li, B., Payne, W.L., and Cebula, T.A. 1996. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science*, **274**, 1208–1211.
- Leff, J.W. and Fierer, N. 2013. Bacterial Communities Associated with the Surfaces of Fresh Fruits and Vegetables. *PLoS One*, **8**, e59310. doi: 10.1371/journal.pone.0059310.
- Lemon, K.P., Higgins, D.E., and Kolter, R. 2007. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *Journal of Bacteriology*, **189**, 4418–4424.
- Lemon, K.P., Freitag, N.E., and Kolter, R. 2010. The virulence regulator PrfA promotes biofilm formation by *Listeria monocytogenes*. *Journal of Bacteriology*, **192**, 3969–3976.
- Liao, C.-H., Cooke, P.H., and Niemira, B.A. 2010. Localization, growth and inactivation of *Salmonella* Saintpaul on jalapeno peppers. *Journal of Food Science*, **75**, M377–M382.
- Lin, C.-M. and Wei, C.-I. 1997. Transfer of *Salmonella* Montevideo onto the interior surfaces of tomatoes by cutting. *Journal of Food Protection*, **60**, 858–863.
- Lu, Y.J. and Wu, C.Q. 2010. Reduction of *Salmonella enterica* contamination on grape tomatoes by washing with thyme oil, thymol, and carvacrol as compared with chlorine treatment. *Journal of Food Protection*, **73**, 2270–2275.

- Luo, Y., He, Q., McEvoy, J.L., and Conway, W.S. 2009. Fate of *Escherichia coli* O157:H7 in the presence of indigenous microorganisms on commercially packaged baby spinach, as impacted by storage temperature and time. *Journal of Food Protection*, **72**, 2038–2045.
- Luo, Y., He, Q., and McEvoy, J.L. 2010. Effect of storage temperature and duration on the behavior of *Escherichia coli* O157:H7 on packaged fresh-cut salad containing romaine and iceberg lettuce. *Journal of Food Science*, **75**, M390–397.
- Maitland, J.E., Boyer, R.R., Eifert, J.D., and Williams, R.C. 2011. High hydrostatic pressure processing reduces *Salmonella enterica* serovars in diced and whole tomatoes. *International Journal of Food Microbiology*, **149**, 113–117.
- Mansur, A.R., Wang, J., Park, M.S., and Oh, D.H. 2014. Growth model of *Escherichia coli* O157:H7 at various storage temperatures on kale treated by thermosonication combined with slightly acidic electrolyzed water. *Journal of Food Protection*, **77**, 23–31.
- Martin-Diana, A.B., Rico, D., Barry-Ryan, C., Frias, J.M., Mulcahy, J., and Henehan, G.T.M. 2005. Calcium lactate washing treatments for salad-cut Iceberg lettuce: effect of temperature and concentration on quality retention parameters. *Food Research International*, **38**, 729–740.
- Mattson, T.E., Johnny, A.K., Amalaradjou, M.A.R., More, K., Schreiber, D.T., Patel, J., and Venkitanarayanan, K. 2011. Inactivation of *Salmonella* spp. on tomatoes by plant molecules. *International Journal of Food Microbiology*, **144**, 464–468.
- McKellar, R.C., LeBlanc, D.I., Lu, J., and Delaquis, P. 2012. Simulation of *Escherichia coli* O157:H7 behavior in fresh-cut lettuce under dynamic temperature conditions during distribution from processing to retail. *Foodborne Pathogens and Disease*, **9**, 239–244.
- Mody, R.K., Greene, S.A., Gaul, L., Sever, A., et al. 2011. National outbreak of *Salmonella* serotype Saintpaul infections: importance of Texas restaurant investigations in implicating Jalapeno peppers. *PLoS One*, **6**.
- Moore, K.L., Patel, J., Jaroni, D., Friedman, M., and Ravishankar, S. 2011. Antimicrobial activity of apple, hibiscus, olive, and hydrogen peroxide formulations against *Salmonella enterica* on organic leafy greens. *Journal of Food Protection*, **74**, 1676–1683.
- Moretro, T. and Langsrud, S. 2004. *Listeria monocytogenes*: biofilm formation and persistence in food-processing environments. *Biofilms*, **1**, 107–121.
- Mount, D.W. 2008. Using PAM matrices in sequence alignments. *Cold Spring Harbor Protocols*, **2008**. doi: 10.1101/pdb.top38.
- Neal, J.A., Marquez-Gonzalez, M., Cabrera-Diaz, E., Lucia, L.M., et al. 2012. Comparison of multiple chemical sanitizers for reducing *Salmonella* and *Escherichia coli* O157:H7 on spinach (*Spinacia oleracea*) leaves. *Food Research International*, **45**, 1123–1128.
- Oliveira, M., Usall, J., Viñas, I., Anguera, M., Gatiús, F., and Abadias, M. 2010. Microbiological quality of fresh lettuce from organic and conventional production. *Food Microbiology*, **27**, 679–684.
- Orue, N., Garcia, S., Feng, P., and Heredia, N. 2013. Decontamination of *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7 from leafy green vegetables using edible plant extracts. *Journal of Food Science*, **78**, M290–M296.
- Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., and Griffin, P.M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, **19**, 407–415.
- Pao, S., Long III, W., Kim, C., and Rafie, A.R. 2012. *Salmonella* population rebound and its prevention on spray washed and non-washed jalapeno peppers and roma tomatoes in humid storage. *Foodborne Pathogens and Disease*, **9**, 362–366.
- Rico, D., Martin-Diana, A.B., Barat, J.M., and Barry-Ryan, C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science and Technology*, **18**, 373–386.

- Riedel, C.U., *et al.* 2009. AgrD-dependent quorum sensing affects biofilm formation, invasion, virulence and global gene expression profiles in *Listeria monocytogenes*. *Molecular Microbiology*, **71**, 1177–1189.
- Rieu, A., *et al.* 2007. Agr system of *Listeria monocytogenes* EGD-e: role in adherence and differential expression pattern. *Applied and Environmental Microbiology*, **73**, 6125–6133.
- Rodríguez-Caturla, M.Y., Valero, A., García-Gimeno, R.M., Zurera, G. 2012. Development of a risk-based methodology for estimating survival and growth of enteropathogenic *Escherichia coli* on iceberg-lettuce exposed at short-term storage in foodservice centers. *Journal of Microbiology Methods*, **90**, 273–279.
- Sahu, S.N., Zink, D., and Datta, A.R. 2014. Growth potential of *Listeria monocytogenes* in artificially contaminated chicken salad. IAFP Annual Meeting, Indianapolis, IN, USA. Abstract.
- Salleh, N.A., Rusul, G., Hassan, Z., Reezal, A., Isa, S.H., Nishibuchi, M., and Radu, S. 2003. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. *Food Control*, **14**, 475–479.
- Sant'Ana, S.A., Landgraf, M., Destro, M.T., and Franco, B.D.G.M. 2011. Prevalence and counts of *Salmonella* spp. in minimally processed vegetables in Sao Paulo, Brazil. *Food Microbiology*, **28**, 1235–1237.
- Sant'Ana, A.S., *et al.* 2012. Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature conditions during shelf-life. *International Journal of Food Microbiology*, **157**, 52–58.
- Sant'Ana, A.S., *et al.* 2013. Growth potential of *Salmonella* and *Listeria monocytogenes* in ready-to-eat lettuce and collard greens packaged under modified atmosphere and in perforated film. *Journal of Food Protection*, **76**, 888–891.
- Sanz, S., Gimenez, M., Olarte, C., Lomas, C., and Portu, J. 2002. Effectiveness of chlorine washing disinfection and effects on the appearance of artichoke and borage. *Journal of Applied Microbiology*, **93**, 986–993.
- Sapers, G.M. and Simmons, G.F. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. *Food Technology*, **52**, 48–52.
- Sela, S., *et al.* 2006. A mutation in the luxS gene influences *Listeria monocytogenes* biofilm formation. *Applied and Environmental Microbiology*, **72**, 5653–5658.
- Shen, C., Luo, Y., Nou, X., Wang, Q., and Millner, P.D. 2013. The effects of free chlorine concentration, organic load, and exposure time on the inactivation of *Salmonella*, *Escherichia coli* O157:H7 and non-O157 STEC. *Journal of Food Protection*, **76**, 386–393.
- Shi, X., Namvar, A., Kostrzynska, M., Hora, R., and Warriner, K. 2007. Persistence and growth of different *Salmonella* serovars on pre- and post-harvest tomatoes. *Journal of Food Protection*, **70**, 2725–2731.
- Shi, X., Wu, Z., Namvar, A., Kostrzynska, M., Dunfield, K., and Warriner, K. 2009. Microbial population profiles of the microflora associated with pre- and postharvest tomatoes contaminated with *Salmonella typhimurium* or *Salmonella montevideo*. *Journal of Applied Microbiology*, **107**, 329–338.
- Silva, S., *et al.* 2008. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. *Journal of Food Protection*, **71**, 1379–1385.
- Singh, N., Singh, R.K., Bhunia, A.K., and Stroshine, R.L. 2002. Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157:H7 on lettuce and baby carrots. *Lebensm.-Wiss. Technology*, **35**, 720–729.
- Skalina, L. and Nikolajeva, V. 2010. Growth potential of *Listeria monocytogenes* strains in mixed ready-to-eat salads. *International Journal of Food Microbiology*, **144**, 317–321.
- Smith, S.M., Scott, J.W., and Bartz, J.A. 2006. The effect of time after harvest on stem scar water internalization in tomato. *Proceedings of Florida State Horticultural Society*, **119**, 272–274.

- Sofos, J.N. 2008. *Listeria monocytogenes* – Enemy No. 1 for the ready-to-eat industry. *Troy: The National Provisioner*, pp. 70–72.
- Somers, E.B., Schoeni, J.L., and Wong, A.C.L. 1994. Effect of trisodium phosphate on biofilm and planktonic cells of *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella*-Typhimurium. *International Journal of Food Microbiology*, **22**, 269–276.
- Sospedra, I., Rubert, J., Soriano, J.M., Manes, J. 2013. Survey of microbial quality of plant-based foods served in restaurants. *Food Control*, **30**, 418–422.
- Stafford, R.J., McCall, B.J., Neill, A.S., Leon, D.S., Dorricott, G.J., Towner, C.D., and Micalizzi, G.R. 2002. A statewide outbreak of *Salmonella* Bovismobificans phage type 32 infection in Queensland. *Communications in Disease Intelligence*, **26**, 568–573.
- Sy, K.V., Murray, M.B., Harrison, M.D., Beuchat, L.R. 2005. Evaluation of gaseous chlorine dioxide as a sanitizer for killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and yeasts and molds on fresh and fresh-cut produce. *Journal of Food Protection*, **68**, 1176–1187.
- Takeuchi, K. and Frank, J.F. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *Journal of Food Protection*, **63**, 434–440.
- Tamaki, S., Bui, V.N., Ngo, L.H., Ogawa, H., and Imai, K. 2014. Virucidal effect of acidic electrolyzed water and neutral electrolyzed water on avian influenza viruses. *Archives of Virology*, **159**, 405–412.
- Teplitski, M., Warriner, K., Bartz, J., and Schneider, K.R. 2011. Untangling metabolic and communication networks: interactions of enterics with phyto bacteria and their implications in produce safety. *Trends in Microbiology*, **19**, 121–127.
- Tian, J.Q., et al. 2012. Survival and growth of food-borne pathogens in minimally processed vegetables at 4 and 15 degrees C. *Journal of Food Science*, **77**, M48–M50.
- Todd, E.C.D., Michaels, B.S., Greig, J.D., Smith, D., and Bartleson, C.S. 2010. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 8. Gloves as barriers to prevent contamination of food by workers. *Journal of Food Protection*, **73**, 1762–1773.
- Uyttendaele, M., Neyts, K., Vanderswalmen, H., Notebaert, E., and Debevere, J. 2004. Control of *Aeromonas* on minimally processed vegetables by decontamination with lactic acid, chlorinated water, or thyme essential oil solution. *International Journal of Food Microbiology*, **90**, 263–271.
- Valentin-Bon, I., Jacobson, A., Monday, S.R., and Feng, P.C.H. 2008. Microbiological quality of bagged cut spinach and lettuce mixes. *Applied and Environmental Microbiology*, **74**, 1240–1242.
- Vandamm, J.P., Li, D., Harris, L.J., Schaffner, D.W., and Danyluk, M.D. 2013. Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on fresh-cut celery. *Food Microbiology*, **34**, 151–157.
- Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Ragaert, P., and Van Camp, J. 2009. Optimization and evaluation of a decontamination step with peroxyacetic acid for fresh-cut produce. *Food Microbiology*, **26**, 882–888.
- Vital, P.G., Dimasuay, K.G.B., Widmer, K.W., and Rivera, W.L. 2014. Microbiological quality of fresh produce from open air markets and supermarkets in the Philippines. *The Scientific World Journal*, **2014**, article ID 219534, 7 pp. doi: 10.1155/2014/219534.
- Waitt, J.A., Kuhn, D.D., Welbaum, G.E., and Ponder, M.A. 2013. Postharvest transfer and survival of *Salmonella enterica* serotype enteritidis on living lettuce. *Letters in Applied Microbiology*, **58**, 95–101.
- Wijnands, L.M., Delfgou-van Asch, E.H.M., Beerepoot-Mensink, M.E., van der Meij-Florijn, A., Fitz-James, I., van Leusden, F.M., and Pielaat, A. 2014. Prevalence and concentration of bacterial pathogens in raw produce and minimally processed packaged salads produced in and for the Netherlands. *Journal of Food Protection*, **77**, 388–394.

- Zeng, W., Vorst, K., Brown, W., Marks, B.P., Jeong, S., Pérez-Rodríguez, F., and Ryser, E.T. 2014. Growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in packaged fresh-cut Romaine mix at fluctuating temperatures during commercial transport, retail storage, and display. *Journal of food protection*, **77**, 197–206.
- Zheng, J., Allard, S., Reynolds, S., Millner, P., Arce, G., Blodgett, R.J., and Brown, E.W. 2013. Colonization and internalization of *Salmonella enterica* in tomato plants. *Applied and Environmental Microbiology*, **79**, 2494–2502.
- Zhou, B., Luo, Y., Nou, X., Yang, Y., Wu, Y., and Wang, Q. 2014. Effects of postharvest handling conditions on internalization and growth of *Salmonella enterica* in tomatoes. *Journal of Food Protection*, **77**, 365–370.
- Zhuang, R.Y., Beuchat, L.R., and Angulo, F.J. 1995. Fate of *Salmonella*-Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, **61**, 2127–2131.

CHAPTER 18

Microbial ecology of fruits and fruit-based products

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18.1 Introduction

Fruits constitute a rather diverse substrate regarding their properties that affect microbial growth. The main reservoirs of microorganisms that contaminate fruits are the soil and the irrigation water while domestic and wild animals may be considered as the main vectors of contamination. The presence of protective peels acts as a barrier and prevents entrance of the microorganisms into the nutritious inner tissues. In intact fruits the microbial community is dominated by fungi of the genera *Rhizopus*, *Aspergillus*, *Penicillium*, *Eurotium*, and *Wallemia*, yeasts of the genera *Saccharomyces*, *Zygosaccharomyces*, *Hanseniaspora*, *Candida*, *Debaryomyces*, and *Pichia*, as well as Gram-negative bacteria such as *Pseudomonas* and *Erwinia*. Human pathogens are not usually members of this microcommunity. However, their ability to survive on the surface of many types of fruits has been thoroughly studied. It is very likely that small cuts or bruises may occur during post-harvest operations. In that case, the microorganisms may gain access not only to the nutrients of the inner tissues but also to an acidic environment with pH values that may range from 1.6 in lime to 6.7 in melon and watermelon, and organic acids such as citric, malic, tartaric, and benzoic acids. These conditions then determine the composition of the microecosystem that will be further developed out of the initial indigenous microbiota.

Processing that a fruit may receive may be as minimal as removal of protective tissues, cutting into pieces, and packaging or may be more intense in order to obtain frozen products, juices, products of reduced water activity (e.g., dried fruits), as well as acidified or fermented products. The microbial ecology is then affected by several factors such as the initial microbial load, the efficacy of decontamination procedures, the hygienic conditions during processing, the type of packaging, and the storage temperature. Research has been focused on decontamination strategies aiming to reduce the initial microbial load and packaging conditions able to limit microbial growth. However, the importance of the quality of raw materials is always highlighted. Regarding the fruit products that have received more intense processing (e.g., heating), the processing itself as well as the addition of chemical preservatives usually ensures the absence of any microbial growth.

Until recently, nearly all data regarding the microbial ecology, not only of fruits but also of all food commodities, were based almost exclusively on the application of culture-dependent techniques. However, many microorganisms cannot be cultivated under the conditions used in a laboratory and therefore only a fraction of a microcommunity under study was revealed (Akkermans *et al.*, 1994). It has been reported that less than 1% of the bacterial species present

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in soil may be culturable (Malik *et al.*, 2008); this percentage may increase to more than 10% when nutrient availability is improved, e.g., in the rhizosphere (Hirsch *et al.*, 2010). The advent of modern molecular techniques and especially next-generation sequencing has offered alternative and more integrated approaches to characterize microbial diversity both in qualitative and quantitative terms. Although important limitations still exist, a new insight, especially on environmental microecosystems rather than food-associated ones, has been provided.

The most common human pathogens associated with fresh produce are the soil-associated *Clostridium botulinum*, and *Listeria monocytogenes*, the feces-associated *Salmonella* spp., *Shigella* spp., and *Escherichia coli* O157:H7, the parasites *Cryptosporidium* spp. and *Cyclospora* spp., as well as the hepatitis A virus and the norovirus. In Table 18.1 outbreaks in the United States from 1998 to 2012 associated with the consumption of fruits are presented. A total of 25 fruits were implicated in the outbreaks with tomatoes reported in 33 cases and fruit salads in 21. The most common cause was *Salmonella* spp. (in 54 outbreaks) and the second most common was norovirus (in 47 outbreaks). Regarding the former, *S. Newport* was identified in 14 outbreaks, *S. Javiana* in 7, and *S. Saintpaul* and *S. Typhimurium* in 6. In Figure 18.1 the pathogens responsible for the outbreaks caused by the consumption of fruit salad and tomatoes are shown. In the first case, half of them were caused by norovirus whereas in the latter *Salmonella* was held responsible for the majority of them with norovirus being the second cause.

In the following paragraphs, the factors affecting the microbial ecology of fresh whole, minimally processed, and processed fruits are discussed with a specific focus on microbial food safety, given that this is the leading concern in this food category. Particular emphasis is given on areas of active research interest, such as fresh whole and minimally processed fruits, since the necessary procedures to ensure safety

are under development and evaluation, and less on processed fruit products for which these procedures currently exist.

18.2 Fresh whole fruits

Contamination of the fruits may occur in various ways. The primary source of contamination is the environment, in which the fruits are grown. The soil and any soil amendment that may have been incorporated as well as the irrigation water may be considered as sources of contamination, with animals, either wild or domestic, as vectors of contamination.

The soil itself is a reservoir of various pathogenic microorganisms. There are two important aspects referring to the possibility of contamination of fruits from the soil. First is the level of soil contamination; former use of the land is very important, so, for example, prior use as a grazing field may result in extensive fecal contamination of the land. A similar result may be improper use of animal manures (Beuchat, 1999). Then the ability of the pathogen to survive in the soil actually reflects the possibility of fruit contamination. Extensive research has taken place in order to study the fate of the pathogens after soil contamination and the factors that affect their survival. Survival times as long as three years for *Salmonella*, more than a year for *E. coli* O157:H7, 6 months for *Listeria* spp., 3 months for *Campylobacter* spp., and more than a year for hepatitis A virus have been reported (Nicholson *et al.*, 2005; Kudva *et al.*, 1998; Avery *et al.*, 2005; Seymour and Appleton, 2001).

Irrigation water is a very important means of both chemical and microbiological contamination, generally of fresh produce. It has been reported that pre-harvest contamination with *E. coli* may occur through plant roots as well as by repeated application of contaminated water directly to plant leaves (Solomon *et al.*, 2003; Wachtel *et al.*, 2002a, 2002b). In a study by Oliveira *et al.* (2011) transfer of *L. innocua* from contaminated compost and water to lettuce leaves was assessed. It was reported

Table 18.1 Outbreaks in the United States associated with the consumption of fruits (1998–2012).

year	State	Fruit	Pathogen
1998	Multistate	Tomatoes	<i>S. Baidon</i>
1998	Texas	Strawberries	Hepatitis A
1998	Washington	Mango	<i>S. Oranienburg</i>
1998	Wisconsin	Fruit salad	<i>E. coli</i> O157:H7
1999	Florida	Orange juice	<i>S. Anatum</i>
1999	Florida	Mamey shake	<i>S. Typhi</i>
1999	Minnesota	Grapes	Norovirus Genogroup I
1999	Multistate	Orange juice	<i>S. Muenchen</i>
1999	Multistate	Mango	<i>S. Newport</i>
1999	New Jersey	Mixed fruit	Mycotoxins
1999	Oklahoma	Apple cider	<i>E. coli</i> O157:H7
1999	Tennessee	Mixed fruit	Norovirus Genogroup II
1999	Virginia	Mixed fruit	Norovirus Genogroup I
1999	Washington	Mixed fruit	<i>S. Javiana</i>
1999	Wisconsin	Tomato sauce	<i>Clostridium perfringens</i>
1999	Wisconsin	Melon	Norovirus Genogroup I
2000	California	Squash/tomato	<i>Clostridium botulinum</i>
2000	California	Grapes	<i>E. coli</i> O157:H7
2000	Colorado	Melon	<i>S. Heidelberg</i>
2000	Massachusetts	Strawberries	Hepatitis A
2000	Missouri	Mixed fruit	Norovirus Genogroup I
2000	Multistate	Tomatoes	<i>S. Thompson</i>
2000	Multistate	Orange juice	<i>S. Enteritidis</i>
2000	Ohio	Tomatoes	Norovirus Genogroup II
2000	Ohio	Fruit-based salad	Norovirus Genogroup I
2000	Ohio	Fruit salad	Norovirus Genogroup II
2000	Tennessee	cucumber	Norovirus Genogroup I
2000	Utah	Fruit salad	<i>S. Enteritidis</i>
2000	Wisconsin	Watermelon	<i>E. coli</i> O157:H7
2001	California	Fruit salad	Norovirus Genogroup I
2001	Maryland	Fruit salad	<i>Campylobacter jejuni</i>
2001	Multistate	Mango	<i>S. Saintpaul</i>
2001	multistate	Grapes	<i>S. Senftenberg</i>
2001	New York	Tomatoes	<i>Shigella flexneri</i> ser 2a
2001	Washington	Pears	<i>E. coli</i> O157:H7
2002	Colorado	Fruit salad	<i>S. Newport</i>
2002	Connecticut	Grapes	<i>S. Newport</i>
2002	Florida	Tomatoes	<i>S. Javiana</i>
2002	Massachusetts	Tomatoes	<i>S. Javiana</i>
2002	Minnesota	Melon	Norovirus Genogroup I
2002	Multistate	Tomatoes	<i>S. Newport</i>
2003	California	Tomatoes	<i>S. Virchow</i>
2003	California	Strawberries	<i>S. enterica</i>
2003	California	Mango	<i>S. Saintpaul</i>
2003	California	Banana	Norovirus Genogroup I
2003	New Jersey	Fruit-based salad	Norovirus Genogroup I
2003	Ohio	Apple cider	<i>Cryptosporidium parvum</i>
2004	California	Cucumber	Norovirus Genogroup I
2004	Colorado	Fruit salad	Norovirus Genogroup I
2004	Connecticut	Tomatoes	Norovirus Genogroup I

2004	Illinois	Cucumber salad	<i>E. coli</i> enteropathogenic
2004	Iowa	Fruit salad	Norovirus Genogroup I
2004	New York	Apple cider	<i>E. coli</i> O111; <i>Cryptosporidium parvum</i>
2004	Ohio	Tomatoes	<i>Campylobacter</i> spp.
2005	Colorado	Tomatoes	Norovirus Genogroup I
2005	Georgia	Strawberries	Norovirus Genogroup I
2005	Georgia	Grapes	Norovirus Genogroup I
2005	Idaho	Watermelon	Norovirus Genogroup I
2005	Iowa	Fruit salad	Norovirus Genogroup I
2005	Maryland	Mixed fruit	Norovirus Genogroup II
2005	Multistate	Tomatoes	<i>S. Newport</i>
2005	Multistate	Orange juice	<i>S. Saintpaul</i> ; <i>Typhimurium</i>
2005	Multistate	Grapes	<i>E. coli</i> O157:H7
2005	North Carolina	Squash	Norovirus
2005	Ohio	Fruit salad	<i>E. coli</i> O157:H7
2005	Pennsylvania	Tomatoes	Norovirus Genogroup I
2005	Tennessee	Tomatoes	Hepatitis A
2006	California	Watermelon	Norovirus
2006	Maine	Tomatoes	<i>S. Typhimurium</i>
2006	Minnesota	Fruit salad	<i>Cyclospora cayatenensis</i>
2006	Minnesota	Cucumber salad	Norovirus genogroup I
2006	Multistate	Tomatoes	<i>S. Newport</i>
2006	Multistate	Tomatoes	<i>S. Typhimurium</i>
2006	Multistate	Fruit salad	<i>S. Oranienburg</i>
2006	New York	Watermelon	<i>S. Newport</i>
2006	North Carolina	Mixed fruit	Norovirus
2006	North Dakota	Mixed fruit	Norovirus
2006	Pennsylvania	Tomatoes	<i>S. Berta</i>
2006	Virginia	Watermelon	<i>Campylobacter jejuni</i>
2006	Washington	Mixed fruit	Norovirus Genogroup I
2006	Wisconsin	Mixed fruit	Norovirus Genogroup I
2007	California	Grapes	Norovirus
2007	California	Fruit salad	Norovirus
2007	Colorado	Tomatoes	Norovirus Genogroup I
2007	Florida	Acai, bananas, strawberries, sugar cane juice	Hepatitis A
2007	Georgia	Strawberries	Norovirus Genogroup I
2007	Iowa	Melon	Norovirus Genogroup I
2007	Massachusetts	Apple cider	<i>E. coli</i> O157:H7
2007	Minnesota	Tomatoes	<i>S. Typhimurium</i>
2007	multistate	Tomatoes	<i>S. Newport</i>
2007	New York	Tomatoes	<i>S. Newport</i>
2007	Wisconsin	Fruit salad	Norovirus Genogroup II
2008	California	Mixed berries	<i>Cyclospora cayatenensis</i>
2008	California	Fruit salad	<i>S. Panama</i>
2008	California	Watermelon	<i>S. enterica</i>
2008	Georgia	Grapes	Norovirus Genogroup II
2008	Iowa	Apple cider	<i>E. coli</i> O157:H7
2009	Michigan	Tomatoes	<i>S. Saintpaul</i>
2009	multistate	Melon	<i>S. Carrau</i>
2009	New York	Lemon; lime	<i>Shigella sonnei</i>
2009	Washington	Tomatoes	Norovirus
2010	Florida	Cucumber; tomato	Norovirus Genogroup II
2010	Maryland	Apple cider	<i>E. coli</i> O157:H7

(Continued)

Table 18.1 (Continued)

year	State	Fruit	Pathogen
2010	Michigan	Watermelon	<i>S. Saintpaul</i>
2010	Missouri	Fruit salad	Norovirus Genogroup II
2010	Multistate	Mamey	<i>S. Typhi</i>
2010	Multistate	Tomatoes	<i>S. Javiana</i>
2010	Multistate	Tomatoes	<i>S. Newport</i>
2010	Ohio	Grapes	Hepatitis A
2011	California	Mixed fruit	Norovirus
2011	Colorado	Mixed fruit	Norovirus
2011	Florida	Avocado	<i>S. Braenderup</i>
2011	Georgia	Cucumber; tomato	<i>S. Saintpaul</i>
2011	Minnesota	Strawberries; watermelon	<i>E. coli</i> O157:H7
2011	Minnesota	Cucumber	<i>S. Javiana</i>
2011	Minnesota	Apple cider	<i>E. coli</i> O111; <i>Cryptosporidium parvum</i>
2011	Minnesota	Watermelon	<i>S. Typhimurium</i>
2011	Multistate	Tomatoes	<i>S. Newport</i>
2011	Multistate	Papaya	<i>S. Agona</i>
2011	Multistate	Cantaloupe	<i>L. monocytogenes</i>
2011	Multistate	Cantaloupe	<i>S. Panama</i>
2011	Nevada	Lemon; lime	Norovirus genogroup II
2011	New York	Tomatoes	<i>S. Newport</i>
2011	Oregon	Strawberries	<i>E. coli</i> O157:H7
2011	Oregon	Fruit salad	<i>S. Typhimurium</i>
2012	Michigan	Grape salad	Norovirus Genogroup II
2012	Michigan	Apple cider	<i>E. coli</i> O157:NM (H-)
2012	Multistate	Mango	<i>S. Worthington</i> ; <i>Braenderup</i>
2012	Multistate	Cucumber	<i>S. Javiana</i>
2012	New York	Mixed fruit	<i>S. Javiana</i>
2012	Ohio	Fruit salad	<i>Campylobacter</i> spp.
2012	Oregon	Fruit salad	Norovirus Genogroup II
2012	Pennsylvania	Tomatoes	<i>S. Newport</i>
2012	Virginia	Strawberries	Norovirus

Data from Foodborne Outbreak Online Database (FOOD). Available at: <http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx>.

that transfer of the microorganism from contaminated soil mainly to the outer leaves took place and the survival profiles in both lettuce and soil samples were similar, irrespective of the contamination method. Internalization of *Salmonella* spp. in tomato plants through contaminated irrigation water has also been extensively studied. Jablasone *et al.* (2004) reported failure to detect *Salmonella* Enteritidis in the leaf, stems, and fruits of tomato plants irrigated from soil with artificially contaminated water. In two studies by Miles *et al.* (2009) and Hintz *et al.* (2010), the ability of *S. Montevideo* and *S. Newport*, respectively, for internaliza-

tion in the roots, stems, leaves, and fruit through contaminated irrigation water was assessed. In the former study, *S. Montevideo* was only detected in 16% of the root samples whereas in the latter, the presence of *S. Newport* was confirmed in all tissues with a significant difference according to the tissue (roots > stems > leaves and fruit). It was therefore concluded that *S. Newport* may be capable of contaminating aerial tomato plant tissues provided that the contamination of the irrigation water is persistent and of a high level (7 log cfu/ml). Nevertheless, several outbreaks have been linked with the use of contaminated

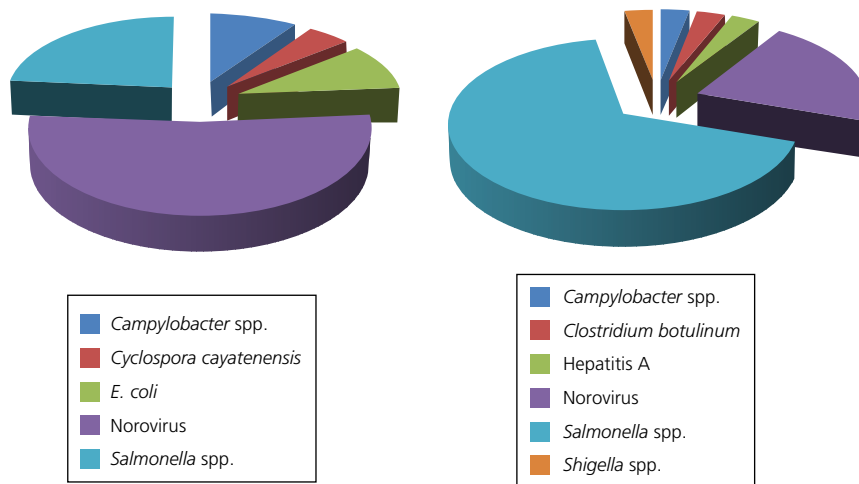


Figure 18.1 Distribution of the pathogens responsible for the outbreaks after consumption of fruit salad (left) and tomatoes (right).

water for irrigation and subsequent spread of *S. Typhimurium* and *E. coli* O157:H7, as well as viruses (Takkinen *et al.*, 2005; Soderstrom *et al.*, 2005; Beuchat, 1996; Josefson, 2003).

Another important aspect that may affect the microbial load of the phyllosphere is the ability of the microorganisms to invade the inner leaf tissues through lesions or open stomata. Indeed, the ability of romaine lettuce leaf tip burn lesions to promote multiplication of *E. coli* O157:H7 over a short period of time and to harbor a rather dense microbial cell population has been exhibited (Brandl, 2008). Moreover, aggregation of *Salmonella enterica* near open stomata of iceberg lettuce leaves and invasion into the inner tissue has been attributed to attraction by nutrients produced by photosynthetically active plant cells (Kroupitski *et al.*, 2009). Therefore, it is becoming a general belief that plant tissues do not simply represent a vehicle for the transmission of human pathogenic microorganisms but rather constitutes a responsive biological system with active and specific interactions; however, this is based on studies mostly on the surface of leafy vegetables and less on fruits.

The importance of produce type and farming practice on the microbial communities associated with the surface of fresh fruits has been

highlighted by Ottesen *et al.* (2009) and Leff and Fierer (2013). In the former study, the impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop was assessed. A quite impressive bacterial diversity was revealed: members of eight phyla and 14 classes were identified. It was reported that in four out of six time points in which this study was performed, significant differences in the respective microcommunities were observed. However, this was not the case regarding the occurrence of human pathogens since no significant differences were reported between either treatment. In all cases, classes Alpha-, Beta-, and Gamma-proteobacteria were by far the most abundant taxonomic groups. In the latter study, the differences in the composition of the microbial communities of various fresh fruits and vegetables were assessed. Gamma-proteobacteria were found to prevail over other classes on the surface of tomatoes, strawberries, and peppers with *Enterobacteriaceae* being the most abundant family. On the contrary, Actinobacteria dominated the surface of apples and peaches and Bacilli the surface of grapes. Similar differences were also observed when comparing organically and conventionally produced fruits. However, these differences could

also be attributed to a number of factors such as growing location, agricultural practices, and logistic conditions. Finally, in an integrated approach by Ottessen *et al.* (2013), the anatomical microbial ecology of a tomato plant was studied. The remarkable biodiversity was accompanied by a spatial distribution of the different microbial communities: different tomato plant organs harbored different microecosystems. Moreover, a gradient of compositional similarity between them was observed and correlated to the distance of a part of the plant from the soil. Regarding *Salmonella*, no definitive presence could be confirmed in any of the samples, despite the presence of closely related genera on leaves, fruits, and roots.

The majority of fresh whole fruits are characterized by the presence of a protective peel that acts as a natural barrier and prevents the microorganisms from reaching the nutritious internal tissues. The fate of several *Salmonella* serovars inoculated on the surface of tomato fruits or applied in a manner that allows internalization in the tomato fruits has been studied to some extent due to the association between consumption of tomato fruits and several salmonellosis outbreaks. *Salmonella* serovars associated with food-borne outbreaks as well as ones isolated regularly from animal or clinical infections were introduced on to the flowers of growing plants in order to study their recovery from the surface and within the developing tomato fruit (Shi *et al.*, 2007). All serovars examined were able to persist and grow on the surface of unripe tomato fruits with *Salmonella* Enteritidis, Typhimurium, and Dublin exhibiting less capacity to grow than serovars Hadar, Montevideo, or Newport. Regarding serovar Montevideo, it appeared to be more adapted to survival within tomatoes. Moreover, it has been exhibited that *S. Montevideo* may remain on the surfaces of inoculated tomatoes stored at 10°C without any significant population change for at least 18 days. On the contrary, when the tomatoes were stored at 20 and 30°C a significant increase in the population was observed within 7 days and 1 day, respectively. Dipping of the

tomatoes into a water suspension of *S. Montevideo* cells resulted in a significant uptake of the pathogen by the core tissue in a temperature-dependent manner, most probably through the stem scar (Zhuang *et al.*, 1995). The ability of *S. Montevideo* to persist on or in tomatoes was also exhibited by Guo *et al.* (2001). In that study, tomato plants were inoculated before and after fruits set, either by injecting stems or by brushing flowers with the inoculum. The pathogen was then detected in the 37% of the tomatoes examined; 82% of them on the surface, 73% in the stem tissue, and 55% in the pulp of the tomatoes. The significance of the stem scar in the internalization of *E. coli* O157:H7 and *Salmonella* Enteritidis was also exhibited in the case of oranges (Shawn Eblen *et al.*, 2004). In that study, 5 out of 200 oranges (i.e., 2.5%) became internally contaminated when the initial thermal equilibration at 37°C was followed by surface inoculation at the stem scar with the pathogen and transfer at 4°C for 3 h. The average internalized population was enumerated at 3.82 log cfu per orange, approximately 0.1% of the initial inoculum. Similarly, 6 out of 200 oranges (3%) became internally contaminated by *Salmonella* Enteritidis when the oranges were subjected to a 13°C temperature differential (from 37 to 24°C) by a mean population of 2.34 log cfu per orange, approximately 0.01% of the initial inoculum. Similar results have been reported by Buchanan *et al.* (1999) regarding the internalization of *E. coli* O157:H7 into intact apples; temperature differential promoted infiltration of the pathogen to the outer core region, most probably through stem and blossom ends.

Another aspect that affects pathogen survival and growth in the nutritious inner fruit tissues is the acidic environment. In Figure 18.2 the pH values of a variety of fruits are shown. The lowest pH value have been reported for lime (1.6–3.2) and passion fruit (1.9–3.3) and the highest for watermelon (5.2–6.7) and melon (6.2–6.7). Regarding the type of acid, almost all fruits contain citric and malic acids; tartaric acid is present in fruits such as grapes and pineapples; benzoic

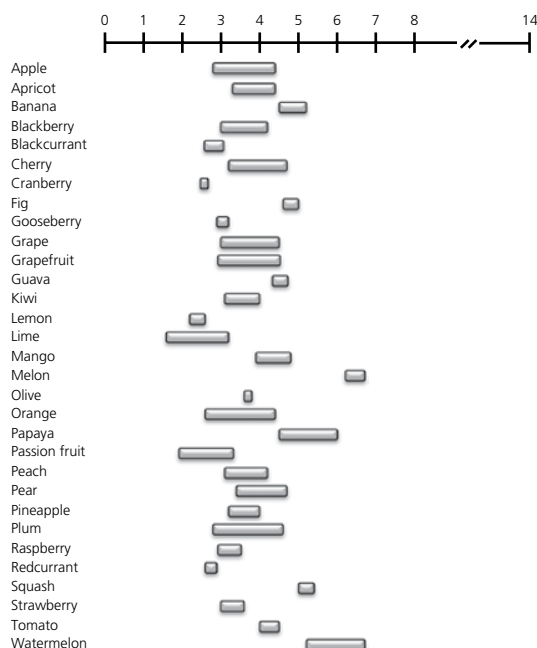


Figure 18.2 pH values of fruits (Jay *et al.*, 1992; Basset and McClure, 2008).

acid in cranberries, raspberries, and plums (FAO, 2003). The antimicrobial potential of all these acids has been extensively studied; they are currently in use as chemical preservatives with very specific attributes and applications. However, all these may not reflect the actual conditions that a microorganism may face upon entrance in the inner tissues; organic acids are usually held within the eukaryotic cell's vacuoles and the pH value of the surrounding tissue is therefore higher. Despite that, microbial invasion requires, most of the time, the presence of some type of physical damage, which may result in the disruption of the vacuole's integrity and a local release of the organic acids.

The uneven distribution of the microorganisms on the surface of field-grown cantaloupes has been revealed by Dobhal *et al.* (2015). The cantaloupe surface that was in direct contact with soil contained more cfu of total aerobic bacteria, coliforms, yeasts, and molds than the area exposed to air. Subsequent washing with a commercial sodium hypochlorite solution

removed a significant portion of the yeasts and molds but failed to reduce the bacterial counts.

Generally survival of pathogens on a fruit surface is improved at storage temperatures of 10°C or below. This was also the case for *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* on intact strawberries (Flessa *et al.*, 2005; Knudsen *et al.*, 2001; Yu *et al.*, 2001), *E. coli* on apples (Abadias *et al.*, 2009), as well as *L. monocytogenes* on peeled Hamlin oranges (Pao *et al.*, 1998) and whole tomatoes (Beuchat and Brackett, 1991). In the case of cut strawberries, reduction of the *L. monocytogenes* population stored for 48 h or 7 days at 24 and 4°C, respectively, was no more than 0.5 log cfu per fruit (Flessa *et al.*, 2005). Uchima *et al.* (2008) and Rezende *et al.* (2009) reported that *Salmonella* spp. and *L. monocytogenes* were able to grow on the peel and pulp of persimmon fruit stored at 10, 20, and 30°C. However, the authors mention the possibility of surface contamination with pulp particles due to experimental mishandling and therefore this result might not reflect the actual *in situ* conditions.

The survival and growth of *Salmonella* Salford, *E. coli*, and *L. innocua* on the surface of passion fruit, banana, cantaloupe, and honeydew melon inoculated at low (ca. 10³ cfu/ml) and high (ca. 10⁵–10⁶ cfu/ml) levels and stored for 13 days at 18°C (bananas), for 6 days at 10°C (passion fruit), for 7 days at 8°C (cantaloupes), and for 1 day at 12°C then 5 days at 8°C (honeydew melons) was studied by Behrsing *et al.* (2003). *E. coli* was the less capable in surviving; after storage time it was not detected on banana, it was detected on passion fruit and on honeydew only after enrichment, and a significant reduction of the population was observed for cantaloupe. The population of *Salmonella* Salford was stable on banana and cantaloupe; on the contrary, enrichment was necessary in order to detect it on passion fruit and honeydew. In the case of *L. innocua*, growth was observed on cantaloupe, a stable population on banana, and a significant reduction of the population on passion fruit and honeydew.

In Table 18.2 data on the prevalence of *Salmonella* spp., *Shigella* spp., *E. coli* O157:H7,

Table 18.2 Prevalence of pathogenic microorganisms on fresh fruits.

	Salmonella spp.	Shigella spp.	E. coli O157:H7	L. monocytogenes	Staphylococcus sp.	Reference
Cantaloupe	8/151 (5.29) ¹	3/151 (1.98)				FDA, 2001
Cantaloupe	4/164 (2.43)	1/164 (0.60)	0/164			FDA, 2003
Cantaloupe	24/2220 (1.06)					Madden, 1992
Cantaloupe	11/1440 (0.76)					Madden, 1992
Cantaloupe	7/9985 ² (0.07)		15/9985 ^{2,3} (0.15)			USDA, 2011
Fresh-cut fruits ⁴	0/21		0/21 ⁵	0/21		Abadias <i>et al.</i> , 2008
Persimmon	5/582 (0.9%)			0/582		Rezende <i>et al.</i> , 2009; Uchima <i>et al.</i> , 2008
Strawberries	1/143 (0.69)	0/151				FDA, 2001
Strawberries	0/139					FDA, 2003
Strawberries	0/173		0/173	1/173 (0.57)	26/173 ⁶ (15.02)	Johannessen <i>et al.</i> , 2002
Strawberries			1/26 (3.84)	1/26 (3.84)		Hadjilouka <i>et al.</i> , 2013
Tomatoes	0/198					FDA, 2003
Tomatoes	2/11582 ² (0.01)		0/11582 ²			USDA, 2011

¹Percentage is given in parenthesis.

²Sum of the data obtained from 2004 to 2009 during the still ongoing microbiological data program.

³Data refer to pathogenic *E. coli*.

⁴Apple, pineapple, orange, mango, peach.

⁵Generic *E. coli*.

⁶Only four of the isolates were identified as *S. aureus*, all of them non-toxinogenic.

L. monocytogenes, and *Staphylococcus* spp. on fresh fruits are given. Only a limited amount of studies regarding this subject have taken place. Most of the data come from surveillance programs by the FDA and the USDA and therefore the fruits that have been studied are associated with food-borne outbreaks. Interestingly, in the case of cantaloupes, the more samples analyzed the less prevalence of a pathogen is reported. This trend is not the case for tomato samples; however, it should be noted that the two positive samples to *Salmonella* reported one in 2005 and one in 2009.

18.3 Minimally processed fruits

Minimal processing procedures have been developed to meet the market demands for safe, like-fresh, convenient products of high nutritional value and less preservatives. The term “minimal processing” thus refers to the minimal necessary interventions in order to obtain products with the above-mentioned characteristics. In the case of fruits it may include coring, cutting, dicing, peeling, shredding, slicing, and finally packaging, depending on the type of fruit.

The microbiological quality and safety of the minimally processed products, throughout shelf life, is defined by the:

- 1 Microbiological quality of the raw materials
- 2 Effectiveness of sanitation treatments that take place before removing of the protective peel
- 3 Hygienic conditions during processing
- 4 Effectiveness of the protective environment (e.g., MAP, active/intelligent packaging)
- 5 Maintenance of the cold chain

The quality of the raw materials is of the utmost importance and largely determines the efficacy of the decontamination procedure applied. Therefore, a number of good agricultural practice (GAP) guidelines have been proposed and may help reduce the risk of contamination; despite that, decontamination strategies are still necessary.

Surface sanitation aims at reducing the microbial load prior to any treatment in order to

minimize contamination of the final product. This usually takes place by washing with water containing an antimicrobial agent, usually chlorine (usually 100 mg free chlorine per liter at pH 6.5–7.0 for 20 min). Chlorine is the most widely used sanitizing agent generally of fresh produce since it is easy to use and relatively inexpensive. However, it is characterized by several disadvantages: it is irritating to skin, corrosive to metal surfaces, it is decomposed by organic residues, and the application may only result to a 2 log reduction of the microbial population (Beuchat *et al.*, 1998; Sapers *et al.*, 1999). The use of several alternative antimicrobials such as ozone, chlorine dioxide, peroxyacetic acid, hydrogen peroxide, trisodium phosphate, and lactic and acetic acids has been extensively studied (Kim and Yousef, 2000; Kim *et al.*, 1999; Wisniewsky *et al.*, 2000; Sapers *et al.*, 1999; Aguayo *et al.*, 2014; Najafi and Khodaparast, 2009). Each method is characterized by specific advantages and disadvantages that should be taken into consideration upon application and are finely reviewed by Sapers (2003).

Several studies currently exist comparing the efficacy of various decontamination strategies. In a study by Sapers *et al.* (1999), a comparison between several commercial washing formulations, chlorine, H₂O₂, or combinations of H₂O₂ with commercial formulations for decontamination of Golden Delicious apples inoculated with *E. coli* was reported. Reduction of 1 to 2 log was achieved in all cases when application took place at 20 °C and a 2.5 log reduction when it took place at 50 °C. The combination of H₂O₂ with acidic surfactants, at 50 °C, proved to be more effective, leading to a 3–4 log reduction. The importance of the quality of the raw materials on the effectiveness of the decontamination procedure has been highlighted by Sapers *et al.* (2000). In that study, H₂O₂-based treatments were used for the decontamination of punctured and non-punctured Golden Delicious apples inoculated with approximately 5 log cfu/g *E. coli*. Application of 5% H₂O₂ resulted in only a

0.58 log reduction of the *E. coli* population on punctured apples compared to the 2.34 log reduction on the non-punctured apples. Similar results were obtained by a two-stage treatment involving 1% APL-Kleen® 245 and 5% H₂O₂; reduction of the *E. coli* population on punctured apples was improved and estimated at 1.62 log cfu/g but was still less than the reduction obtained on non-punctured ones, which was enumerated at 2.83 log cfu/g. In the study by Wisniewsky *et al.* (2000), the efficacy of peroxyacetic acid, chlorine dioxide, and chlorine-phosphate buffer solutions to reduce the population of *E. coli* O157:H7 that was inoculated at approximately 6–8 log cfu/g on whole Braeburn apples was reported. Both peroxyacetic acid and chlorine-phosphate buffer solutions achieved a 5 log reduction when used at 2.1 to 14 times and 3 to 15 times the recommended concentration, respectively, depending on the length of the wash time. On the contrary, chlorine dioxide could not achieve such a population reduction. Finally, Abadias *et al.* (2011) evaluated carvacrol, vanillin, peroxyacetic acid, H₂O₂, *N*-acetyl-L-cysteine and Citrox for their effectiveness to reduce the population of a mixture of *E. coli* O157:H7, *Listeria* spp., and *Salmonella* spp. on fresh-cut apple. It has been concluded that peroxyacetic acid, H₂O₂, and *N*-acetyl-L-cysteine may replace chlorine once the effect on sensorial properties as well as the effectiveness under commercial processing conditions is evaluated.

In Table 18.3 new and emerging technologies that have been used for fruit decontamination are presented. Some of them, such as gamma irradiation and the use of silver nitrate, have been studied for many years and their advantages and limitations are well known, while others, such as pulsed light and cold atmospheric plasma, have only recently emerged and therefore more experimental data on their effectiveness are required. A strategy to increase the effectiveness and possibly reduce the limitations of the individual techniques is their combination. The effectiveness

of the combination of vanillin with conventional preservation systems has been studied by Cerrutti *et al.* (1997). Strawberry puree was effectively stored for at least 60 days at room temperature by a combination of mild heat treatment, calcium lactate (1000 ppm), ascorbic acid (500 ppm), *a_w* (to 0.95), and pH reduction (to 3 with citric acid) and vanillin addition (3000 ppm). This combination inhibited growth of *B. coagulans*, *Lb. delbrueckii* as well as *Z. bailii*, *Z. rouxii*, and *P. membranifaciens*. Better color preservation was obtained at temperatures below 10 °C. Similar results were obtained for banana puree with the addition of ascorbic acid (0.2 ml/g), reduction of *a_w* (to final value of 0.97) and pH (to final value of 3.3), and addition of potassium sorbate (1000 ppm) and vanillin (1000 or 3000 ppm). Addition of both potassium sorbate and vanillin (3000 ppm) resulted in optimum preservation since no microbial growth was detected for 60 days at 15, 25, and 35 °C. Only browning limited the shelf-life of the product (Castanon *et al.*, 1999). In both cases vanillin affected the organoleptic properties of the products.

A combination of UV-C irradiation with heat treatment to improve post-harvest life of strawberries was studied by Pan *et al.* (2004). A reduction of total phenolic compounds but no significant changes in the total sugar content and titratable acidity was reported. Regarding microbial stability, the combined treatment reduced fungal infections and delayed *in vitro* germination of *Botrytis cinerea* conidia.

The combined effect of high hydrostatic pressure (100 to 300 MPa) and temperature (–5 to 45 °C) on the inactivation of *Z. bailii* and *L. monocytogenes* in artificially contaminated smoothies was studied by Scolari *et al.* (2015). It was reported that when 300 MPa was combined with either a 45 or –5 °C reduction of the population more than 5 log cfu/ml was obtained. On the contrary, a reduction of only 2–3 log cfu/ml was obtained at temperatures closer to room temperature. In a similar study by Zimmermann *et al.* (2013) aiming at the inactivation of *B. coagulans* spores in tomato pulp the

Table 18.3 New and emerging technologies studied for fruit decontamination.

Method	Advantages	Limitations	References
Biological control	Is considered as a "natural" method	No more than 1–2 log reduction of pathogen population Safety considerations	Trias <i>et al.</i> , 2008; Allende <i>et al.</i> , 2007; Randazzo <i>et al.</i> , 2009; Daniel <i>et al.</i> , 2006; Kocharunchitt <i>et al.</i> , 2009; Viazis <i>et al.</i> , 2011
Cold atmospheric plasma	Significant potential for sanitation	Not thoroughly studied yet Variety and complexity of the necessary equipment	Critzer <i>et al.</i> , 2007; Perni <i>et al.</i> , 2008a, 2008b; Niemira and Sites, 2008
Electrolyzed water	Improvement of visual acceptability	Similar effectiveness to chlorine Negative effects on browning, texture, and nutritional value	Gomez-Lopez <i>et al.</i> , 2007; Rico <i>et al.</i> , 2008
Essential oils	Is considered as a "natural" method	Similar effectiveness to chlorine Negative effect on organoleptic properties	Tzortzakis 2009; Gunduz <i>et al.</i> , 2009, 2010
Gamma-irradiation	Very effective on microbial inactivation Delays maturation	Consumer disapproval Negative effects on texture and nutritional properties	Mohacsi-Farkas <i>et al.</i> , 2014; Cabo Verde <i>et al.</i> , 2013; Prakash <i>et al.</i> , 2000
High-pressure processing	Microbial and enzymatic inactivation No effect on nutritional value and flavor	Possible negative effect on texture High cost of application	Considine <i>et al.</i> , 2008; Guerrero-Beltran <i>et al.</i> , 2005; Hsu <i>et al.</i> , 2014
Pulsed light	Very effective on microbial inactivation	Negative effects on color, texture, headspace gas composition, and nutritional value	Ramos-Villarroel <i>et al.</i> , 2012; Oms-Oliu <i>et al.</i> , 2010
Silver nitrate UV light	More effective than chlorine Induction of health promoting compound synthesis More than 1 log reduction in microbial population	Residual toxicity May result in off-flavors and color changes Low penetration depth	Gopal <i>et al.</i> , 2010; WHO, 2003 Neves <i>et al.</i> , 2012; Fonseca and Rushing, 2006; Fernandez-Suarez <i>et al.</i> , 2013

importance of higher temperatures was highlighted. When 300 MPa was combined with 50 and 60°C the reduction was 2 and 2.4 log, respectively. The reduction was more pronounced when the pressure was increased to 600 MPa and combined with 50 and 60°C, 3.1 and 5.7 log cfu, respectively.

Application of modified atmosphere packaging is not new to the fresh produce industry. However, due to the increasing number of food-borne outbreaks related to such products and the market trend towards minimally processed products, this topic has re-emerged.

Generally, there are two approaches to modified atmosphere packaging: passive and active MAP. Passive MAP is typically applied to fresh produce; the product is sealed under ambient atmosphere using a permeable film. The product will consume O₂ through respiration and produce CO₂, thus changing the atmosphere within the packaging towards an equilibrium of high CO₂ and low O₂ that depends on the permeability of the film. This type of equilibrium reduces the respiration rate of the produce, thus delaying senescence and extending shelf life. Care should be taken to observe the levels of both O₂ and CO₂; adequate amounts of O₂ are necessary in order to avoid fermentative catabolism that will result in the formation of off-flavors and rapid physical degradation of the produce, whereas tolerance to high CO₂ levels differs according to the fruit. In the active MAP, a gas mixture is introduced into the container and then sealed with a permeable film. The permeability of the film remains a critical aspect that defines the effectiveness of the packaging. In both cases, optimization of the packaging should take place according to the type of product.

In a study by Abadias *et al.* (2012) *E. coli* O157:H7 grew well on the surface of fresh-cut melon stored under passive MAP using a micro-perforated film (O₂ and CO₂ permeability of 3500 cm³/m² day atm at 23°C) at 25°C, reaching a final population of 8.5 log cfu/g, while the respective under air was 8.9 log cfu/g. On the contrary, no growth was observed during

storage at 5°C; however, the pathogen survived throughout the studied period. When the pathogen was inoculated on the surface of fresh-cut pineapple no growth was observed but the pathogen survived throughout the studied period. Gunes and Hotchkiss (2002) reported that modified atmosphere high in CO₂ (>15%) and low in O₂ (<1%) inhibited growth of *E. coli* O157:H7 inoculated on fresh-cut apple at both 15 and 20°C compared to the respective in air, which increased 1 log after 2 weeks.

Alegre *et al.* (2010a) studied the effect of apple variety (Golden Delicious, Granny Smith, Shampion), storage temperature (10, 20, 25°C), treatment with antioxidants (ascorbic acid 2%; NatureSeal® 6%), and packaging in passive MAP (polypropylene film with O₂ transfer rate of 40 000 cm³/m² 24h) on the growth of *E. coli* O157:H7, *Salmonella*, and *L. innocua*. It was concluded that the storage temperature actually defined the population level of the pathogens: the lower the temperature the lower the generation time. All cultivars promoted growth of the pathogens at 25°C. Treatment with antioxidant compounds as well as passive MAP packaging had no effect on pathogen growth. In a similar study, the effect of peach variety (Diana, Royal Glory, Elegant Lady, and Placido), storage temperature (5, 25°C), treatment with 2% ascorbic acid, and packaging in passive MAP (polypropylene film with O₂ transfer rate of 40 000 cm³/m² 24h) on the growth of *E. coli* O157:H7, *Salmonella*, and *L. innocua* was assessed by Allegre *et al.* (2010b). Differences in the growth of the pathogens was observed between the varieties tested with higher population increases in Royal Glory and Diana varieties and assigned to their higher pH values. Passive MAP as well as ascorbic acid treatment had no effect on the growth of the pathogens.

The effect of citron EO, hexanal, 2-(E)-hexenal, citral, and carvacrol alone or in combination on the shelf life of minimally processed apples packaged in active MAP 7% O₂ and 0% CO₂ (CO₂ permeability: 2720 cm³/m² day, O₂ permeability 986 cm³/m² day, both at 22°C) and stored at 6°C was assessed by Siroli *et al.*

(2014). It was exhibited that product shelf life was determined by changes in color and texture rather than microbial growth. Samples treated with a combination of 2-(E)-hexenal with citral or hexanal exhibited good retention of color, while the latter promoted the best retention of firmness throughout the storage time.

Active and intelligent packaging, although not new as a concept, is a field that has advanced considerably over the last few years. The term “active” refers to substances added in or on the packaging material in order to enhance the performance of the packaging system. Active packaging systems usually contain oxygen scavengers, carbon dioxide emitters, moisture absorbers, ethanol emitters, ethylene scavengers, odor absorbers, or natural preservatives and antioxidants. The term “intelligent” refers to indicators that are incorporated in the packaging system and are able to inform the consumer about the quality of the product. Intelligent packaging devices include sensors, time–temperature indicators, gas sensing dyes, microbial growth indicators, physical shock indicators, etc. (Pereira de Abreu *et al.*, 2012). Application of this type of packaging has been extensively studied for muscle-based products. In the case of fruits and fruit-based products, only a limited amount of studies have been published. Abe and Watada (1991) used an ethylene scavenger based on palladium chloride and managed to reduce the rate of softening in kiwi fruits and bananas. A combination of MAP with granular activated carbon impregnated with palladium as an ethylene absorber was effectively used to maintain tomato fruit quality during cold storage (Bailen *et al.*, 2006). Similarly, ethylene production and thus prolongation of shelf life of climacteric fresh produce like bananas and avocados was controlled by a palladium-based ethylene absorber developed by Smith *et al.* (2009). Jawaheer *et al.* (2003) reported the development of a sensor for the detection of metabolites – markers of maturity stage and quality of pineapple, mango, and papaya. The optimization of an active packaging

system based on the release of 2-nonanone in order to increase the shelf life of wild strawberries was reported by Almenar *et al.* (2007, 2009). Finally, the development of a glucose biosensor for rapid assessment of strawberry quality was reported by Bordonaba and Terry (2009). The biosensor was constructed using glucose oxidase immobilized on to Meldolas Blue mediated screen printed electrodes and was able to discriminate and rank eight different strawberry cultivars according to their glucose content.

A very interesting alternative for protection of fresh produce from mechanical, physicochemical, and microbiological deterioration is the edible coating, which may act as a water and/or oxygen barrier as well as a vehicle of antimicrobial and antioxidant compounds. Ready-to-eat, minimally processed fresh-cut fruits are a field in which a lot of research has taken place regarding this approach over the last decade.

Prolongation of pineapple shelf life by using an alginate-based coating containing *trans*-cinnamaldehyde or lemongrass essential oil has been studied by Mantilla *et al.* (2013) and Azarakhsh *et al.* (2014), respectively. In both cases the shelf life was extended, growth of yeasts/molds was inhibited, and organoleptic properties were negatively affected. In the first study no effect on color, texture, and pH value was reported, while in the latter a decrease in firmness was evident. Similar results were obtained by Sipahi *et al.* (2013) that used an alginate-based edible coating containing *trans*-cinnamaldehyde on fresh-cut watermelon. Firmness was significantly enhanced; pH and °Brix were unaffected while flavor remained at an acceptable level throughout the time of the study. Regarding the microbiological quality, growth of psychrotrophs, coliforms, yeasts, and molds were inhibited. Incorporated into a chitosan-based matrix, *trans*-cinnamaldehyde has been effectively used for the extension of melon (group Inodorous) shelf life as well; coated samples were reported to be firmer and maintained color and total carotenoid content and showed less weight loss than the controls (Moreira *et al.*, 2014).

Similar results were also obtained by Martinon *et al.* (2014) concerning cantaloupe. In the latter study *trans*-cinnamaldehyde concentration of 2g/100g was proposed in order to combine the antimicrobial action with acceptable sensorial attributes. Finally, the effect of different concentrations of chitosan coating applied pre- or post-harvest on raspberry shelf life was studied by Tezotto-Uliana *et al.* (2014). Application of 1 or 2% chitosan post-harvest and 2% pre-harvest was successful in maintaining the titratable acidity and in delaying respiration, ethylene production, and weight loss. Firmness was only retained when 2% chitosan was applied pre-harvest.

18.4 Processed fruits

In this section, the factors affecting the microbiota of fruit products that have undergone a more intense treatment will be discussed. Such products are frozen, dried, fermented, and acidified fruits as well as dried fruits.

18.4.1 Dried fruits

Drying is a very important procedure that alters both physicochemical properties and nutritional value of the product. The effect on the microbiota depends upon the type of fruit, the severity of the process itself, the final water activity value, and treatment with sulfur dioxide prior to drying. Regarding the latter, fruits such as apricots, bananas, peaches, and pears require treatment with SO₂ to prevent non-enzymatic browning; this will practically eliminate all microbiota throughout shelf life. On the other hand, fruits such as dates, figs, and prunes are not treated with SO₂ and therefore are more interesting from a microbiological point of view. Such products are susceptible to spoilage by xerophilic fungi such as *Xeromyces bisporus* that may limit the shelf life of the product.

18.4.2 Frozen fruits

Before freezing, fruits may undergo a pre-treatment such as blanching, which aims at inactivating enzymes, treatment with citric

acid, dipping into sulfate solution to control browning, or treatment with ascorbic acid to control oxidation. All these pre-treatments greatly reduce the initial microbial load of the fruits. However, it has been shown that pathogenic bacteria and viruses may survive in frozen fruits for extended periods of time. Indeed, an outbreak of typhoid fever in the US has been associated with contamination of frozen mamey with *S. Typhi* (Katz *et al.*, 2002), outbreaks of hepatitis A in the US have been linked to frozen strawberries (Ramsay and Upton, 1989; CDC, 1997), and a Calicivirus outbreak in Finland to frozen raspberries (Ponka *et al.*, 1999). Moreover, *L. monocytogenes* and *E. coli* O157:H7 exhibited similar survival capacity in frozen strawberries; both pathogens were able to survive for at least one month at -20°C. An *L. monocytogenes* population reduction was estimated at 0.5 and 1.8 log cfu per sample and *E. coli* O157:H7 at 0.7 and 2.2 log, with or without the addition of 20% sucrose, respectively (Knudsen *et al.*, 2001; Flessa *et al.*, 2005). Similarly, a cocktail of four strains of *E. coli* O157:H7 and five serotypes of *Salmonella* (Michigan, Montevideo, Muenchen, Newport, and Saintpaul) were inoculated on cut pineapples, mangoes, and papayas and frozen at -20°C. In the case of pineapples, reduction of the population of both pathogens was enumerated at ca. 2.5 log cfu/g and in the case of mangoes and papayas at ca. 2.0 log cfu/g after 180 days of storage (Strawn and Danyluk, 2010a, 2010b).

18.4.3 Fruit juices

Fruit juices are the aqueous solutions that are obtained from the edible part of appropriately matured fruits and may be used as such or as ingredients in beverages. The most important properties that affect microbial growth are the pH value and the type of acid present. Decontamination of the raw materials as well as pasteurization of the final product are the two most important steps in the respective production procedure. Survival or even growth of human pathogens in fruit juices and apple cider

has been well documented (Miller and Kaspar, 1994; Ceylan *et al.*, 2004; Parish *et al.*, 1997; Ingham *et al.*, 2006; Raybaudi-Massilia *et al.*, 2006; Caggia *et al.*, 2009). Three major multi-state outbreaks in the US have been linked with the consumption of unpasteurized juices: the 1996 outbreak of *E. coli* O157:H7 that was linked with the consumption of unpasteurized apple juice (CDC, 1996), the 1999 outbreak of *S. Muenchen* that was linked to the consumption of unpasteurized orange juice (CDC, 1999) and the 2005 outbreak of *S. Typhimurium* and *S. Saintpaul* associated with unpasteurized orange juice (Jain *et al.*, 2009). Moreover, the more recent outbreak of hepatitis A among tourists from Egypt that occurred in 2004 with orange juice as the most likely infection vehicle may serve as an example of a general failure to the hygienic conditions during processing (Frank *et al.*, 2007).

Despite the relative importance of pasteurization, there is still a consumer trend towards unpasteurized juices whose safety depends solely on the establishment of proper GMP as well as design and implementation of an HACCP system. Alternatively, application of non-thermal treatments, such as high hydrostatic pressure (Jordan *et al.*, 2001; Parish *et al.*, 1998; Zook *et al.*, 1999), pulsed electric fields (Yeom *et al.*, 2000; Evrendilek *et al.*, 1999; Noci *et al.*, 2008), ozone (Williams *et al.*, 2004; Steenstrup and Floros, 2004; Torlak, 2014a), neutral electrolyzed water (Torlak, 2014b), UV light (Guerrero-Beltran and Barboza-Canovas, 2005; Murakami *et al.*, 2006; Gabriel and Nakano, 2009), and irradiation (Buchanan *et al.*, 1998) and their effect on the inactivation of both spoilage and pathogenic microorganisms has been examined and their relative advantages and limitations have been exhibited.

Regarding the spoilage microbiota, *Alicyclobacillus* spp. has been reported to be the most important spoilage bacterium regarding juices; because of its thermoacidophilic nature and its spore-forming capacity it can readily grow in high acidic juices and result in characteristic off-flavors and taste deterioration. Inactivation of

these bacteria has been extensively studied and several chemical, physical, and combined methods have been proposed for their control (Tianli *et al.*, 2014). Spoilage may also occur by lactic acid bacteria belonging to the genera *Lactobacillus* and *Leuconostoc*, acetic acid bacteria such as *Acetobacter aceti* and *A. pasteurianus*, and yeasts of the genera *Candida*, *Pichia*, *Rhodotorula*, *Torulopsis*, *Saccharomyces*, *Zygosaccharomyces*, *Hansenula*, and *Trichosporon*, which have been associated with spoilage of fruit juices (Tribst *et al.*, 2009).

Regarding molds, particular interest lies in the production of mycotoxins. Only patulin and ochratoxin A are currently considered to be important for fruit products. The former is produced by *Penicillium expansum* in apples and pears while the latter from *Aspergillus carbonarius* in grapes. Although many studies reporting the occurrence of these mycotoxins in products throughout the world currently exist, literature is lacking data regarding the specific conditions that affect their *in situ* production.

18.4.4 Fermented and acidified fruits

A wide variety of fruits has been traditionally utilized as a substrate for lactic acid fermentation. Among them, olive and cucumber fermentation have met worldwide commercial significance, whereas the production of regional products, such as tempoyak and fermented caper berries, has been recently studied.

Olive and cucumber fermentations are usually dominated by *Lb. plantarum* and secondarily by *Lb. brevis* and *Ln. mesenteroides*. The microbial stability of the final product is ensured by the low pH values (typically 3.6–4.2), the increase of the NaCl concentration to 8% or higher after the end of fermentation, and the pasteurization that takes place. This is also the case for acidified fruits and thus an excellent safety record has been so far achieved. Despite that, recent studies by Cho *et al.* (2011) and Paramithiotis *et al.* (2012) have exhibited the ability of *E. coli* O157:H7 and *L. monocytogenes* to survive during spontaneous cabbage and

radish kimchii fermentation as well as the ability of *L. monocytogenes* and *S. Typhimurium* to survive during spontaneous cauliflower fermentation. In a study by Kanellou *et al.* (2013), the pH value was identified as the only factor significantly affecting survival of *L. monocytogenes* and *S. Typhimurium* survival in acidified fruits and vegetables products; an increase by 1 unit in the pH value of the product resulted in an increase of the relative risk for *L. monocytogenes* and *S. Typhimurium* survival by a factor of ca. 24 and 40, respectively.

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References

- Abadias, M., Usall, J., Anguera, M., Solsona, C., and Vinas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, **123**, 121–129.
- Abadias, M., Usall, J., Alegre, I., Torres, R., and Vinas, I. 2009. Fate of *Escherichia coli* in apple and reduction of its growth using the postharvest bio-control agent *Candida sake* CPA-1. *Journal of the Science of Food and Agriculture*, **89**, 1526–1533.
- Abadias, M., Alegre, I., Usall, J., Torres, R., and Vinas, I. 2011. Evaluation of alternative sanitizers to chlorine disinfection for reducing food-borne pathogens in freshcut apple. *Postharvest Biology and Technology*, **59**, 289–297.
- Abadias, M., Alegre, I., Oliveira, M., Altisent, R., and Vinas, I. 2012. Growth potential of *Escherichia coli* O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control*, **27**, 37–44.
- Abe, K. and Watada, A. 1991. Ethylene absorbent to maintain quality of lightly processed fruits and vegetables. *Journal of Food Science*, **56**, 1589–1592.
- Aguayo, E., Escalona, V., Silveira, A.C., and Artes, F. 2014. Quality of tomato slices disinfected with ozonated water. *Food Science and Technology International*, **20**, 227–235.
- Akkermans, A.D.L., Mirza, M.S., Harmse, H.J.M., Blok, H.J., Herron, P.R., Sessitsch, A., and Akkermans, W.M. 1994. Molecular ecology of microbes: a review of promises, pitfalls and true progress. *FEMS Microbiology Reviews*, **15**, 185–194.
- Alegre, I., Abadias, M., Anguera, M., Oliveira, M., and Vinas, I. 2010a. Factors affecting growth of foodborne pathogens on minimally processed apples. *Food Microbiology*, **27**, 70–76.
- Alegre, I., Abadias, M., Anguera, M., Usall, J., and Vinas, I. 2010b. Fate of *Escherichia coli* O157:H7, *Salmonella* and *Listeria innocua* on minimally-processed peaches under different storage conditions. *Food Microbiology*, **27**, 862–868.
- Allende, A., Martínez, B., Selma, V., Gil, M.I., Suarez, J.E., and Rodríguez, A. 2007. Growth and bacteriocin production by lactic acid bacteria in vegetable broth and their effectiveness at reducing *Listeria monocytogenes* *in vitro* and in freshcut lettuce. *Food Microbiology*, **24**, 759–766.
- Almenar, E., Catala, R., Hernandez-Munoz, P., and Gavara, R. 2009. Optimization of an active package for wild strawberries based on the release of 2-nonanone. *LWT – Food Science and Technology*, **42**, 587–593.
- Almenar, E., Del Valle, V., Catala, R., and Gavara, R. 2007. Active package for wild strawberry fruit (*Fragaria vesca* L.). *Journal of Agricultural and Food Chemistry*, **55**, 2240–2245.
- Avery, L.M., Killham, K., and Jones, D.L. 2005. Survival of *E. coli* O157: H7 in organic wastes destined for land application. *Journal of Applied Microbiology*, **98**, 814–822.
- Azarakshsh, N., Osman, A., Ghazali, H.M., Tan, C.P., and Adzahan, N.M. 2014. Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality

- retention of fresh-cut pineapple. *Postharvest Biology and Technology*, **88**, 1–7.
- Bailen, G., Guillen, F., Castillo, S., Serrano, M., Valero, D., and Martinez-Romero, D. 2006. Use of activated carbon inside modified atmosphere packages to maintain tomato fruit quality during cold storage. *Journal of Agricultural and Food Chemistry*, **54**, 2229–2235.
- Bassett, J. and McClure, P. 2008. A risk assessment approach for fresh fruits. *Journal of Applied Microbiology*, **104**, 925–943.
- Behrsing, J., Jaeger, J., Horlock, F., Kita, N., Franz, P., and Premier, R. 2003. Survival of *Listeria innocua*, *Salmonella* Salford and *Escherichia coli* on the surface of fruit with inedible skins. *Postharvest Biology and Technology*, **29**, 249–256.
- Beuchat, L.R. 1996. Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection* **59**, 204–216.
- Beuchat, L.R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *Journal of Food Protection*, **62**, 845–849.
- Beuchat, L.R. and Brackett, R.E. 1991. Behavior of *Listeria monocytogenes* inoculated into raw tomatoes and processed tomato products. *Applied and Environmental Microbiology*, **57**, 1367–1371.
- Beuchat, L.R., Nail, B.V., Adler, B.B., and Clavero, M.R.S. 1998. Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes, and lettuce. *Journal of Food Protection*, **61**, 1305–1311.
- Bordonaba, J.G. and Terry, L.A. 2009. Development of a glucose biosensor for rapid assessment of strawberry quality: relationship between biosensor response and fruit composition. *Journal of Agricultural and Food Chemistry*, **57**, 8220–8226.
- Brandl, M.T. 2008. Plant lesions promote the rapid multiplication of *Escherichia coli* O157:H7 on postharvest lettuce. *Applied and Environmental Microbiology*, **74**, 5285–5289.
- Buchanan, R.L., Edelson, S.G., Snipes, K., and Boyd, G. 1998. Inactivation of *Escherichia coli* O157:H7 in apple juice by irradiation. *Applied and Environmental Microbiology*, **64**, 4533–4535.
- Buchanan, R.L., Edelson, S.G., Miller, R.L., and Sapers, G.M. 1999. Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *Journal of Food Protection*, **62**, 444–450.
- Cabo Verde, S., Trigo, M.J., Sousa, M.B., Ferreira, A., et al. (2013) Effects of gamma radiation on raspberries: safety and quality issues. *Journal of Toxicology and Environmental Health, Part A*, **76**, 291–303.
- Caggia, C., Scifo, G.O., Restuccia, C., and Randazzo C.L. 2009. Growth of acid-adapted *Listeria monocytogenes* in orange juice and in minimally processed orange slices. *Food Control*, **20**, 59–66.
- Castanon, X., Argaz, A., and Lopez-Malo, A. 1999. Effect of storage temperature on the microbial and color stability of banana puree with addition of vanillin or potassium sorbate. *Food Science and Technology International*, **5**, 53–60.
- CDC. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice. *Morbidity and Mortality Weekly Report*, **48**, 975.
- CDC. 1997. Hepatitis A associated with consumption of frozen strawberries – Michigan 1997. *Morbidity and Mortality Weekly Report*, **46**, 295.
- CDC. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice. *Morbidity and Mortality Weekly Report*, **48**, 582–585.
- Cerrutti, P., Alzamora, S.M., and Vidales, S.L. 1997. Vanillin as an antimicrobial for producing shelf-stable strawberry puree. *Journal of Food Science*, **62**, 608–610.
- Ceylan, E., Fung, D.Y.C., and Sabah, J.R. 2004. Antimicrobial activity and synergistic effect of cinnamon with sodium benzoate or potassium sorbate in controlling *Escherichia coli* O157:H7 in apple juice. *Journal of Food Science*, **69**, FMS102–FMS106.
- Cho, G.-Y., Lee, M.H., and Choi, C. 2011. Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during kimchi fermentation supplemented with raw pork meat. *Food Control*, **22**, 1253–1260.

- Considine, K.M., Kelly, A.L., Fitzgerald, G.F., Hill, C., and Sleator, R.D. 2008. High pressure processing – effects on microbial food safety and food quality. *FEMS Microbiology Letters*, **281**, 1–9.
- Critzer, F., Kelly-Wintenberg, K., South, S., and Golden, D. 2007. Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces. *Journal of Food Protection*, **70**, 2290–2296.
- Daniel, C., Poiret, S., Goudercourt, D., Dennin, V., Leyer, G., and Pot, B. 2006. Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Applied and Environmental Microbiology*, **72**, 5799–5805.
- Dobhal, S., Zhang, G., Gautam, D., Fletcher, J., and Ma, L.M. 2015. Uneven distribution of microorganisms on the surface of field-grown cantaloupes. *Food Control*, **47**, 185–189.
- Evrendilek, G.A., Zhang, Q.H., and Richter, E.R. 1999. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. *Journal of Food Protection*, **62**, 793–796.
- FAO. 2003. General considerations for preservation of fruits and vegetables. In: *Handling and Preservation of Fruits and Vegetables by Combined Methods for Rural Areas*, pp. 39–54. Food and Agriculture Organization of the United Nations, Rome, Italy.
- FDA. 2001. FDA survey of imported fresh produce. FY 1999 Field Assignment. Available at: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118891.htm>, accessed September 2014.
- FDA. 2003. FDA survey of imported fresh produce. FY 2000/2001 Field Assignment. Available at: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm118306.htm>, accessed September 2014.
- FDA. 2008. Irradiation in the production, processing and handling of foods. 21 CFR 179.26. Code of Federal Regulations. Available at: <http://www.fda.gov/Food/FoodIngredientsPackaging/IrradiatedFoodPackaging/ucm074734.htm>, accessed September 2014.
- Fernandez-Suarez, R., Ramirez-Villatoro, G., Diaz-Ruiz, G., Eslava, C., Calderon, M., Navarro-Ocana, A., Trejo-Marquez, A., and Wachter C. 2013. Effect of postharvest UV-C treatment on the bacterial diversity of Ataulfo mangoes by PCR-DGGE, survival of *E. coli* and antimicrobial activity. *Frontiers in Microbiology*, **4**, 134.
- Flessa, S., Lusk, D.M., and Harris, L.J. 2005. Survival of *Listeria monocytogenes* on fresh and frozen strawberries. *International Journal of Food Microbiology*, **101**, 255–262.
- Fonseca, J.M. and Rushing, J.W. 2006. Effect of ultraviolet-C light on quality and microbial population of fresh-cut watermelon. *Postharvest Biology and Technology*, **40**, 256–261.
- Frank, C., Walter, J., Muehlen, M., Jansen, A., et al. 2007. Major outbreak of hepatitis A associated with orange juice among tourists Egypt 2004. *Emerging Infectious Diseases*, **13**, 156–158.
- Gabriel, A.A. and Nakano, H. 2009. Inactivation of *Salmonella*, *E. coli* and *Listeria monocytogenes* in phosphate-buffered saline and apple juice by ultraviolet and heat treatments. *Food Control*, **20**, 443–446.
- Gomez-Lopez, V.-M., Ragaert, P., Ryckeboer, J., Jeyachandran, V., Debevere, J., and Devlieghere, F. 2007. Shelf-life of minimally processed cabbage treated with neutral electrolysed oxidising water and stored under equilibrium modified atmosphere. *International Journal of Food Microbiology*, **117**, 91–98.
- Gopal, A., Coventry, J., Wan, J., Roginski, H., and Ajlouni, S. 2010. Alternative disinfection techniques to extend the shelf-life of minimally processed iceberg lettuce. *Food Microbiology*, **27**, 210–219.
- Guerrero-Beltran, J.A. and Barboza-Canovas, G.V. 2005. Reduction of *Saccharomyces cerevisiae*, *Escherichia coli* and *Listeria innocua* in apple juice by ultraviolet light. *Journal of Food Process Engineering*, **28**, 437–452.
- Guerrero-Beltran, J.A., Barbosa-Canovas, G.V., and Swanson, B.G. 2005. High hydrostatic pressure processing of fruit and vegetable products. *Food Reviews International*, **21**, 411–425.
- Gunduz, G.T., Gonul, S.A., and Karapinar, M. 2009. Efficacy of myrtle oil against *Salmonella*

- Typhimurium on fresh produce. *International Journal of Food Microbiology* **130**, 147–150.
- Gunduz, G.T., Gonul, S.A., and Karapinar, M. 2010. Efficacy of sumac and oregano in the activation of *Salmonella* Typhimurium on tomatoes. *International Journal of Food Microbiology*, **141**, 39–44.
- Gunes, G.G. and Hotchkiss, J.H. 2002. Growth and survival of *Escherichia coli* O157:H7 on fresh-cut apples in modified atmospheres at abusive temperatures. *Journal of Food Protection*, **65**, 1641–1645.
- Guo, X., Chen, J., Brackett, R.E., and Beuchat, L.R. 2001. Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Applied and Environmental Microbiology*, **67**, 4760–4764.
- Hadjilouka, A., Mantzourani, K.-S., Koubou, V., Paramithiotis, S., Mataragas, M and Drosinos, E.H. 2013. Prevalence of *Listeria monocytogenes* and *E. coli* O157:H7 in rocket (*Eruca sativa*), cucumber (*Cucumis sativus*) and strawberry (*Fragaria ananassa*) samples. In: *XI International Controlled and Modified Atmosphere Research Conference*, Trani, 3–7 June 2013.
- Hintz, L.D., Boyer, R.R., Ponder, M.A., Williams, R.C., and Rideout, S.L. 2010. Recovery of *Salmonella enterica* Newport introduced through irrigation water from tomato (*Lycopersicon esculentum*) fruit, roots, stems, and leaves. *Hortiscience*, **45**, 675–678.
- Hirsch, P.R., Mauchline, T.H., and Clark, I.M. 2010. Culture-independent molecular techniques for soil microbial ecology. *Soil Biology and Biochemistry*, **42**, 878–887.
- Hsu, H.Y., Sheen, S., Sites, J., Huang, L., and Wu, J.S.-B. 2014. Effect of high pressure treatment on the survival of Shiga toxin producing *Escherichia coli* in strawberry puree. *Food Microbiology*, **40**, 25–30.
- Ingham, S.C., Schoeller, E.L., and Engel, R.A. 2006. Pathogen reduction in unpasteurized apple cider: adding cranberry juice to enhance the lethality of warm hold and freeze–thaw steps. *Journal of Food Protection*, **69**, 293–298.
- Jablasone, J., Brovko, L.Y., and Griffiths, M.W. 2004. A research note: the potential for transfer of *Salmonella* from irrigation water to tomatoes. *Journal of the Science of Food and Agriculture*, **84**, 287–289.
- Jain, S., Bidol, S.A., Austin, J.L., Berl, E., et al. 2009. Multistate outbreak of *Salmonella* Typhimurium and Saintpaul infections associated with unpasteurized orange juice – United States, 2005. *Clinical Infectious Diseases*, **48**, 1065–1071.
- Jawaheer, S., White, S.F., Rughooputh, D.V., and Cullen, D.C. 2003. Development of a common biosensor format for an enzyme based biosensor array to monitor fruit quality. *Biosensors and Bioelectronics*, **18**, 1429–1437.
- Jay, J.M. 1992. Spoilage of fruits and vegetables. In: *Modern Food Microbiology*, 4th edn, pp. 187–198. Chapman & Hall, New York, USA.
- Johannessen, G.S., Loncarevic, S., and Kruse, H. 2002. Bacteriological analysis of fresh produce in Norway. *International Journal of Food Microbiology*, **77**, 199–204.
- Jordan, S.L., Pascual, C., Bracey, E., and Mackey, B.E. 2001. Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. *Journal of Applied Microbiology*, **91**, 463–469.
- Josefson, D. 2003. Three die in US outbreak of hepatitis. *British Medical Journal*, **327**, 1188.
- Kanellou, G., Paramithiotis, S., Mataragas, M., and Drosinos, E.H. 2013. Field study on the microbiological quality of pickles in brine and survival of *Salmonella* Typhimurium and *Listeria monocytogenes* during storage at 4°C. *European Food Research and Technology*, **236**, 391–396.
- Katz, D.J., Cruz, M.A., Trepka, M.J., Suarez, J.A., Fiorella, P.D., and Hammond, R.M. 2002. An outbreak of typhoid fever in Florida associated with an imported frozen fruit. *Journal of Infectious Diseases*, **186**, 234–239.
- Kim, J.-G. and Yousef, A.E. 2000. Inactivation kinetics of foodborne spoilage and pathogenic bacteria by ozone. *Journal of Food Science*, **65**, 521–528.
- Kim, J.-G., Yousef, A.E., and Chism, G.W. 1999. Use of ozone to inactivate microorganisms on lettuce. *Journal of Food Safety*, **19**, 17–34.

- Knudsen, D.M., Yamamoto, S.A., and Harris, L.J. 2001. Survival of *Salmonella* spp. and *Escherichia coli* O157:H7 on fresh and frozen strawberries. *Journal of Food Protection*, **64**, 1483–1488.
- Kocharunchitt, C., Ross, T., and McNeil, D.L. 2009. Use of bacteriophages as biocontrol agents to control *Salmonella* associated with seed sprouts. *International Journal of Food Microbiology*, **128**, 453–459.
- Kroupitski, Y., Golberg, D., Belausov, E., Pinto, R., Swartzberg, D., Granot, D., and Sela, S. 2009. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. *Applied and Environmental Microbiology*, **75**, 6076–6086.
- Kudva, I.T., Blanch, K., and Hovde, C.J. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology*, **64**, 3166–3174.
- Leff, J.W. and Fierer, N. 2013. Bacterial communities associated with the surfaces of fresh fruits and vegetables. *Plos One*, **8**, e59310.
- Madden, J.M. 1992. Microbial pathogens in fresh produce—the regulatory perspective. *Journal of Food Protection*, **55**, 821–823.
- Malik, S., Beer, M., Megharaj, M., and Naidu, R. 2008. The use of molecular techniques to characterize the microbial communities in contaminated soil and water. *Environmental International*, **34**, 265–276.
- Mantilla, N., Castell-Perez, M.E., Gomes, C., and Moreira, R.G. 2013. Multilayered antimicrobial edible coating and its effect on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*). *LWT – Food Science and Technology*, **51**, 37–43.
- Martinon, M.E., Moreira, R.G., Castell-Perez, M.E., and Gomes, C. 2014. Development of a multilayered antimicrobial edible coating for shelf-life extension of fresh-cut cantaloupe (*Cucumis melo* L.) stored at 4°C. *LWT – Food Science and Technology*, **56**, 341–350.
- Miles, J.M., Sumner, S.S., Boyer, R.R., Williams, R.C., Latimer, J.G., and McKinney, J.M. 2009. Internalization of *Salmonella enterica* serovar Montevideo into greenhouse tomato plants through contaminated irrigation water or seed stock. *Journal of Food Protection*, **72**, 849–852.
- Miller, L.G. and Kaspar, C.W. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. *Journal of Food Protection*, **57**, 460–464.
- Mohacsi-Farkas, C., Nyiro-Fekete, B., Daoood, H., Dalmadi, I., and Kisko, G. 2014. Improving microbiological safety and maintaining sensory and nutritional quality of pre-cut tomato and carrot by gamma irradiation. *Radiation Physics and Chemistry*, **99**, 79–85.
- Moreira, S.P., de Carvalho, W.M., Alexandrino, A.C., de Paula, H.C.B., et al. 2014. Freshness retention of minimally processed melon using different packages and multilayered edible coating containing microencapsulated essential oil. *International Journal of Food Science and Technology*, **49**, 2192–2203.
- Murakami, E.G., Jackson, L., Madsen, K., and Schickedanz, B. 2006. Factors affecting the ultra-violet inactivation of *Escherichia coli* K12 in apple juice and a model system. *Journal of Food Process Engineering*, **29**, 53–71.
- Najafi, M.B.H. and Khodaparast, M.H.H. 2009. Efficacy of ozone to reduce microbial populations in date fruits. *Food Control*, **20**, 27–30.
- Neves, F.I.G., Vieira, M.C., and Silva, C.L.M. 2012. Inactivation kinetics of peroxidase in zucchini (*Cucurbita pepo* L.) by heat and UV-C radiation. *Innovative Food Science and Emerging Technologies*, **13**, 158–162.
- Nicholson, F.A., Groves, S.J., and Chambers, B.J. 2005. Pathogen survival during livestock manure storage and following land application. *Bioresource Technology*, **96**, 135–143.
- Niemira, B.A. and Sites, J. 2008. Cold plasma inactivates *Salmonella* Stanley and *Escherichia coli* O157:H7 inoculated on golden delicious apples. *Journal of Food Protection*, **71**, 1357–1365.
- Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D.A., Morgan, D.J., and Lyng, J.G. 2008. Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering*, **85**, 141–146.
- Oliveira, M., Usall, J., Vinas, L., Solsona, C., and Abadias, M. 2011. Transfer of *Listeria innocua*

- from contaminated compost and irrigation water to lettuce leaves. *Food Microbiology*, **28**, 590–596.
- Oms-Oliu, G., Aguilo-Aguayo, I., Martin-Belloso, O., and Soliva-Fortuny, R. 2010. Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology*, **56**, 216–222.
- Ottesen, A.R., White, J.R., Skaltsas, D.N., Newell, M.J., and Walsh, C.S. (2009) Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *Journal of Food Protection*, **72**, 2321–2325.
- Ottesen, A.R., Gonzalez Pena, A., White, J.R., Pettengill, J.B., *et al.* 2013. Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). *BMC Microbiology*, **13**, 114.
- Pan, J., Vicente, A.R., Martinez, G.A., Chaves, A.R., and Civello, P.M. 2004. Combined use of UV-C irradiation and heat treatment to improve post-harvest life of strawberry fruit. *Journal of the Science of Food and Agriculture*, **84**, 1831–1838.
- Pao, S., Brown, E., and Schneider, K.R. 1998. Challenge studies with selected pathogenic bacteria on freshly peeled Hamlin orange. *Journal of Food Science*, **63**, 359–362.
- Paramithiotis, S., Doulgeraki, A.I., Tsilikidis, I., Nychas, G.J.E., and Drosinos, E.H. 2012. Fate of *Listeria monocytogenes* and *Salmonella* sp. during spontaneous cauliflower fermentation. *Food Control*, **27**, 178–183.
- Parish, M.E. 1998. High pressure inactivation of *Saccharomyces cerevisiae*, the endogenous microflora and pectinmethylesterase in orange juice. *Journal of Food Safety*, **18**, 57–65.
- Parish, M.E., Narciso, J.A., and Friedrich, L.M. 1997. Survival of *Salmonella* in orange juice. *Journal of Food Safety*, **17**, 273–281.
- Pereira de Abreu, D.A., Cruz, J.M., and Paseiro Losada, P. 2012. Active and intelligent packaging for the food industry. *Food Reviews International*, **28**, 146–187.
- Perni, S., Liu, D.W., Shama, G., and Kong, M.G. 2008a. Cold atmospheric plasma decontamination of the pericarps of fruit. *Journal of Food Protection*, **71**, 302–308.
- Perni, S., Shama, G., and Kong, M.G. 2008b. Cold atmospheric plasma disinfection of cut fruit surfaces contaminated with migrating microorganisms. *Journal of Food Protection*, **71**, 1619–1625.
- Ponka, A., Maunula, L., von Bonsdorff, C.H., and Lyytikäinen, O. 1999. Outbreak of calicivirus gastroenteritis associated with eating frozen raspberries. *Eurosurveillance Monthly*, **4**, 66–69.
- Prakash, A., Inthajak, P., Huibregtse, H., Caporaso, F., and Foley, D.M. 2000. Effects of low-dose gamma irradiation and conventional treatments on shelf-life and quality characteristics of diced celery. *Journal of Food Science*, **65**, 1070–1075.
- Ramos-Villarroel, A.Y., Aron-Maftei, N., Martin-Belloso, O., and Soliva-Fortuny, R. 2012. Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, **69**, 32–39.
- Ramsay, C.N. and Upton, P.A. 1989. Hepatitis A and frozen raspberries. *Lancet*, **8628**, 43–44.
- Randazzo, C.L., Pitino, I., Scifo, G.O., and Caggia, C. 2009. Biopreservation of minimally processed iceberg lettuces using a bacteriocin produced by *Lactococcus lactis* wild strain. *Food Control*, **20**, 756–763.
- Raybaudi-Massilia, R., Mosqueda-Melgar, J., and Martín-Belloso, O. 2006. Antimicrobial activity of essential oils on *Salmonella* Enteritidis, *Escherichia coli* and *Listeria innocua* in fruit juices. *Journal of Food Protection*, **69**, 1579–1586.
- Rezende, A.C.B., de Castro, M.F.P.M., Porto, E., Uchima, C.A., Benato, E., and Penteado, A.L. 2009. Occurrence of *Salmonella* spp. in persimmon fruit (*Diospyrus kaki*) and growth of *Salmonella* Enteritidis on the peel and in the pulp of this fruit. *Food Control*, **20**, 1025–1029.
- Rico, D., Martín-Diana, A.B., Barry-Ryan, C., Frias, J.M., Henehan, G.T.M., and Barat, J.M. 2008. Use of neutral electrolysed water (EW) for quality maintenance and shelf-life extension of minimally processed lettuce. *Innovative Food Science and Technologies*, **9**, 37–48.

- Sapers, G.M. 2003. Washing and sanitizing raw materials for minimally processed fruit and vegetable products. In: *Microbial Safety of Minimally Processed Foods* (eds Novak, J.S., Sapers, G.M., and Juneja, V.K.), pp. 221–254. Taylor and Francis, London.
- Sapers, G.M., Miller, R.L., and Mattrazzo, A.M. 1999. Effectiveness of sanitizing agents in inactivating *Escherichia coli* in Golden Delicious apples. *Journal of Food Science*, **64**, 734–737.
- Sapers, G.M., Miller, R.L., Jantschke, M., and Mattrazzo, A.M. 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. *Journal of Food Science*, **65**, 529–532.
- Scolari, G., Zacconi, C., Busconi, M., and Lambri, M. 2015. Effect of the combined treatments of high hydrostatic pressure and temperature on *Zygosaccharomyces bailii* and *Listeria monocytogenes* in smoothies. *Food Control*, **47**, 166–174.
- Seymour, I.J. and Appleton, H. 2001. A review, foodborne viruses and fresh produce. *Journal of Applied Microbiology*, **91**, 759–773.
- Shawn Eblen, B., Walderhaug, M.O., Edelson-Mammel, S., Chirtel, S.J., De Jesus, A., Merker, R.I., Buchanan, R.L., and Miller, A.J. 2004. Potential for internalization, growth, and survival of *Salmonella* and *Escherichia coli* O157:H7 in oranges. *Journal of Food Protection*, **67**, 1578–1584.
- Shi, X., Namvar, A., Kostrzynska, M., Hora, R., and Warriner, K. 2007. Persistence and growth of different *Salmonella* serovars on pre- and post-harvest tomatoes. *Journal of Food Protection*, **12**, 2725–2731.
- Sipahi, R.E., Castell-Perez, M.E., Moreira, R.G., Gomes, C., and Castillo, A. 2013. Improved multilayered antimicrobial alginate-based edible coating extends the shelf-life of fresh-cut watermelon (*Citrullus lanatus*). *LWT – Food Science and Technology*, **51**, 9–15.
- Sirolì, L., Patrignani, F., Serrazanetti, D.I., Tabanelli, G., Montanari, C., Tappi, S., Rocculi, P., Gardini, F., and Lanciotti, R. 2014. Efficacy of natural antimicrobials to prolong the shelf-life of minimally processed apples packaged in modified atmosphere. *Food Control*, **46**, 403–411.
- Smith, A.W.J., Poulston, S., Rowsell, L., Terry, L.A., and Anderson, J.A. 2009. A new palladium-based ethylene scavenger to control ethylene-induced ripening of climacteric fruit. *Platinum Metals Review*, **53**, 112–122.
- Soderstrom, A., Lindberg, A., and Andersson, Y. 2005. EHEC O157 outbreak in Sweden from locally produced lettuce, August–September 2005. *Eurosurveillance Weekly*, **10**, E050922.1. Available at: <http://www.eurosurveillance.org/ew/2005/050922.asp#1>, accessed September 2014.
- Solomon, E.B., Pang, H., and Mathews, K.R. 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. *Journal of Food Protection*, **66**, 2198–2202.
- Steenstrup, L.D. and Floros, J.D. 2004. Inactivation of *E. coli* O157:H7 in apple cider by ozone at various temperatures and concentrations. *Journal of Food Processing and Preservation*, **28**, 103–116.
- Strawn, L.K. and Danyluk, M.D. 2010a. Fate of *Escherichia coli* O157:H7 and *Salmonella* on fresh and frozen cut pineapples. *Journal of Food Protection*, **73**, 418–424.
- Strawn, L.K. and Danyluk, M.D. 2010b. Fate of *Escherichia coli* O157:H7 and *Salmonella* spp. on fresh and frozen cut mangoes and papayas. *International Journal of Food Microbiology*, **138**, 78–84.
- Takkinen, J., Nakari, U.-M., Johansson, T., Niskanen, T., Siitonen, A., and Kuusi, M. 2005. A nationwide outbreak of multiresistant *Salmonella* Typhimurium var. Copenhagen DT104B infection in Finland due to contaminated lettuce from Spain, May 2005. *Eurosurveillance Weekly*, **10**. Available at <http://www.eurosurveillance.org/ew/2005/050630.asp>, accessed September 2014.
- Tezotto-Uliana, J.V., Possati Fargoni, G., Geerdink, G.M., and Kluge, R.A. 2014. Chitosan applications pre- or postharvest prolong raspberry shelf-life quality. *Postharvest Biology and Technology*, **91**, 72–77.
- Tianli, Y., Jiangbo, Z., and Yahong, Y. 2014. Spoilage by *Alicyclobacillus* bacteria in juice and beverage products: chemical, physical, and combined

- control methods. *Comprehensive Reviews in Food Science and Food Safety*, **13**, 771–797.
- Torlak, E. 2014a. Efficacy of ozone against *Alicyclobacillus acidoterrestris* spores in apple juice. *International Journal of Food Microbiology*, **172**, 1–4.
- Torlak, E. 2014b. Inactivation of *Alicyclobacillus acidoterrestris* spores in aqueous suspension and on apples by neutral electrolyzed water. *International Journal of Food Microbiology*, **185**, 69–72.
- Trias, R., Baneras, L., Badosa, E., and Montesinos, E. 2008. Bioprotection of golden delicious apples and iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *International Journal of Food Microbiology*, **123**, 50–60.
- Tribst, A.A.L., Sant'Ana, A.S., and de Massaguer, P.R. 2009. Review: microbiological quality and safety of fruit juices-past, present and future perspectives. *Critical Reviews in Microbiology*, **35**, 310–339.
- Tzortzakis, N.G. 2009. Impact of cinnamon oil-enrichment on microbial spoilage of fresh produce. *Innovative Food Science and Emerging Technologies*, **10**, 97–102.
- Uchima, C.A., de Castro, M.F.P.M., Gallo, C.R., Rezende, A.C.B., Benato, E.R., and Penteado, A.L. 2008. Incidence and growth of *Listeria monocytogenes* in persimmon (*Diospyros kaki*) fruit. *International Journal of Food Microbiology*, **126**, 235–239.
- USDA. 2011. Agricultural Marketing Service, Microbiological Data Program. Available at: <http://www.ams.usda.gov/AMSV1.0/ams.fetchTemplateData.do?template=TemplateO&topNav=&leftNav=ScienceandLaboratories&page=MDPPProgramReports&description=MDP+Program+Reports&acct=microbiodataprg>, accessed September 2014.
- Viazis, S., Akhtar, M., Feirtag, J., and Diez-Gonzalez, F. 2011. Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. *Food Microbiology*, **28**, 149–157.
- Wachtel, M.R., Whitehand, L.C., and Mandrell, R.E. 2002a. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. *Journal of Food Protection*, **65**, 18–25.
- Wachtel, M.R., Whitehand, L.C., and Mandrell, R.E. 2002b. Prevalence of *Escherichia coli* associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater. *Journal of Food Protection*, **65**, 471–475.
- WHO. 2003. Silver in drinking-water. Guidelines for drinking-water quality. In: *Health Criteria and Other Supporting Information*, 2nd edn, vol. 2. WHO, Geneva, Switzerland.
- Williams, R.C., Sumner, S.S., and Golden, D.A. 2004. Survival of *Escherichia coli* O157:H7 and *Salmonella* in apple cider and orange juice as affected by ozone and treatment temperature. *Journal of Food Protection*, **67**, 2381–2386.
- Wisniewsky, M.A., Glatz, B.A., Gleason, M.L., and Reitmeier, C.A. 2000. Reduction of *Escherichia coli* O157:H7 counts on whole fresh apples by treatment with sanitizers. *Journal of Food Protection*, **63**, 703–708.
- Yeom, H.W., Streaker, C.B., Zhang, Q.H., and Min, D.B. 2000 Effects of pulsed electric fields on the activities of microorganisms and pectin methyl esterase in orange juice. *Journal of Food Science*, **65**, 1359–1363.
- Yu, K., Newman, C., Archbold, D.D., and Hamilton-Kemp, T.R. 2001. Survival of *Escherichia coli* O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. *Journal of Food Protection*, **64**, 1334–1340.
- Zhuang, R.Y., Beuchat, L.R., and Angulo, F.J. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Applied and Environmental Microbiology*, **61**, 2127–2131.
- Zimmermann, M., Schaffner, D.W., and Aragao, G.M.F. 2013. Modeling the inactivation kinetics of *Bacillus coagulans* spores in tomato pulp from the combined effect of high pressure and moderate temperature. *LWT – Food Science and Technology*, **53**, 107–112.
- Zook, C.D., Parish, M.E., Braddock, R.J., and Balaban, M.O. 1999. High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juices. *Journal of Food Science*, **64**, 533–535.

CHAPTER 19

Microbial ecology of cereal and cereal-based foods

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19.1 Introduction

Cereals and cereal products are one of the most important sources of food (FAO, 2002); cereal grains are food staples all over the world and constitute the raw materials for many foods (Bullerman and Bianchini, 2009).

The main cereals used as livestock feeds include maize, wheat, barley, rice, oats, rye, millet, and sorghum; examples of cereal products from the grains are wheat, rye, oat flour, semolina, cornmeal, corn grits, doughs, bread, breakfast cereals, pasta, snack foods, dry mixes cakes, pastries, and tortillas. Moreover, cereals are used as ingredients in many products, like infant foods, processed meats, confectionery products, and alcoholic beverages such beer and sake (Bullerman and Bianchini, 2009).

The microflora of harvested cereal grains contains up to millions of bacteria and molds per gram, but the low water activity effectively inhibits many microorganisms and in some conditions only molds can effectively grow (Hayes, 1992). Thus, for grains and many cereal products the microbiology is the description of the spoiling microorganisms and safety issues; however, there are some cereal products that undergo a beneficial fermentation by bacteria and/or yeasts.

Therefore, the main goal of this chapter is a focus on the dual role of microorganisms in cereal and cereals products: starter cultures for

a beneficial and desired food fermentation or responsible for some spoiling events. For the first topic (beneficial role of microorganisms in cereal products) we focused on sourdough, as it represents probably the most important fermented cereal product in Europe, and some ethnic fermented foods from “Warm Countries”, for their impact as a source of carbohydrates for the diet.

On the other hand, the topic of the second part is the description of the most important spoiling events (moldy and mycotoxins production; rope and sour spoilage) for some categories of cereal products.

19.2 Sourdough

19.2.1 Description

Nowadays sourdough represents the most important fermentation for cereal-based products; a sourdough consists of a mixture of flour and water, fermented by lactic acid bacteria (LAB) and yeasts (Corsetti and Settanni, 2007), which are responsible for acidification (LAB), leavening (mainly yeasts and secondarily heterofermentative lactobacilli), and flavor formation (LAB and yeasts). The most important parameters of this system are the following (De Vuyst *et al.*, 2014): (a) low pH; (b) high carbohydrate concentration; (c) oxygen limitation; and (d) the concentration of LAB twofold

higher than that of yeasts (9 log cfu/g versus 7 log cfu/g).

Sourdoughs are generally designed for the production of bread, with some favorable traits in terms of shelf life, flavor, nutritional properties, and health effects if compared to the bread produced through leavening by yeasts (Delcour *et al.*, 2012; De Vuyst *et al.*, 2014).

Sourdoughs can be classified by either focusing on the kind of inoculum or on the production protocol. Concerning the production protocol, a sourdough could be classified as 0, I, II, or III (Huys *et al.*, 2013). *Type 0 doughs* are pre-doughs in which flour and water are mixed and left at room temperature for some time; thus the mix gets sour and starts to bubble. LAB of “0 sourdough” are mainly homofermentative. *Type I or traditional sourdoughs* are firm doughs with a low dough yield and daily backslopped at ambient temperature to keep LAB and yeasts viable (the backslopping is a repeated and cycling addition of water and flour). *Type II sourdoughs* are industrial and semi-liquid preparations produced in a bioreactor. Finally, *type III sourdoughs* are dried products to be used as a non-living acidifier supplement or flavor carrier. Whilst type I sourdoughs do not require the addition of leavening agents, yeast inoculum is compulsory for types II and III.

The second classification of sourdough is based on the kind of inoculum (Corsetti and

Settanni, 2007; De Vuyst *et al.*, 2014); thus a *first type* represents a spontaneous fermentation process based on backslopping. These preparations harbor yeasts and LAB species, which can be regarded as a kind of imprinting of the place of production/preparation (region or country) (for an exhaustive review see the paper by De Vuyst *et al.*, 2014). A *second type* of sourdough is based on the addition of starter cultures to a flour/water mix; this process is performed at 30°C or at higher temperatures and lasts 1–3 days. The finished products are marketed as semi-liquid, heat treated, or dried preparations (Gaggiano *et al.*, 2007) and generally contain *Lactobacillus fermentum*, *Lb. plantarum*, or *Lb. sanfranciscensis*, with or without *Saccharomyces cerevisiae*. A *third type* of sourdough is a dough initiated with a starter inoculum, followed by backslopping.

19.2.2 Microbiota

Generally, the stable microbiota of sourdough consists of particular yeasts (mainly ascomycetes) and lactic acid bacteria (Table 19.1). Focusing on the yeast role, most yeast species are ubiquitous and have also been found in other ecosystems, whilst the recovery of *S. cerevisiae* might be ascribed to its presence in the bakery environment (De Vuyst *et al.*, 2014). To the best of our knowledge the only species originally recovered from sourdough was *S. exiguus* (now *Kazachstania exigua*).

Table 19.1 Microbiota of sourdough (from De Vuyst *et al.*, 2014).

Lactic acid bacteria (LAB)	Predominant and/or characteristic of sourdough <i>Lactobacillus plantarum</i>, <i>Lb. fermentum</i>, <i>Lb. sanfranciscensis</i>, <i>Lb. reuteri</i>, <i>Lb. amylovorus</i>, <i>Lb. panis</i>, <i>Lb. pontis</i> Less predominant <i>Weissella cibaria</i>, <i>W. confusa</i>, <i>Pediococcus acidilactici</i>, <i>P. pentosaceus</i>, <i>Leuconostoc mesenteroides</i>, <i>L. citreum</i> Subdominant Lactococci, enterococci, streptococci
Yeasts	<i>Saccharomyces cerevisiae</i>, <i>Kazachstania exigua</i>, <i>Candida humilis</i>, <i>Pichia kudriavzevii</i>, <i>Torulaspora delbrueckii</i>, <i>Wickerhamomyces anomalus</i>
Stable yeast–LAB association	<i>K. exigua</i> (maltose negative, acid tolerant)–<i>Lb. sanfranciscensis</i> (maltose positive) <i>K. barnetti</i> (maltose negative)–<i>Lb. sanfranciscensis</i> <i>C. humilis</i> (maltose negative)–<i>Lb. sanfranciscensis</i> <i>S. cerevisiae</i> (maltose positive)–<i>Lb. plantarum</i> (prefers glucose and fructose) <i>S. cerevisiae</i>–<i>C. milleri</i>–<i>Lb. sanfranciscensis</i>

Concerning LAB microflora, there are some species always found in sourdough (predominant microflora, Table 19.1), whilst other microorganisms are less predominant (found in few samples) or subdominant (if their presence is occasional) (De Vuyst *et al.*, 2014). The presence of the dominant species could be ascribed to some peculiarities: (a) highly adapted carbohydrate metabolism (fermentation of maltose by *Lb. fermentum*, *Lb. reuteri*, or *Lb. sanfranciscensis*); (b) dedicated amino acid assimilation (arginine deiminase pathway in *Lb. fermentum* and *Lb. reuteri*); (c) stress resistance and stress response, competitiveness (*Lb. plantarum*) (De Vuyst *et al.*, 2014).

Apart from the most important LAB, after 2000 many new species have been isolated and identified, i.e., *Lb. crustorum*, *Lb. mindensis*, *Lb. nantensis*, and *Lb. nodensis* (homofermentative); *Lb. spicheri* (facultatively heterofermentative); *Lb. acidifarinae*, *Lb. frumenti*, *Lb. hammesii*, *Lb. namurensis*, *Lb. rossiae*, *Lb. secaliphilus*, *Lb. siliginis*, and *Lb. zymae* (De Vuyst *et al.*, 2014).

An important trait of sourdough is the stable association of LAB with some species of yeasts. Table 19.1 reports the most important associations and the reasons leading to the formation of these joint ventures.

It is well known that the use of certain raw materials and some peculiar technological protocols affect, determine, and lead to the stability of LAB and yeast microflora. Other important parameters able to influence the qualitative and the quantitative composition of sourdough microbiota are temperature, dough yield, pH, redox potential, backslipping procedure, and resting time (storage time of the mother sponge) (De Vuyst *et al.*, 2014).

Flour plays a crucial role in establishing the microbial consortia, as it can direct the microbiota in different ways (e.g., the prevalence of *Lb. helveticus* in sorghum sourdough, *Lb. amylovorus*, or *Lb. fermentum* in rye, *Lb. sakei* in amaranth, *Lb. pontis* in teff, *Lb. brevis* in barley). On the other hand, it has been reported that

some robust and ubiquitous species, like *Lb. plantarum* and *Lb. fermentum*, dominate the majority of sourdough. This evidence suggests that they come from the grain surface (De Vuyst *et al.*, 2014).

On the other hand, the prevalence of the most characteristic species of sourdough, *Lb. sanfranciscensis*, relies on some environmental parameters, supposing its presence in the flour.

Finally, some species (*Lb. acidophilus*, *Lb. reuteri*, and *Lb. rossiae*) might be of intestinal origin: for example, *Lb. reuteri* might originate from rodent intestines (Su *et al.*, 2012).

19.3 Ethnic fermented foods

The tropical world, as well as countries of South-East Asia and South America, has a long history of fermenting raw materials, with a special focus on starchy materials because they are the major contributors to human energy intakes in these countries (Guyot, 2012). It is not possible for a general description of the so-called ethnic fermented products from cereals, due to the different kinds of products; however, a general trait is the prevalence of LAB (7/11 log cfu/ml or g of product). Yeasts are also frequently recovered but at lower levels (4/6 log cfu/ml or g).

Although many LAB species were recovered from these kinds of products, there are at least 10 species prevailing over the others and belonging to the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*, being *Lb. plantarum* and *Lb. fermentum* as the main actors (Guyot, 2012).

The microbiota of these foods is a mix of heterofermentative and homofermentative strains, and the end-products include lactic acid, ethanol, acetic acid, and carbon dioxide and the ratio of lactic acid to ethanol provides useful information on the dominant microorganisms. An interesting trait of the LAB of these ecological niches is the ability to degrade the starch, which has been recovered in at least 10%

Table 19.2 Description of two traditional cereal-based products.

Product	Raw material	Country	Description/microbiota
Boza	Wheat, rye, millet, maize	Bulgaria, Romania, Turkey, Albania	Fermented non-alcoholic beverage; the microflora is mainly represented by lactobacilli (<i>Lb. acidophilus</i> , <i>Lb. brevis</i> , <i>Lb. coryniformis</i> , <i>Lb. fermentum</i> , <i>Lb. graminis</i> , <i>Lb. paracasei</i> , <i>Lb. pentosus</i> , <i>Lb. plantarum</i>), lactococci (<i>Lc. lactis</i> subsp. <i>lactis</i>), <i>W. confusa</i> , <i>Pediococcus</i> spp., <i>Candida</i> spp., <i>Pichia</i> spp., <i>Saccharomyces</i> spp. The production of this beverage relies upon some different steps: preparation of raw materials, boiling, filtration, cooling, sugar addition, fermentation, and bottling (Altay <i>et al.</i> , 2013)
Poto poto	Maize	Congo	Poto poto is a traditional fermented maize dough prepared in Congo homes for use directly as a weaning food. The process includes a soaking step of the grains (first fermentation step), which in average lasts 55 h, milling and settling of the paste in water (second fermentation step, 10–11 h), followed by cooking to produce a gruel. The fermented maize paste is dewatered and modeled into poto poto balls. The microbiota includes <i>Lb. plantarum</i> , <i>Lb. paraplantarum</i> , <i>Lb. reuteri</i> , <i>Lb. gasseri</i> , <i>Lb. fermentum</i> , <i>Lb. brevis</i> , <i>Enterococcus</i> spp., <i>Escherichia coli</i> , <i>Bacillus</i> spp. (Abriouel <i>et al.</i> , 2006)

of the lactic population (Guyot, 2010), above all in some strains of *Lb. plantarum*, *Lb. fermentum*, *Lactococcus lactis*, *Streptococcus* spp., and *L. mesenteroides* (Guyot, 2012).

Concerning yeast microbiota, Guyot (2010) reported the following genera: *Kluyveromyces*, *Pichia*, *Candida*, *Saccharomyces*, *Debaryomyces*, and *Geotrichum*.

The traditional cereal products include both dough and simil-bread foods as well as beverages, gruel, pancake, and fritter. Table 19.2 reports a brief description for two traditional products (a beverage and a food).

19.4 Spoilage of cereals and cereal products

The sources of microbial contamination of cereals and cereal products are all traceable from the environment (air, dust, soil, water, insects) as well as from the processing conditions (shipping containers, handling and processing equipments) (Bullerman and Bianchini, 2009).

The microbiota involved in the spoiling phenomena is variable and composite and includes bacteria (lactic acid bacteria, rope-forming bacteria, coliforms and enterococci, pathogens), yeasts and molds. Table 19.3 reports a short overview of the microorganisms recovered in some spoiling events for some kinds of products.

Bacteria are often involved in surface contamination. They do not generally represent a problem for dry grains and pose a significant issue only after an extensive deterioration or when the content of moisture increases (Bullerman and Bianchini, 2009). Many times bacteria survive the processing conditions, contaminate the flour, and spoil the dough prepared with it. The role of yeasts as spoiling microorganisms is controversial, although they are responsible for “yeasty” phenomena in bakery products and refrigerated dough.

Nevertheless, molds represent the main source of contamination for cereals and cereal products, both for their visible growth as well as for the production of mycotoxins. Thus, the

Table 19.3 Kinds of spoiling phenomena associated with different cereal products (compiled using the data reported by Bullermann and Bianchini, 2009, and Cook and Johnson, 2009).

Grain	Description of the category	Spoilage
		Molds
Bakery products	Leavened and unleavened breads, rolls, buns, croissants, muffins, crumpets, cakes, pastries, biscuits, etc. They are typically baked at temperatures able to destroy vegetative bacteria, yeasts, and molds; they are rarely involved in food poisoning phenomena and thus they are not considered high-risk foods. They are highly perishable, due to pH (5.4–7.5) and a_w (0.75–0.98)	Molds (<i>Aspergillus</i> , <i>Penicillium</i> , <i>Fusarium</i> , <i>Monilia</i> , <i>Cladosporium</i> , <i>Endomyces</i> , <i>Rhizopus</i> , and <i>Mucor</i> species) Yeasts (<i>Saccharomyces</i> , <i>Debaryomyces</i> , <i>Kluyveromyces</i> , <i>Pichia</i> , <i>Candida</i> , and <i>Zygosaccharomyces</i> species) Rope spoilage Sour spoilage
Refrigerated dough	Refrigerated cereal products (unbaked biscuits, dinner rolls, breakfast rolls, bread, pie crust dough, and cookie dough) are marketed as convenient products requiring less preparation (i.e., cooking) before consumption	Osmophilic yeasts (<i>Hansenula anomala</i> , producing off-flavors described as fruity or acetone) Lactobacilli and leuconostocs
Dried products	They include ready-to-eat grain-based foods (breakfast cereals, crackers, snack foods), dry bakery mixes, and grain ingredients, prepared by some main protocols: flaking, puffing, or extrusion. They are considered as convenience foods designed for quick “on-the-go” consumption. Microbial spoilage is rarely recovered	Molds (<i>Rhizopus</i> , <i>Penicillium</i> , <i>Absidia</i> , <i>Mucor</i> and <i>Eurotium</i> species)
Compound cereal products	They contain a non-grain-based component (tarts filled with fruit or meat, sausage rolls, cream-filled pastries, cream cakes, cheesecakes, pizza); the non-grain component can be added either before or after baking	<i>Clostridium botulinum</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i>

following sections report a brief description of the most important spoiling events (moldy and yeasty, sour and rope spoilage) as well as on the significance of mycotoxin contamination in cereals.

19.4.1 Molds on grains

There are at least 150 different species of filamentous fungi and yeasts on cereal grains. They can be grouped into two different classes:

Field fungi, which contaminate grains in the field when the moisture of the raw material is 18 to 30% and the relative humidity ca. 90–100%. They include fungi from the species *Alternaria*, *Cladosporium*, *Fusarium*, and *Helminthosporium* (Bullerman and Bianchini, 2009).

Storage fungi, which contaminate grains and cereal products at lower moisture content (14–16%) and lower relative humidity (65–90%). The most important genera are *Eurotium*, *Aspergillus*, and *Penicillium* (Bullerman and Bianchini, 2009).

The most important effects of fungal deterioration of grains are the following:

- a** decreased germination of seeds;
- b** discoloration;
- c** development of a visible mycelium;
- d** musty odors;
- e** production of mycotoxins.

The only effective way for preventing mold growth on the grains is to maintain the moisture content below 14% (Bullerman and Bianchini, 2009).

19.4.2 Mold contamination of cereal products

Mold growth can contribute to the spoilage of cereal products, namely bakery and dried products. On the bakery products mold spoilage is evident as white, filamentous, and fuzzy colonies that gradually turn from blue-green to black as conidia are produced (Cook and Johnson, 2009); typically, untreated bread becomes moldy after 5–6 days (Vora and Sidhu, 1987). As reported, for the grains a major concern is related to the production of mycotoxins.

The genera involved in the fungal spoilage of cereal products are *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Monilia*, *Endomyces*, *Rhizopus*, and *Mucor* (Legan and Voysey 1991).

19.4.3 Mycotoxins

Mycotoxins are toxic secondary metabolites (*toxins*) produced by molds (*myco*). They are different from the primary metabolites as they are not required for growth and have no apparent link with the metabolism (Bullerman and Bianchini, 2009; Cook and Johnson, 2009). Thus it has been suggested that they could be waste products or defense mechanisms (Cook and Johnson, 2009).

Contamination by mycotoxin is a great challenge for grains, but they also pose a strong safety issue in cereal products because they are heat-stable and resist destruction by food processing (Cook and Johnson, 2009).

Mycotoxins exhibit both acute toxicity or poisoning, which can cause the death, or chronic toxicity, responsible for a gradual weakening and a lowering of the immune response (Chen *et al.*, 2008; Girish *et al.*, 2008); in addition, some mycotoxins are carcinogenic, mutagenic, teratogenic, and embryo toxic (Bennet and Klich, 2003).

Unfortunately the contamination can be controlled only by inhibiting mold growth on cereals and cereal products, as to the best of our knowledge there are not effective methods to detoxify the products if they contain the toxins.

The most important toxins for cereals are the following ones (the producing molds are

given in parentheses) (Bullerman and Bianchini, 2009):

- 1 Aflatoxins (*A. flavus* and other *aspergilli*)
- 2 Ochratoxin A (*A. ochraceus*, *A. niger*, and *P. verrucosum*)
- 3 Fumosins (*F. verticilloides*, *F. proliferatum*, and *F. subglutinas*)
- 4 Moniliformin (*F. proliferatum*, *F. subglutinas*)
- 5 Deoxynivalenol (*F. graminearum*, *F. culmorum*, and *F. crookwellense*)
- 6 Zearalenone (*F. graminearum*, *F. culmorum*, and *F. crookwellense*).

19.4.4 Spoilage by yeasts

This kind of spoilage is rare in cereal and cereal products, although it could be found in bakery products as a surface event. The genera mostly involved are *Saccharomyces*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Candida*, and *Zygosaccharomyces* (Cook and Johnson, 2009). They are responsible for the chalky appearance of surfaces and off-odours (Doyle, 2007). A yeasty event can also be found on refrigerated dough, as a result of the metabolism of *Hansenula anomala*. This yeast produces the so-called “fruity” or “acetome” off-odour and causes the bubbling or the expansion of the flexible packaging (Membre *et al.*, 1999).

19.4.5 Rope spoilage

Rope spoilage is primarily due to *Bacillus subtilis* and *B. licheniformis*, and secondarily to *B. pumilus*, *B. cereus*, *B. clausii*, *B. amyloliquefaciens*, and *B. firmus* (Pepe *et al.*, 2003; Andersson *et al.*, 1995; Valerio *et al.*, 2012). The spoiling event is the result of spore germination under favorable conditions (temperature >25°C; pH >5; a_w >0.95) (Valerio *et al.*, 2012). Thus the cells cause a loss of bread structure due to slime formation as a result of the combination of both proteolytic and amilolytic activities (Viedma *et al.*, 2011).

The visible sign of the occurring spoilage is enzymatic degradation of crumb and stickiness because of the production of extra-cellular mucilaginous polysaccharides (Thompson *et al.*, 1998).

Bacilli are common in the soil, where they are active in decomposition of the organic matter. Thus from the soil they can be recovered in the flour and survive the baking process in the center of the loaves (Cook and Johnson, 2009). Although the quality of the raw material is an important parameter, rope contamination rarely occurs or occurs at low levels, and poor milling conditions and inadequately cleaned and sanitized dough equipment are the primary source of contamination during baking (Cook and Johnson, 2009).

19.4.6 Sour spoilage

This kind of spoilage is associated with a *sour* odour, due to the production of lactic acid as a result of bacterial metabolism of carbohydrates. This phenomenon is a desired trait in sourdough, whilst it is a spoiling event for many bakery products (Salovaara and Savoianen, 1984; Cook and Johnson, 2009).

Generally the sour spoilage is caused by some species of the genus *Lactobacillus* (*Lb. plantarum*, *Lb. curvatus*, *Lb. casei*, *Lb. sanfranciscensis*, *Lb. fermentum*, *Lb. buchneri*, *Lb. acidophilus*, and many other species) (Cook and Johnson, 2009), although Stiles and Holzapfel (1997) reported the recovery of some species of the genera *Pediococcus*, *Enterococcus*, *Carnobacterium*, *Streptococcus*, *Tetragenococcus*, and *Vagococcus*. This kind of spoilage could be prevented by sufficient baking to destroy the vegetative cells as well as by moisture control at the product surface (Cook and Johnson, 2009).

References

- Abriouel, H., Omar, N.B., López, R.L., Martínez-Cañamero, M., Keleke, S., and Gálvez, A. 2006. Culture-independent analysis of the microbial composition of the African fermented foods *poto poto* and *dégue* by using three different DNA extraction methods. *International Journal of Food Microbiology*, **111**, 228–233.
- Altay, F., Karbancioglu-Güler, Daskaya-Dikmen, C., and Heperkan D. 2013. A review on traditional Turkish fermented non alcoholic beverages: microbiota, fermentation process and quality characteristics. *International Journal of Food Microbiology*, **167**, 44–56.
- Andersson, A., Rönner, U., and Granum, P.E. 1995. What problems does the food industry have with spore-forming pathogens *Bacillus cereus* and *Clostridium perfringens*? *International Journal of Food Microbiology*, **28**, 145–155.
- Bennet, J.W. and Klich, M. 2003. Mycotoxins. *Clinical Microbiology Reviews*, **16**, 497–516.
- Bullerman, L.B. and Bianchini, A. 2009. Food safety issues and the microbiology of cereals and cereal products. In: *Microbiologically Safe Foods* (eds Heredia, N., Wesley, I., and García, S.), pp. 315–335. John Wiley & Sons, New York, USA.
- Chen, D.C., Lee, Y.Y., Yeh, P.Y., Lin, J.C., Chen, Y.L., and Hung, S.L. 2008. Eugenol inhibited the antimicrobial functions of neutrophils. *Journal of Endodontics*, **34**, 176–180.
- Cook, F.K. and Johnson, B.L. 2009. Microbiological spoilage of cereal products. In: *Compendium of the Microbiological Spoilage of Foods and Beverages* (eds Sperber, W.H. and Doyle, M.P.), pp. 223–244. Springer, Berlin.
- Corsetti, A. and Settanni, L. 2007. Lactobacilli in sourdough fermentation. *Food Research International*, **40**, 439–558.
- Delcour, J.A., Rouau, X., Courtin, C.M., Poutanen, K., and Ranieri, R. 2012. Technologies for enhanced exploitation of the health-promoting potential of cereals. *Trends in Food Science and Technology*, **25**, 78–86.
- De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, S., Daniel, H.-M., and Weckx, S. 2014. Microbial ecology of sourdough fermentations: diverse or uniform? *Food Microbiology*, **37**, 11–29.
- Doyle, M.E. 2007. Microbial food spoilage – losses and control strategies. A brief overview of the literature. Available at: http://fri.wisc.edu/docs/pdf/FRI_Brief_Microbial_Food_Spoilage_7_07.pdf (accessed 2 June 2014).
- FAO. 2002. World Agriculture: Towards 2015/2030 Summary Report. Available at: <http://www.fao.org/docrep/004/y3557e/y3557e00.htm>.

- Gaggiano, M., Di Cagno, R., De Angelis, M., Arnault, P., Tossut, P., Fox, P.F., and Gobbetti, M. 2007. Defined multi-species semi-liquid ready-to-use sourdough starter. *Food Microbiology*, **24**, 15–24.
- Girish, C.K., Smith, T.K., Boermans, H.J., and Karrow, N.A. 2008. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, hematology, metabolism, and immunocompetence of turkeys. *Poultry Science*, **87**, 421–432.
- Guyot, J.P. 2010. Fermented cereal products. In: *Fermented Foods and Beverages of the Worlds* (ed. Tamang, J.P.), pp. 247–261. CRC Press (Taylor & Francis Group), New York, USA.
- Guyot, J.P. 2012. Cereal-based fermented foods in developing countries: ancient foods for modern research. *International Journal of Food Science and Technology*, **47**, 1109–1114.
- Hayes, P.R. 1992. *Food Microbiology and Hygiene*, 2nd edn, p. 150. Chapman & Hall, CITTA'.
- Huys, G., Daniel, H.-M., and De Vuyst, L. 2013. Taxonomy and biodiversity of sourdough yeasts and lactic acid bacteria. In: *Handbook of Sourdough Biotechnology* (eds Gobbetti, M. and Gänzle, M.), pp. 105–154. Springer, New York, USA.
- Legan, J.D. and Voysey, P.A. 1991. Yeast spoilage of bakery products and ingredients. *Journal of Applied Bacteriology*, **70**, 361–371.
- Membre, J.M., Kubaczka, M., and Chene, C. 1999. Combined effects of pH and sugar on growth rate of *Zygosaccharomyces rouxii*, a bakery product spoilage yeast. *Applied and Environmental Microbiology*, **65**, 4921–4925.
- Pepe, O., Blaiotta, G., Moschetti, G., Greco, T., and Villani, F. 2003. Rope-producing strain of *Bacillus* spp. from wheat bread and strategy for their control by lactic acid bacteria. *Applied and Environmental Microbiology*, **69**, 2321–2329.
- Salovaara, H. and Savoianen, J. 1984. Yeast types isolated from Finnish sourdough starters. *Acta Alimentaria Polonica*, **10**, 242–246.
- Stiles, M.E. and Holzapfel, W.H. 1997. Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology*, **36**, 1–29.
- Su, M.S.-W., Oh, P.L., Walter, J., and Gänzle, M.J. 2012. Intestinal origin of sourdough *Lactobacillus reuteri* isolates as revealed by phylogenetic, genetic, and physiological analysis. *Applied and Environmental Microbiology*, **78**, 6777–6780.
- Thompson, J.M., Waites, W.M., and Dodd, C.E.R. 1998. Detection of rope spoilage in bread caused by *Bacillus* species. *Journal of Applied Microbiology*, **85**, 481–486.
- Valerio, F., De Bellis, P., Di Biase, M., Lonigro, S.L., Giussani, B., Visconti, A., Lavermicocca, P., and Sisto, A. 2012. Diversity of spore-forming bacteria and identification of *Bacillus amyloliquefaciens* as a species frequently associated with rope spoilage of bread. *International Journal of Food Microbiology*, **156**, 278–285.
- Viedma, P.M., Abriouel, H., Omar, N.B., López, R.I., and Gálvez A. 2011. Inhibition of spoilage and toxigenic *Bacillus* species in dough from wheat flour by the cyclic peptide enterocin AS-48. *Food Control*, **22**, 756–761.
- Vora, H.M., and Sidhu, J.S. 1987. Effect of varying concentration of ethyl alcohol and carbon dioxide on the shelf life of bread. *Chemie, Mikrobiologie, Technologie der Lebensmittel*, **11**, 56–59.

CHAPTER 20

Microbial ecology of nuts, seeds, and sprouts

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20.1 Introduction

Nuts, seeds, and sprouts are generally known as wholesome foods rich in antioxidants, fiber, high-quality vegetable protein, minerals, and unsaturated fatty acids (Abadias *et al.*, 2008; Blomhoff *et al.*, 2006; King *et al.*, 2008; Kwak *et al.*, 2011; Ros, 2010; Viswanathan and Kaur, 2001). These commodities can be found in almost all retail markets and are used in a variety of foods such as dressings, sandwiches, salads, and soups (King *et al.*, 2008; Saroj *et al.*, 2006). However, nuts, seeds, and sprouts are well-documented primary sources of food-borne pathogen outbreaks (Yang *et al.*, 2013). Controlling pathogen outbreaks is a primary concern for the food industry.

The microbiological safety of nuts, seeds, and sprouts has been in the spotlight since several large outbreaks attributed to pathogenic bacterial infection of these commodities occurred. Two large consecutive *Salmonella* outbreaks associated with consumption of almonds were reported in Canada and the United States during 2000–2001 (168 cases) and 2003–2004 (29 cases) (CDC, 2004; Isaacs *et al.*, 2005). Radish sprouts were implicated in the world's largest *Escherichia coli* O157:H7 outbreak, which occurred in Japan in 1996 and caused 9451 cases, 1808 hospitalized patients, and 12 deaths (NIID, 1997). From May to July 2011, there was a serious outbreak of *E. coli* O104:H4 producing Shiga toxin, primarily in northern Germany. The World Health

Organization (WHO) reported that the infections and subsequent complications caused 4075 cases, including 908 cases complicated by hemolytic uremic syndrome (HUS), and 50 deaths in 16 countries (WHO, 2011). Sprouts were identified as the most likely vehicle of this outbreak (Buchholz *et al.*, 2011).

Effective management of food safety for nuts, seeds, and sprouts requires an understanding of microbial communities associated with these products. This chapter presents an overview of microbial ecology for nuts, seeds, sprouts, and their products in retail markets or food manufacturers. We discuss quantitative and qualitative approaches used to assess pathogenic bacterial contamination of nuts, seeds, and sprouts and also analyze the published data about microbiological changes occurring in seeds and sprouts during production in a real manufacturing plant.

20.2 Definition and classification of nuts, seeds, and sprouts

Edible seeds are defined as seeds that can be ingested by humans as foodstuffs, including all cereals, legumes, and nuts. According to the classification of foods and animal feeds by CODEX (2006), nuts and seeds include tree nuts, oilseeds, and seeds for beverages and sweets. Tree nuts are defined as seeds of trees and shrubs, which are characterized by a hard

inedible shell enclosing an oily seed (e.g., almonds, hazelnuts, macadamia nuts, pine nuts, and pistachios). Oilseeds are plant seeds used in the production of edible vegetable oils (e.g., sesame and sunflower oil) and seed meals for animal feed; some oilseeds are directly consumed or slightly processed as food (e.g., peanuts, pumpkin seed, sunflower seed) or for food flavoring (e.g., sesame seed). Seeds for beverages and sweets are produced by tropical and subtropical trees and shrubs (e.g., coffee and cacao beans). This chapter focuses on nuts and seeds as defined by the CODEX classification, but excludes cereals and legumes. Particular focus is given to edible nuts and seeds that can be directly consumed by humans, excluding seeds for beverages and sweets. Sprouts are generally defined as young plant growth such as a bud or shoot. Several seeds are commonly used for sprout production. Bean, alfalfa, and soy sprouts have been highly consumed in the global sprout produce industry (Warriner and Smal, 2014).

20.3 Microbial ecology of nuts and seeds

20.3.1 Edible nuts

Edible nuts were generally believed to contain few microorganisms due to their inherent dry characteristics (moisture content: 0.7–8.5%), which provide unfavorable environments for bacterial survival and growth (Jeong *et al.*, 2012; Rosengarten, 2004). Unexpectedly, as shown in Table 20.1, both raw and processed edible tree nuts had large numbers of microorganisms, including aerobic bacteria (up to >5.4 log cfu/g), yeasts (up to 4.5 log cfu/g), and molds (up to >5.2 log cfu/g). The detection and contamination level of each microorganism differed according to product type, harvest origins, and distribution channels. Eglezos *et al.* (2008) and Eglezos (2010) examined roasted kernels as a raw material for ready-to-eat (RTE) nut production and RTE nuts, and reported that raw materials contained relatively higher levels of aerobic

bacteria (average 2.5–4.5 log cfu/g, 45–84%) than RTE products (average 2.4–2.8 log cfu/g, 36–62%). Molds were detected in almost all of the tested edible nuts at a considerable level.

Some reports identified dominant mold species that naturally exist in edible nuts, including *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Rhizopus* (Fernane *et al.*, 2010; Heperkan *et al.*, 1994; Jimenez *et al.*, 1991; Rodrigues *et al.*, 2012). *Aspergillus flavus* and *A. parasiticus* have been mainly investigated because they produce toxic metabolites (mycotoxins) such as hepatotoxic and/or carcinogenic aflatoxins and nephrotoxic ochratoxins (Eaton and Gallagher, 1994; Molyneux *et al.*, 2007). Many studies report the incidence of mycotoxins in edible nuts such as almonds (Schatzki, 1996), peanuts (Abdulkadar *et al.*, 2002; Chun *et al.*, 2007; Hepsag *et al.*, 2014; Juan *et al.*, 2008; Luttfullah and Hussain, 2011), pine nuts (Luttfullah and Hussain, 2011), pistachios (Abdulkadar *et al.*, 2000, 2002; Al-Moghazy *et al.*, 2014; Chun *et al.*, 2007; Doster and Michailides, 1994a, 1994b; Hepsag *et al.*, 2014; Juan *et al.*, 2008; Luttfullah and Hussain, 2011), walnuts (Juan *et al.*, 2008; Luttfullah and Hussain, 2011), and other nuts (e.g., Brazil nuts, cashew nuts, hazelnuts, etc.) (Imperato *et al.*, 2011). It is very difficult to remove mycotoxins during the production of edible nuts; depending on the level of contamination, they can be harmful to human health after ingestion. The potential health risk of mycotoxins has been recognized by food safety institutions and organizations in several countries, such as the Food and Agriculture Organization of the United Nations (FAO), European Commission (EU), and US Food and Drug Administration (FDA) (Imperato *et al.*, 2011). The EU established a regulatory limit of 2.0 µg/kg for aflatoxin B₁ and 4.0 µg/kg for total aflatoxins (B₁, B₂, G₁, and G₂) in nuts and groundnuts intended for direct human consumption or use as an ingredient in food (EU, 2006).

A comprehensive understanding of the quantitative microbial ecology of edible nuts is still emerging. McGarvey *et al.* (2014) analyzed

Table 20.1 Microorganisms in edible nuts.

Samples	Location	Sample number	Bacterial counts (log cfu/g) (positive %)				Reference
			Aerobic plate counts	Yeasts	Molds	Coliforms	
Almonds	Raw inshell	7	2.6–4.9 (100%)	<1.0–3.1 (28.6%)	3.5 to > 5.2 (100%)		Bansal <i>et al.</i> (2010)
	Raw kernel	81	2.0 to > 5.4, Average = 3.3 (100%)	1.0 to > 3.0 (8.9%)	1.0 to > 5.2, Average = 3.5 (98.7%)		Danyluk <i>et al.</i> (2007)
		46	2.5 to > 5.4 (100%)	<1.0–4.5 (37%)	2.8 to > 5.2 (100%)		Bansal <i>et al.</i> (2010)
	Roasted kernel	40	Average = 4.4 (78%)				Eglezos <i>et al.</i> (2008)
Brazil nuts	RTE ^a	15	Average = 4.2	Average = 2.9	Average = 2.9	ND ^b	Vural and Erkan (2008)
		42	Average = 2.4 (36%)				Eglezos (2010)
	Roasted kernel	40	Average = 3.8 (45%)				Eglezos <i>et al.</i> (2008)
	RTE	40	Average = 2.8 (56%)				Eglezos (2010)
Cashews	Roasted kernel	77	Average = 3.1 (74%)				Eglezos <i>et al.</i> (2008)
	RTE	45	Average = 2.4 (62%)				Eglezos (2010)
Hazelnus	Roasted kernel	34	Average = 2.5 (50%)				Eglezos <i>et al.</i> (2008)
	RTE	15	Average = 4.9	Average = 1.4	Average = 2.2	Average = 2.6	Vural and Erkan (2008)
		51	Average = 2.6 (36%)				Eglezos (2010)

Peanuts	Roasted kernel	Australia	644	Average=4.5 (84%)				Eglezos et al. (2008)
	RTE	Diyarbakir, Turkey	18	Average=4.1	Average=0.6	Average=1.5	Average=0.6	Vural and Erkan (2008)
		Australia	343	Average=2.7 (48%)				Eglezos (2010)
Pistachio	RTE	Diyarbakir, Turkey	15	Average=5.7	Average=0.5	Average=0.9	Average=1.5	Vural and Erkan (2008)
Walnuts	RTE	Diyarbakir, Turkey	15	Average=4.6	Average=0.9	Average=1.9	Average=2.6	Vural and Erkan (2008)
Mixed nuts	RTE	Diyarbakir, Turkey	16	Average=4.8	Average=1.7	Average=1.9	ND	Vural and Erkan (2008)
		Australia	43	Average=2.7 (44%)				Eglezos (2010)

^a Ready-to-eat.

^b Not detected.

bacterial composition and dynamics during the development of almond drupes. Immature almonds had relatively few bacterial species, and these were primarily plant-associated bacteria (e.g., *Pseudomonas*, *Pantoea*, *Methylobacterium*, and *Sphingomonas*). The number of non-plant-associated bacterial species multiplied as the drupes split and matured (e.g., *Brachybacterium*, *Frontrahabitan*, *Hymenobacter*, *Rhodocytophaga*, *Salmonella*, and *Sanguibacter*). These species have been isolated primarily from soil, sediment, and wind.

Reports on salmonellosis outbreaks associated with edible nuts started in the early 2000s (CDC, 2004; Isaacs *et al.*, 2005; Ledet *et al.*, 2007). Since then, most microbiologists and food safety technicians have focused on *Salmonella* contamination in raw and/or processed edible nuts. Almond is one of the main products associated with *Salmonella enterica* serovar Enteritidis outbreaks (Parker *et al.*, 2010). Danyluk *et al.* (2007) surveyed four raw almond products from manufacturing lots responsible for the 2000–2001 salmonellosis outbreak, and reported that 84% of the samples contained *Salmonella* with an average most-probable-number (MPN) of 8.3 per 100 g (data not shown). Peanuts (FDA, 2009a; Kirk *et al.*, 2004), pine nuts (CDC, 2011; Irvin, 2012), and pistachios (CDC, 2009; FDA, 2009b) also have been intermittently associated with *Salmonella* outbreaks and voluntary manufacturer recalls due to possible contamination.

Table 20.2 presents *Salmonella* contamination identified in edible nuts (in-shell nuts or kernels), including raw materials in processing plants and/or commercially prepared RTE products sold in retail markets. *Salmonella* was detected in almonds, Brazil nuts, peanuts, pistachios, and mixed nuts. The occurrence of *Salmonella* was higher in samples of raw peanuts (0.7–6.7%), RTE pistachios (4.0%), and almonds (0.9–1.7%) than other edible nut products (not detected (ND) to 0.4%). The quantitative levels of *Salmonella* ranged from ND to 2.4 MPN/g, which is much lower than the infective dose (10^5 – 10^{10} organisms) (Kothary and Babu, 2001).

Raw in-shell nuts or kernels generally had higher *Salmonella* contamination. Calhoun *et al.* (2013) investigated the major peanut-producing areas in the US. They reported that Virginia and North Carolina (6.7%) had the highest incidence of *Salmonella* in peanut products, followed by southwestern US (New Mexico, Oklahoma, and Texas; 3.6% incidence) and southeastern US (Alabama, Florida, Georgia, Mississippi, and South Carolina; 1.4% incidence). By contrast, no *Salmonella* was detected in RTE retail products in the UK (Little *et al.*, 2009, 2010). Raw almond kernels or in-shell nuts in Australia and the US also were positive for *Salmonella* (0.9–1.5%) (Bansal *et al.*, 2010; Danyluk *et al.*, 2007), whereas RTE almonds in Australia and the UK were negative for *Salmonella* (Eglezos *et al.*, 2008; Little *et al.*, 2009, 2010). The isolate serotypes from many of these samples were too diverse to identify specific trends. Some isolates displayed antibiotic resistance (e.g., ampicillin, cephalothin, streptomycin, and tetracycline), and a few exhibited multidrug resistance to three or more antibiotics (Danyluk *et al.*, 2007; Little *et al.*, 2010). Some studies quantified aerobic plate counts (also described as aerobic mesophilic counts, total plate counts, and total aerobic bacteria), yeasts, and mold levels in *Salmonella*-positive nuts, and concluded that there was no significant correlation between microbial populations and *Salmonella* prevalence (Bansal *et al.*, 2010; Danyluk *et al.*, 2007).

The Almond Board of California (ABC) recognized the potential *Salmonella* risk for almonds and instituted an industry-wide food safety program in association with the US Department of Agriculture (USDA). This program became mandatory for California almond producers in 2007 (ABC, 2007; USDA, 2007). All Californian almonds are pasteurized under this program, which results in a minimum 4 log reduction in *Salmonella* prior to shipment (ABC, 2007; USDA, 2007). Four pasteurization methods are used for nuts, including roasting, blanching, steaming, and propylene oxide treatment (ABC, 2007; USDA, 2007). The US

Table 20.2 Prevalence of *Salmonella* spp. in raw and processed edible nuts.

Samples		Location	Sample number	Positive %	Serotype (% in positive samples) ^a	Level (MPN ^b)	Reference
Almonds	Raw in shell	California, USA	455	1.5	Muenchen (28.6%), Give (14.3%), Newport (14.3%), Typhimurium (14.3%), Thompson (14.3%), Illa 18:z32 (14.3%)	Not tested	Bansal et al. (2010)
	Raw kernel	California, USA	9274	0.9	Montevideo (12.3%), Thompson (9.9%), Enteritidis (7.4%), Typhimurium (7.4%), Senftenberg (4.9%)	1.2–2.9/100g	Danyluk et al. (2007)
Brazil nuts	Roasted kernel RTE ^c	California, USA	3698	1.2	Muenchen (13%), Newport (10.9%), Untypeable (10.9%), Enteritidis PT 30 (8.7%), Typhimurium (6.5%)	ND–18.3/100g	Bansal et al. (2010)
		Australia	60	1.7	Fremantle (100%)		Eglezos et al. (2008)
	Roasted kernel RTE	Australia	42	ND			Eglezos (2010)
		UK	83	ND			Little et al. (2009)
		UK	359	ND			Little et al. (2010)
Cashews	Roasted kernel RTE	Australia	60	ND			Eglezos et al. (2008)
		Australia	40	ND			Eglezos (2010)
	Roasted kernel RTE	UK	218	ND			Little et al. (2009)
		UK	469	0.4	Senftenberg (66.7%), Tennessee (33.3%)	0.1–0.2/g	Little et al. (2010)
		Australia	100	ND			Eglezos et al. (2008)
Hazelnuts	Roasted kernel RTE	Australia	45	ND			Eglezos (2010)
		UK	130	ND			Little et al. (2009)
	Roasted kernel RTE	UK	459	ND			Little et al. (2010)
		Australia	48	ND			Eglezos et al. (2008)
		Australia	51	ND			Eglezos (2010)
Macadamia	Roasted kernel RTE	UK	38	ND			Little et al. (2009)
		UK	195	ND			Little et al. (2010)
	Roasted kernel RTE	UK	14	ND			Little et al. (2009)
		UK	65	ND			Little et al. (2010)

(Continued)

Table 20.2 (Continued)

Samples	Location	Sample number	Positive %	Serotype (% in positive samples)^a	Level (MPN^b)	Reference
Peanuts	Raw shelled	656	1.4	Dessau (44.4%), Anatum (11.1%), Hartford (11.1%), Meleagridis (11.1%), Tennessee (11.1%), Tornow (11.1%)	<0.03–0.04/g	Calhoun <i>et al.</i> (2013)
	Southwest, USA	194	3.6	sp. 7:m,t (28.6%), sp. 13:b,- (14.3%), Anatum (14.3%), Braenderup (14.3%), Muenchen (14.3%), Rodepoort (14.3%)	<0.03–0.04/g	Calhoun <i>et al.</i> (2013)
Pecans	Virginia/North Carolina, USA	90	6.7	Agona (100%)	<0.03–2.4/g	
	Georgia and Texas, USA	10,162	0.7	Not given	0.7–5.3/350 g	Miksch <i>et al.</i> (2013)
Pine nuts	Roasted kernel	653	ND			Eglezos <i>et al.</i> (2008)
	RTE	343	ND			Eglezos (2010)
Pistachio		26	ND			Little <i>et al.</i> (2009)
		148	ND			Little <i>et al.</i> (2010)
Walnuts		25	ND			Little <i>et al.</i> (2009)
		151	ND			Little <i>et al.</i> (2010)
Mixed nuts		29	ND			Little <i>et al.</i> (2009)
		76	ND			Little <i>et al.</i> (2010)
Peanuts		25	4.0	Havana (100%)	Not tested	Little <i>et al.</i> (2009)
		184	ND			Little <i>et al.</i> (2010)
Pine nuts		74	ND			Little <i>et al.</i> (2009)
		441	ND			Little <i>et al.</i> (2010)
Pistachio		43	ND			Eglezos (2010)
		63	ND			Little <i>et al.</i> (2009)
Walnuts		329	0.3	Anatum (100%)	<0.01/g	Little <i>et al.</i> (2010)

^a Only top five serotypes in each publication were presented in this table.

^b Most probable number.

^c Ready-to-eat.

FDA approved blanching, propylene oxide (also called fumigation), and steam processes for almonds produced by the ABC (ABC, 2007; USDA, 2007). The thermal resistance of *Salmonella* is greater in foods with low-moisture content than in those with high-moisture content (Podolak *et al.*, 2010). Several studies have compared the antimicrobial efficacy of existing sanitizing methods (Beuchat and Mann, 2011; Beuchat *et al.*, 2012; Danyluk *et al.*, 2005; Du *et al.*, 2010; Harris *et al.*, 2012; Jeong *et al.*, 2012; Lee *et al.*, 2006; Weller *et al.*, 2013) or developed novel techniques to control *Salmonella* on edible nuts (Bari *et al.*, 2009; Bingol *et al.*, 2011; Deng *et al.*, 2007; Gao *et al.*, 2011; Niemira, 2012; Pao *et al.*, 2006; Prakash *et al.*, 2010; Weller *et al.*, 2013).

Other food-borne pathogens in edible nuts can potentially lead to severe illness. The first recognized *E. coli* O157:H7 outbreak was caused by in-shell hazelnuts sold at retail markets in Michigan, Minnesota, and Wisconsin (Miller *et al.*, 2012). Vural and Erkan (2008) and Eglezos (2010) reported the incidence of other food-borne pathogenic bacteria in hazelnuts, peanuts, and mixed nuts (*Bacillus cereus*, *Clostridium perfringens*, and *Listeria monocytogenes*) sold in Turkey and Australia. *E. coli* were isolated from the following RTE nut products in the UK: Brazil nuts, 0.5–2.1%; macadamia nuts, 1.5%; walnuts, 0.7–1.4%; almonds, 0.3–1.2%; pistachios, 1.1%; peanuts, 0.7%; mixed nuts, 0.6%; hazelnuts, 0.5%; and cashews, 0.4% (Little *et al.*, 2009, 2010). *E. coli* were not detected in roasted kernel nuts in Australia (Eglezos *et al.*, 2008). Miksch *et al.* (2013) reported that Shiga toxin-producing *E. coli* were detected in three samples of shelled peanuts (0.03%) in the US.

20.3.2 Potential sources and routes of microbial contamination during manufacturing of edible nut products

Tree nuts are enclosed in hard outer hulls and are harvested when the hull ripens and/or splits open (Perry and Sibbett, 1998; Rosengarten, 2004). Figure 20.1 shows the common manufacturing

process used for edible nut products (e.g., almonds, pecans, pistachios, walnuts, and other tree nuts) and the potential sources and routes of microbial contamination. Peanuts are legumes harvested from plant roots; they are washed and boiled prior to drying.

After the mature hull splits open, the nut can be easily contaminated with microorganisms via birds, insects, and rodents (Doster and Michailides, 1994b; Freitas-Silva and Venâncio, 2011; Perry and Sibbett, 1998). McGarvey *et al.* (2014) suggest that mature nuts cannot elicit the plant immune system to control pathogen proliferation, and any bacteria can multiply on/in the split nuts by utilizing the available nutrients and moisture. Nuts are generally harvested by knocking or shaking trees with long poles to detach the nuts from the tree (Perry and Sibbett, 1998). During harvesting, in-shell nuts are exposed to microorganisms on the contact environment (e.g., harvesting apparatus and devices) or on the ground; these microorganisms are associated with irrigation water, manure, and soil (Doster *et al.*, 1996; Uesugi and Harris, 2006). Long-term survival of *Salmonella*, a prominent pathogen of edible nuts, has been reported in water, manure, sediment, and dry soil (0.8–1.5% water content) (Beuchat and Mann, 2010b; Danyluk *et al.*, 2008b; Winfield and Groisman, 2003). If the outer hull protecting the edible kernel is cracked open by herbivores or physical damage, a variety of molds, mycotoxins, and bacteria can easily penetrate into and colonize the edible kernel (Campbell *et al.*, 2003; Doster and Michailides, 1999; Mehrnezhad and Panahi, 2006; Sheibani Tezerji, 2011).

The outer hull should be removed promptly after harvesting in-shell kernels to prevent mold and/or pathogen growth (Perry and Sibbett, 1998). Hulling is performed manually in most home orchards using pocket knives or a metal-top hulling table, whereas some large-scale orchards may use mechanical hulling devices. The food handlers and hulling devices can be potential sources of microbial contamination. After harvesting and hulling, the in-shell kernels

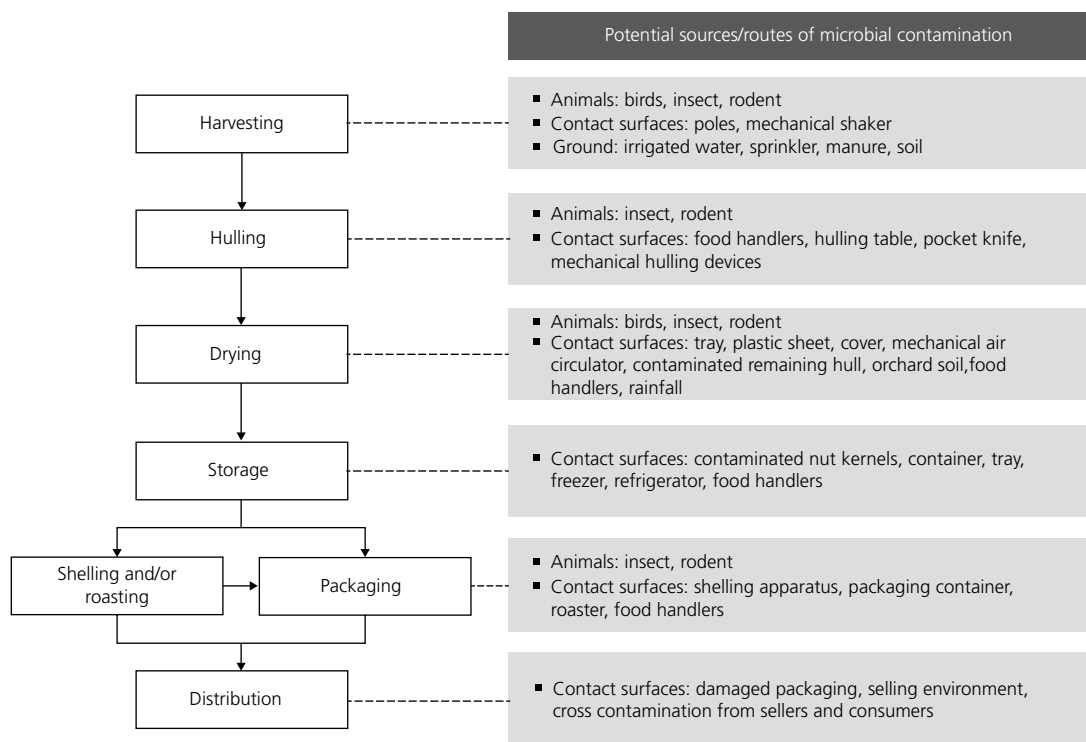


Figure 20.1 Common manufacturing processes used for edible nut products and microbial contamination sources.

are spread in a thin layer on trays or plastic sheets, often on the floor, and dried with regular stirring and good air circulation (mechanical air circulators can be used for rapid drying) to reduce the moisture content to approximately 7% (Pan *et al.*, 2012; Perry and Sibbett, 1998). The duration of drying varies from 2 to 10 days depending on the crop, air temperature, and harvest date. The kernels must be dried sufficiently so that they change from rubbery (fresh kernel) to crispy/brittle (dry kernel) when broken (Perry and Sibbett, 1998). It is recommended to cover nut kernels with screen or plastic netting to deter animal feeding (e.g., birds, insects, and rodents). If the drying process is performed on the orchard ground, the top layers of soil may be unintentionally mixed with the nuts during mechanical sweeping of dried nuts from the ground (Danyluk *et al.*, 2008b). Motile microorganisms can attach to the outer surface of closed hulls/shells and

subsequently migrate to the kernels. Rainfall can be another contributor to microbial contamination, and the salmonellosis outbreak in 2000–2001 was partly caused by significant rainfall during the 2000 harvest and drying. It was experimentally demonstrated that wet hulls can support rapid growth of *Salmonella* on almond hulls and shell slurries (Uesugi and Harris, 2006).

Dried nuts are subjected to long storage at ambient temperature for approximately 8 months, in a refrigerator for approximately 1 year at 0–7.2°C, or in a freezer for approximately 1–3 or more years at –17.8°C (Perry and Sibbett, 1998). These procedures aim to prevent and/or reduce microbial or mold contamination. Storage containers, trays, storage environment, and food handlers can all be potential sources of microbial contamination. Because *Salmonella* can survive for a long time on nut hulls and slurries (Danyluk *et al.*, 2008a; Uesugi

and Harris, 2006), cross-contamination of adjacent nuts can occur. Although low-temperature storage (-20 and 4°C) decreased the *Salmonella* infiltration rate into pecan nuts, large numbers of *Salmonella* contaminants survived well at these temperatures up to 18 months after harvest (Beuchat and Mann, 2010a).

The shell is the primary barrier to microbial contamination of the inner kernel (Pan *et al.*, 2012). Shelling exposes the kernels to cross-contamination from contact surfaces. Shelling may reduce *Salmonella* contamination on the nut kernels by approximately 2 log cfu/g (Pao *et al.*, 2006); however, the remaining pathogens can survive well. This is empirically supported by several publications that report long-term survival of diverse food-borne pathogens (e.g., *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*) in a variety of nut products including in-shell nuts and kernels (e.g., almonds, pecans, pistachios, and walnuts) (Beuchat and Mann, 2010a; Blessington *et al.*, 2012, 2013; Kimber *et al.*, 2012). Nut kernels are shelled depending on the intended use and then subjected to additional processing (e.g., blanching, dicing, grounding, packaging, pasteurization, roasting, salting, and slicing). Packaging can be damaged during shipping and retail handling, which can be another route for microbial contamination of nut products.

20.3.3 Edible seeds

The literature reporting the microbiological quality of edible seeds in the market is limited. Vural and Erkan (2008) report substantial numbers of aerobic plate counts (4.0 – 5.7 log cfu/g), yeasts (ND – 2.5 log cfu/g), molds (0.3 – 2.0 log cfu/g), and coliforms (0.7 – 2.5 log cfu/g) in edible seeds (melon, pumpkin, sunflower, and watermelon seeds) in spite of their dried characteristics. Some food-borne pathogens were detected in edible seeds, ranging from ND – 2.4 log cfu/g (*B. cereus*) and ND – 3.7 log cfu/g (*C. perfringens*).

A few studies surveyed *Salmonella* contamination in edible seeds (Table 20.3). Brockmann *et al.* (2004) were motivated by the 2001 inter-

national salmonellosis outbreak; they examined *Salmonella* in sesame seeds and products including halva, oil, paste, and pastry. They detected *Salmonella* in 12.5% of sesame seeds, 11.3% of halva, and 8.3% of sesame paste. Most of the isolates were serovar Typhimurium DT 104 (6 of 11), and other serovars included Poona and Typhimurium DT 134 isolated from halva and Tennessee and Offa isolated from sesame seed. Willis *et al.* (2009) surveyed *Salmonella* and *E. coli* contamination in 3735 edible seeds products in the UK during 2007–2008. A melon seed (also called egusi) had the highest contamination levels of both *Salmonella* (8.7%) and *E. coli* (12.8%) except sesame seed. *Salmonella* was isolated from 1.7% of sesame and alfalfa seeds, 0.9% of mixed seeds, 0.4% of linseed, and 0.1% of sunflower seed (the *Salmonella* levels in each product were not reported). *E. coli* contamination was detected in 0.4–2.6% of all tested edible seeds except alfalfa seeds.

Sesame seed is the only edible seed product that has been associated with infectious disease outbreaks. Salmonellosis attributed to sesame seed products such as tahini (paste made from ground and hulled sesame seeds) and halva (sweet confections with edible seeds) occurred intermittently in 2001 (27 cases in Sweden, Norway, Germany, and Australia), 2002–2003 (68 patients in Australia and New Zealand), and 2013 (16 cases in the US); these outbreaks were caused by serovar Typhimurium DT 104, Montevideo, and/or Mbandaka, respectively (Andersson *et al.*, 2001; Unicomb *et al.*, 2005). Many investigators have tried to experimentally simulate *Salmonella* survival in edible seeds and relevant foods to understand and manipulate their responses (Komitopoulou and Peñaloza, 2009; Kotzekidou, 1998; Podolak *et al.*, 2010; Torlak *et al.*, 2013).

Edible seeds are generally processed by small-scale farmers and merchants using significantly different methods, which depend on geographical and cultural norms. Therefore, it is challenging to systematically analyze potential sources and routes of microbial contamination. Individual cases need to be independently

Table 20.3 Prevalence of *Salmonella* spp. and *E. coli* in edible seeds.

Samples	Location	<i>Salmonella</i> spp.			<i>E. coli</i> (>2 log cfu/g)	Reference
		Sample number	Positive %	Serotype	Positive %	
Alfalfa	UK	58	1.7	Not given	ND	Willis <i>et al.</i> (2009)
Hemp	UK	121	ND		1.7	
Linseed (flax)	UK	284	0.4	Not given	0.4	
Melon (egusi)	UK	47	8.5	47: z4,z23:- (25%)	12.8	
Poppy	UK	202	ND		1.5	
Pumpkin	UK	886	ND		2.6	
Sesame	Germany	16	12.5	Tennessee (50%), Offa (50%)	Not tested	Brockmann <i>et al.</i> (2004)
	UK	771	1.7	Drypool (30.8%), 47: z4,z23:- (15.4%)	1.0	
Sunflower	UK	976	0.1	Not given	0.4	Willis <i>et al.</i> (2009)
Mixed seeds	UK	350	0.9	Drypool (33.3%)	2.3	

analyzed to identify possible routes of contamination during edible seed harvesting, processing, and manufacturing.

20.4 Microbial ecology of sprouts and their corresponding seeds

20.4.1 Sprouts sold in market

Quantitative analyses of bacterial contamination levels in sprouts and their corresponding seeds sold in retail markets are shown in Tables 20.4 and 20.5, respectively. The target bacteria for analysis and their contamination levels varied in different surveys. Bacteria including aerobic plate counts, yeasts, molds, coliforms, anaerobic bacteria, psychrotrophic bacteria, and lactic acid bacteria have been identified in sprouts (Table 20.4). Sprouts have well-documented high microbiological contamination irrespective of the sanitation methods used during manufacturing. These high bacterial counts may be attributed to endemic bacteria present in the seeds because the environment required for sprouting and germination (25–30°C) provides favorable conditions for bacterial growth (Gabriel *et al.*, 2007;

Taormina *et al.*, 1999). The aerobic plate counts ranged from minimum levels of 4.5 log cfu/g (mung bean sprouts in Muntinlupa, Philippines) (Gabriel *et al.*, 2007) to maximum levels of 11.6 log cfu/g (brown moong sprouts in India) (Viswanathan and Kaur, 2001). Most sprouts had aerobic plate counts of 7–9 log cfu/g, and higher contamination levels of >10 log cfu/g were reported in some samples obtained in India and Philippines. In India, aerobic plate counts on green moong, brown moong, and chana sprouts were greater (10.0–11.6 log cfu/g) (Viswanathan and Kaur, 2001) than those of other samples. A study in Philippines reported that approximately 24% of samples had >10 log cfu/g aerobic plate counts (Gabriel *et al.*, 2007).

Several studies analyzed the yeast and mold counts on sprouts (Abadias *et al.*, 2008; Gabriel *et al.*, 2007; Kim *et al.*, 2009; Saroj *et al.*, 2006; Seow *et al.*, 2012; Tournas, 2005). Yeast and mold contamination levels varied, ranging from 1.2 log cfu/g (mung bean sprouts sold in Taguig, Philippines) (Gabriel *et al.*, 2007) to 7.6 log cfu/g (soybean and alfalfa sprouts in Spain) (Abadias *et al.*, 2008). Tournas (2005) analyzed yeast and mold communities in sprouts. *Alternaria*, *Cladosporium*, *Penicillium*, and *Phoma* were most frequently isolated from sprouts, whereas

Table 20.4 Bacterial contamination levels in retail sprouts.

Samples	Location	Sample number	Bacterial counts (log cfu/g)				Reference
			Aerobic plate counts	Yeast and mold	Coliforms	Others	
Green moong	Mumbai, India	8	11.5		9.3		Viswanathan and Kaur (2001)
Brown moong		8	11.6		10.7		
Chana		8	10.0		8.7		
Soybean	Masan and Changwon, Korea	17	7.8		6.7	Anaerobic microorganisms: 6.5 Psychrotrophic microorganisms: 7.7 Spore-forming microorganisms: 2.7	Kim et al. (2004)
Bean	Washington DC, USA	27		7.2			Tournas (2005)
Alfalfa		27		5.9			
Crunchy		25		7.0			
Broccoli		23		5.0			
Mixed ^a		14		5.3			
Mung	Mumbai and its suburbs, India	46	7.6–8.9	3.6–7.3	5.4–7.9	Staphylococci: 3.3–6.6	Saroj et al. (2006)
Matki		27					
Chana		25					
Vatana		26					
Mung bean	Philippines	100	4.5–11.4 (average=8.3)	1.2–5.9 (average=3.8)	5.1–5.9 (average=6.6)	Generic <i>E. coli</i> : < 4.0–5.5 Lactic acid bacteria: 5.3–10.5	Gabriel et al. (2007)
Soybean and alfalfa	Catalonia, Spain	15	7.9 (7.1–9.2)	5.2 (2.8–7.6)		<i>Enterobacteriaceae</i> : 7.2 (6.3–8.1) Lactic acid bacteria: 5.5 (3.4–7.5) Psychrotrophic microorganisms: 7.3 (6.3–8.9)	Abadías et al. (2008)

(Continued)

Table 20.4 (Continued)

Samples	Location	Sample number	Bacterial counts (log cfu/g)				Reference
			Aerobic plate counts	Yeast and mold	Coliforms	Others	
Alfalfa	Korea	6	7.8–9.0		6.9–8.4		Waje <i>et al.</i> (2009)
Brassica		2	7.7–7.9		7.3–7.3		
Broccoli		7	7.3–8.9		7.1–7.7		
Buckwheat		2	8.5–9.0		7.1–7.9		
Chinese cabbage		4	7.6–8.9		7.2–7.6		
Kale		2	8.2		7.6–7.9		
Mung bean		1	7.1		4.6		
Pak choy		1	7.4		7.0		
Radish		2	7.3–7.6		5.5–6.7		
Rape		5	7.7–8.7		7.1–7.5		
Red cabbage		8	7.4–9.0		7.0–8.0		
Red radish		6	7.7–8.8		7.3–7.9		
Soybean		1	7.0		6.0		
Tatsoi		5	7.3–8.9		7.0–7.8		
Turnip		1	7.5		7.6		
Mixed		2	7.3–7.7		5.7–6.8		
Radish	Seoul, Korea	45	7.0	6.5			Kim <i>et al.</i> (2009)
Mixed ^b		45	7.5	7.4			
Bean	Singapore	19 or 14 ^c	8.0	4.3	5.7	Aerobic psychrotrophic count: 1.6 (ND–8.0)	Seow <i>et al.</i> (2012)
			(7.3–9.1)	(3.7–4.9)	(4.1–6.3)		

^a They included broccoli–cauliflower–kale, clover, lentil, snow pea sprouts, garlic sprouts, onion sprouts, spicy with radish sprouts.

^b They included at least three types of clover, alfalfa, Brussels sprouts, broccoli, kohlrabi, red cover, and Chinese cabbage.

^c Aerobic plate counts and aerobic psychrotrophic count examined for 19 samples while others (coliforms and yeast and molds) examined for 14 samples.

Table 20.5 Prevalence of bacteria in sprouts.

Samples	Location	Sample number	Positive	Negative	Reference
Green moong	Mumbai, India	8	<i>P. aeruginosa</i> (75%), fecal <i>E. coli</i> (62.5%), <i>S. aureus</i> (25%)	<i>Edwardsiella</i> , <i>Enterobacter</i> , <i>Salmonella</i> , <i>Serratia</i>	Viswanathan and Kaur (2001)
Brown moong		8	<i>P. aeruginosa</i> (100%), fecal <i>E. coli</i> (50%), <i>S. aureus</i> (75%), <i>Enterobacter</i> (25%), <i>Salmonella</i> (12.5%)		
Chana		8	<i>P. aeruginosa</i> (87.5%), fecal <i>E. coli</i> (87.5%), <i>S. aureus</i> (62.5%), <i>Enterobacter</i> (62.5%), <i>Serratia</i> (25%)	<i>Edwardsiella</i> , <i>Salmonella</i>	
Soybean	Masan and Changwon, Korea	17	<i>B. cereus</i> (71%)	<i>C. botulinum</i> , <i>C. perfringens</i>	Kim et al. (2004)
Mung	Mumbai and its suburbs, India	46	<i>Salmonella</i> (23%), <i>E. coli</i> (13%), coagulase positive <i>S. aureus</i> (8.7%)	<i>L. monocytogenes</i>	Saroj et al. (2006)
Matki		27	<i>Salmonella</i> (44%), <i>E. coli</i> (26%), coagulase positive <i>S. aureus</i> (11%)	<i>L. monocytogenes</i>	
Chana		25	<i>E. coli</i> (40%), <i>Salmonella</i> (4%), coagulase positive <i>S. aureus</i> (4%)	<i>L. monocytogenes</i>	
Vatana		26	<i>E. coli</i> (19%)	<i>L. monocytogenes</i> , <i>Salmonella</i> , coagulase positive <i>S. aureus</i>	
Mung bean	Philippines	100	<i>Salmonella</i> (94%)		Gabriel et al. (2007)
Soybean and alfalfa	Catalonia, Spain	15	<i>E. coli</i> (40%)	<i>L. monocytogenes</i> , <i>Salmonella</i>	Abadias et al. (2008)

(Continued)

Table 20.5 (Continued)

Samples	Location	Sample number	Positive	Negative	Reference
Alfalfa	Korea	6	<i>L. monocytogenes</i>	<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i>	Waje et al. (2009)
Brassica		2		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Broccoli		7	<i>L. monocytogenes</i>	<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i>	
Buckwheat		2		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Chinese cabbage		4		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Kale		2		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Mungbean		1		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Pak choi		1		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Radish		2		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Rape		5		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Red cabbage		8		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Red radish		6	<i>L. monocytogenes</i>	<i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>B. cereus</i>	
Soybean		1		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Tatsoi		5		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Turnip		1		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Mixed		2		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>Salmonella</i> , <i>L. monocytogenes</i>	
Radish	Seoul, Korea	45		<i>Cronobacter</i> spp., <i>E. coli</i> O157:H7, <i>Salmonella</i>	Kim et al. (2009)
Mixed ^a		45	<i>Cronobacter</i> spp. (13.3%)	<i>E. coli</i> O157:H7, <i>Salmonella</i>	Seow et al. (2012)
Bean	Singapore	14		<i>E. coli</i> O157:H7, <i>Salmonella</i>	

^aThey included at least three types of clover, alfalfa, Brussels sprouts, broccoli, kohlrabi, red cover, and Chinese cabbage.

Fusarium, *Rhizopus*, *Mucor*, and *Geotrichum* were isolated infrequently. Many recovered isolates belonged to toxigenic genera including *Penicillium*, *Alternaria*, and *Phoma*, which can grow at refrigerated temperatures. Tournas (2005) suggested that sprouts are a potential source of mycotoxin production during distribution and marketing; thus high yeast and mold counts in sprouts are a cause for concern.

Coliforms are traditional hygienic indicators used to assess the quality or hygienic status of food. Coliforms were present in most sprouts at levels of >5 log cfu/g. In some surveys (Viswanathan and Kaur, 2001; Waje *et al.*, 2009), coliforms were the predominant microorganisms in sprouts, with populations as high as >7 log cfu/g. Sprouts that had the highest aerobic plate counts also had the highest coliform densities; in India, green moong, brown moong, and chana sprouts had coliform counts of 9.3, 10.7, and 8.7 log cfu/g, respectively (Viswanathan and Kaur, 2001).

Other tested bacteria were identified in sprouts as follows: 6.5 log cfu/g anaerobic bacteria, 7.7 log cfu/g psychrotrophic bacteria, and 2.7 log cfu/g spore-forming bacteria for soybean sprouts in Korea (Kim *et al.*, 2004); 3.3–6.6 log cfu/g *Staphylococci* for mung, matki, chana, and vatana sprouts in India (Saroj *et al.*, 2006); 5.3–10.5 log cfu/g lactic acid bacteria and 4.0–5.5 log cfu/g *E. coli* for mung bean sprouts in Philippines (Gabriel *et al.*, 2007); 7.3 log cfu/g psychrotrophic bacteria, 7.2 log cfu/g *Enterobacteriaceae*, and 5.5 log cfu/g lactic acid bacteria for soybean and alfalfa sprouts in Spain (Abadias *et al.*, 2008); and 1.6 log cfu/g aerobic psychrotrophic bacteria for bean sprouts in Singapore (Seow *et al.*, 2012).

Bacterial communities in four retail samples of alfalfa sprouts were characterized using 16S rDNA profiling and the results detected similar bacterial communities in the four samples (Loui *et al.*, 2008). The dominant phyla in alfalfa sprouts were *Proteobacteria*, *Enterobacteriaceae*, *Oxalobacteraceae*, *Moraxellaceae*, and *Sphingomonadaceae*. Most bacterial communities in sprouts contained saprophytic and

non-pathogenic strains. The bacterial communities associated with four retail samples of alfalfa sprouts were as follows: brand A contained 14.0% *Oxalobacteraceae*, 14.0% *Methylophilaceae*, 13.0% *Comamonadaceae*, and 13.0% *Enterobacteriaceae*; brand B contained 36.6% *Oxalobacteraceae* and 20.4% *Enterobacteriaceae*; brand C contained 31.3% *Enterobacteriaceae* and 17.5% *Sphingomonadaceae*; and brand D contained 52.5% *Enterobacteriaceae* and 35.0% *Moraxellaceae*. The presence of potentially pathogenic bacteria (*Acinetobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Salmonella*, and *Stenotrophomonas*) in alfalfa sprouts also was assessed. *Acinetobacter*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Stenotrophomonas* were detected in three alfalfa sprout samples; *Escherichia* and *Salmonella* were detected in two alfalfa sprout samples (the pathogenicity of recovered bacteria was not reported).

Some studies (Abadias *et al.*, 2008; Gabriel *et al.*, 2007; Kim *et al.*, 2004, 2009; Saroj *et al.*, 2006; Seow *et al.*, 2012; Viswanathan and Kaur, 2001; Waje *et al.*, 2009) qualitatively analyzed the prevalence of food-borne pathogens in sprouts, including *Salmonella*, *E. coli*, *Staphylococcus aureus*, *L. monocytogenes*, and *B. cereus* (Table 20.5). The highest prevalence of *Salmonella* was reported in mung bean sprouts (94%) in Philippines (Gabriel *et al.*, 2007), followed by matki sprouts (44%), mung sprouts (23%), brown moong sprouts (12.5%), and chana sprouts (4%) in India (Saroj *et al.*, 2006; Viswanathan and Kaur, 2001). *Salmonella* was not detected in green moong, chana sprouts, vatana sprouts in India (Saroj *et al.*, 2006; Viswanathan and Kaur, 2001), soybean and alfalfa sprouts in Spain (Abadias *et al.*, 2008), various types of sprouts in Korea (Kim *et al.*, 2009; Waje *et al.*, 2009), or bean sprouts in Singapore (Seow *et al.*, 2012). *E. coli* was detected in the following percentages of sprout samples: 62.5% green moong, 50% brown moong, and 87.5% chana (Viswanathan and Kaur, 2001); 13% mung, 26% matki, 40% chana, and 19% vatana (Saroj *et al.*, 2006); and 40% soybean and alfalfa (Abadias *et al.*, 2008). Pathogenic *E. coli* O157:H7 was not

detected in those sprout samples (Kim *et al.*, 2009; Seow *et al.*, 2012; Waje *et al.*, 2009). Viswanathan and Kaur (2001) and Saroj *et al.* (2006) isolated *S. aureus* in India and reported the prevalence of *S. aureus* in green moong (25%), brown moong (75%), chana (62.5%), mung (8.7%), matki (11%), and chana (4%) sprouts. *L. monocytogenes* was isolated from alfalfa, broccoli, and red radish sprouts in Korea (detection percentages were not mentioned) (Waje *et al.*, 2009). Conversely, other researchers reported that no *L. monocytogenes* was detected in various sprout commodities (Abadias *et al.*, 2008; Saroj *et al.*, 2006; Waje *et al.*, 2009). In Korea, Kim *et al.* (2004) detected *B. cereus* in 71% of soybean sprouts, whereas Waje *et al.* (2009) did not detect *B. cereus* in any samples.

20.4.2 Seeds used for sprouts production

Seeds used for sprouting can have significant levels of endemic bacteria (NCMCF, 1999). Contamination levels and the prevalence of bacteria in seeds used for sprout production are shown in Tables 20.6 and 20.7, respectively. Seeds had much lower bacterial populations than those of sprouts. Most seeds had aerobic plate counts < 4 log cfu/g, yeast and mold < 2.4 log cfu/g, and coliforms < 2 log cfu/g (Kim *et al.*, 2009; Pao *et al.*, 2005; Waje *et al.*, 2009). In the US, Pao *et al.* (2005) reported 0.7–1.0 log cfu/g of presumptive *B. cereus* counts from all tested seed types (alfalfa, broccoli, lentil, mung bean, and radish seed).

No pathogenic bacteria were isolated from seeds used for sprout production, including *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, *B. cereus*, and *Cronobacter* spp. (Kim *et al.*, 2009; Pao *et al.*, 2005; Waje *et al.*, 2009). These studies suggest that pathogen contamination of sprouts may be primarily introduced by other routes during production or distribution. However, Egyptian fenugreek seeds imported to Germany were identified as the source of the contaminated sprouts that caused a huge outbreak in Germany in 2011 (EFSA, 2011). Thus, seeds remain as a possible route for pathogenic contamination of sprouts.

20.4.3 Changes in microbial ecology during sprout processing and manufacture

General processing of seeds and sprouts in a manufacturing plant can be summarized as follows: seeds are delivered to the plant, seeds are placed into storage, pre-germination soak, rinse, germination and growth (utilizing a rotary drum or stationary trays), packaging, cooling and storage, and distribution (NCMCF, 1999). Sprouts can be contaminated from several sources during production, including soil, contact surfaces, environment, water, containers, and food handlers (Sagoo *et al.*, 2003; Yang *et al.*, 2013). To identify the possible contamination routes and pathogens during sprout production, it is necessary to investigate each step of the process during production in real plants.

Kim *et al.* (2013) visited three Korean manufacturing plants for alfalfa and rapeseed sprouts (a total of 20 visits), and collected samples at five different stages of processing including seed storage, pre-germination soak, germination, sprouting, and sprouts storage (Kim *et al.*, 2013). Aerobic plate counts, coliform counts, and *B. cereus* were analyzed, and the presence of *E. coli* and major foodborne pathogens (*E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, and *S. aureus*) was analyzed at each processing step. Changes in bacterial contamination levels of alfalfa and rapeseed sprouts during processing are shown in Figure 20.2. Seeds and sprouts sampled in real plants have similar contamination levels of aerobic plate counts and coliform counts as those of seeds and sprouts sold in retail markets in Korea (Kim *et al.*, 2004; Waje *et al.*, 2009). The germination step was identified as a particularly susceptible stage for bacterial count increases; alfalfa and rapeseed seeds had average 4.1 and 4.7 log CFU/g of aerobic plate counts, which increased after germination to 7.8 and 8.3 log CFU/g, respectively. Those contamination levels were maintained during subsequent processing, but they were slightly reduced by a washing step. The final alfalfa and rapeseed sprout products had bacterial counts of average 7.4 and 8.0 log CFU/g, respectively. *B. cereus*

Table 20.6 Bacterial contamination levels in seeds used for the production of sprouts.

Samples	Location	Sample number	Bacterial counts (log cfu/g or log MPN/g)				Reference
			Aerobic plate counts	Yeast and mold	Coliforms	Others	
Alfalfa	Multistates, USA	12	3.3		-0.3 (log MPN/g)	Presumptive <i>B. cereus</i> counts: 0.7	Pao <i>et al.</i> (2005)
Broccoli		12	4.0		-0.4 (log MPN/g)	Presumptive <i>B. cereus</i> counts: 1.0	
Lentil		12	2.8		-0.5 (log MPN/g)	Presumptive <i>B. cereus</i> counts: 0.8	
Mung bean		12	3.5		0 (log MPN/g)	Presumptive <i>B. cereus</i> counts: 1.0	
Radish		12	3.6		-0.4 (log MPN/g)	Presumptive <i>B. cereus</i> counts: 0.9	
Alfalfa	Korea	5	1.2-4.7		<1-2.0		Waje <i>et al.</i> (2009)
Broccoli		5	2.5-3.3		<1-1.4		
Chinese cabbage		1	4.1		<1-1.3		
Rape sprout		4	3.6-5.5		1.7-3.3		
Red cabbage		3	3.0-3.9		<1-2.0		
Red radish		5	2.2-3.6		<1-1.5		
Tatsoi		5	2.2-5.4		<1-2.2		
Radish		33	4.1	2.4			
Alfalfa	Seoul, Korea	36	2.5	0.8			Kim <i>et al.</i> (2009)
Turnip		27	2.8	1.7			

Table 20.7 Prevalence of bacteria in seed used for the production of sprouts.

Samples	Location	Sample number	Positive	Negative	Reference
Alfalfa	Multistates, USA	12		<i>E. coli</i> O157, fecal coliform, <i>Salmonella</i> , <i>S. aureus</i>	Pao et al. (2005)
Broccoli		12		<i>E. coli</i> O157, fecal coliform, <i>Salmonella</i> , <i>S. aureus</i>	
Lentil		12		<i>E. coli</i> O157, fecal coliform, <i>Salmonella</i> , <i>S. aureus</i>	
Mung bean		12		<i>E. coli</i> O157, fecal coliform, <i>Salmonella</i> , <i>S. aureus</i>	
Radish		12		<i>E. coli</i> O157, fecal coliform, <i>Salmonella</i> , <i>S. aureus</i>	
Alfalfa	Korea	5		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	Waje et al. (2009)
Broccoli		5		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	
Chinese cabbage		1		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	
Rape sprout		4		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	
Red cabbage		3		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	
Red radish		5		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	
Tatsoi		5		<i>B. cereus</i> , <i>E. coli</i> O157:H7, <i>L. monocytogenes</i> , <i>Salmonella</i>	
Radish		33		<i>Cronobacter</i> spp., <i>E. coli</i> O157:H7, <i>Salmonella</i>	
Alfalfa		36		<i>Cronobacter</i> spp., <i>E. coli</i> O157:H7, <i>Salmonella</i>	
Turnip		27		<i>Cronobacter</i> spp., <i>E. coli</i> O157:H7, <i>Salmonella</i>	
	Seoul, Korea				Kim et al. (2009)

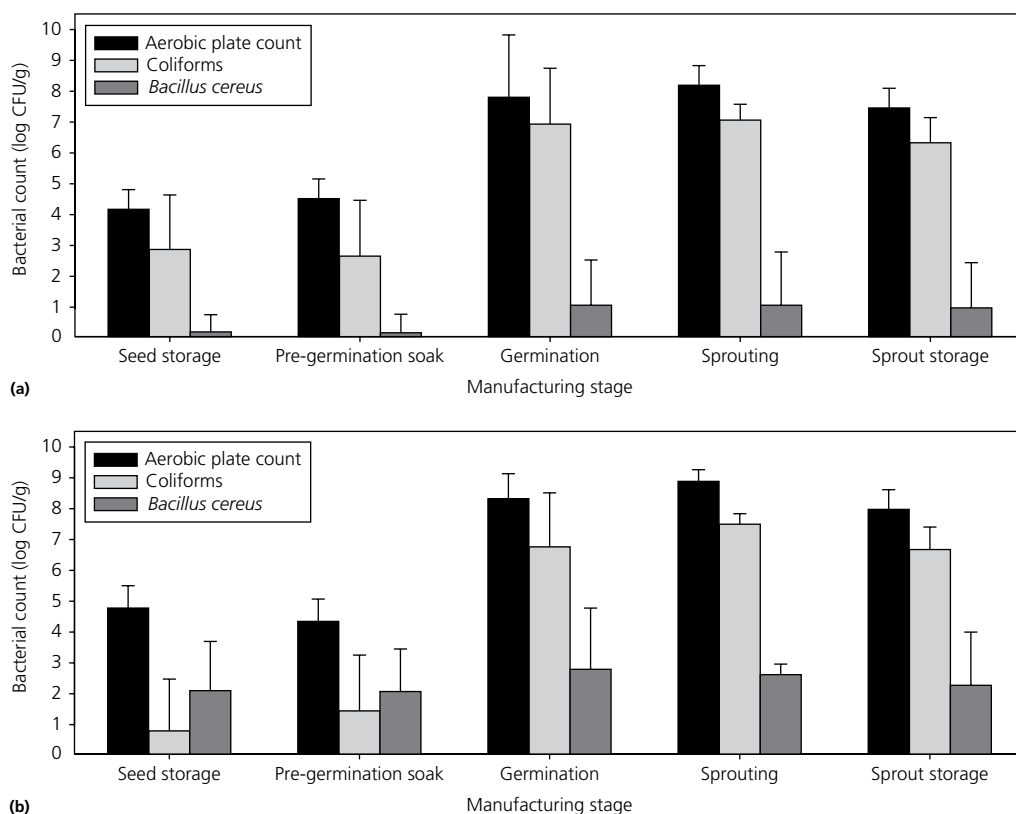


Figure 20.2 Changes in bacteria densities of aerobic plate count, coliforms, and *Bacillus cereus* in a product line sample of (a) alfalfa sprouts and (b) rapeseed sprouts. Data were provided by Kim *et al.* (2013).

isolates were frequently obtained at all processing steps, from seeds to the final products. Therefore, *B. cereus* may be a potential microbiological hazard that should be controlled. The sprout manufacturing process differs according to the type of product, manufacturer, germination conditions, and decontamination methods used for food safety. These processes can affect the microbial prevalence in sprouts. Thus, more investigations in real manufacturing plants are required to clearly identify contamination routes and intervention methods.

20.5 Implications and perspectives

Edible nuts, seeds, and sprouts harbor considerable numbers of microorganisms, and the prevalence of food-borne pathogens has been

reported in a number of publications. Molds and their toxic metabolites and food-borne pathogenic bacteria (especially *Salmonella*) are a major concern for the food safety of edible nuts and seeds. Sprouts contain diverse bacteria including anaerobic bacteria, psychrotrophic bacteria, spore-forming bacteria, lactic acid bacteria, generic *Enterobacteriaceae*, and pathogenic bacteria including *B. cereus*, *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes*. The reported microbiological communities and contamination levels varied among the surveys. Edible nuts, seeds, and sprouts are generally ingested directly as raw products or after slight washing and/or cooking by consumers. These products are ingested along with any pathogenic bacterial contaminants, which elevates potential risks for serious illnesses. To improve the microbial safety of edible nuts, seeds, and sprouts, more empirical data are needed for

where and/or how the products are invaded and colonized by these microorganisms. Many studies have detected microorganisms in the final products, but there is a lack of information on overall microbial ecology in the manufacturing environment, which makes it difficult to predict bacterial contamination routes. It is essential that the following comprehensive counter measures are considered: (1) continuous and regular investigations to determine current microbial composition and densities; (2) identify sources and routes of major microorganism contamination during harvesting to consumption stages; (3) implementation of hygienic measures (such as good agricultural practices or hazard analysis and critical control points (HACCPs)) during manufacturing and distribution with proper intervention techniques; (4) appropriate regulation and establishment of safety criteria; and (5) enhancing consumer awareness about potential hazards and proper treatment (washing or cooking) prior to consumption.

References

- Abadias, M., Usall, J., Anguera, M., Solsona, C., and Viñas, I. 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, **123**, 121–129.
- ABC (Almond Board of California) 2007. Pasteurization: the food safety program and almond pasteurization. Available at: <http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/Pages/Default.aspx> (accessed on July 23, 2014).
- Abdulkadar, A.H.W., Al-Ali, A., and Al-Jedah, J.H. 2000. Aflatoxin contamination in edible nuts imported in Qatar. *Food Control*, **11**, 157–160.
- Abdulkadar, A.H.W., Al-Ali, A., and Al-Jedah, J.H. 2002. Occurrence of aflatoxin in commodities imported into Qatar, 1997–2000. *Food Additives and Contaminants*, **19**, 666–670.
- Al-Moghazy, M., Boveri, S., and Pulvirenti, A. 2014. Microbiological safety in pistachios and pistachio containing products. *Food Control*, **36**, 88–93.
- Andersson, Y., de Jong, B., Hellström, L., Stamer, U., Wollin, R., and Giesecke, J. 2001. *Salmonella* Typhimurium outbreak in Sweden from contaminated jars of helva (or halva). *Eurosurveillance Weekly*, **5**, 19.
- Bansal, A., Jones, T.M., Abd, S.J., Danyluk, M.D., and Harris, L.J. 2010. Most-probable-number determination of *Salmonella* levels in naturally contaminated raw almonds using two sample preparation methods. *Journal of Food Protection*, **73**, 1986–1992.
- Bari, M.L., Nei, D., Sotome, I., Nishina, I., Isobe, S., and Kawamoto, S. 2009. Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate *Salmonella* on almonds. *Foodborne Pathogens and Disease*, **6**, 953–958.
- Beuchat, L.R. and Mann, D.A. 2010a. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. *Journal of Food Protection*, **73**, 1257–1268.
- Beuchat, L.R. and Mann, D.A. 2010b. Survival and growth of *Salmonella* in high-moisture pecan nutmeats, in-shell pecans, inedible nut components, and orchard soil. *Journal of Food Protection*, **73**, 1975–1985.
- Beuchat, L.R. and Mann, D.A. 2011. Inactivation of *Salmonella* on pecan nutmeats by hot air treatment and oil roasting. *Journal of Food Protection*, **74**, 1441–1450.
- Beuchat, L.R., Mann, D.A., and Alali, W.Q. 2012. Evaluation of sanitizers for inactivating *Salmonella* on in-shell pecans and pecan nutmeats. *Journal of Food Protection*, **75**, 1930–1938.
- Bingol, G., Yang, J., Brandl, M.T., Pan, Z., Wang, H., and McHugh, T.H. 2011. Infrared pasteurization of raw almonds. *Journal of Food Engineering*, **104**, 387–393.
- Blessington, T., Mitcham, E.J., and Harris, L.J. 2012. Survival of *Salmonella enterica*, *Escherichia coli* O157: H7, and *Listeria monocytogenes* on inoculated walnut kernels during storage. *Journal of Food Protection*, **75**, 245–254.
- Blessington, T., Theofel, C.G., Mitcham, E.J., and Harris, L.J. 2013. Survival of foodborne pathogens on inshell walnuts. *International Journal of Food Microbiology*, **166**, 341–348.

- Blomhoff, R., Carlsen, M.H., Andersen, L.F., and Jacobs, D.R. 2006. Health benefits of nuts: potential role of antioxidants. *British Journal of Nutrition*, **96**, S52–S60.
- Brockmann, S.O., Piechotowski, I., and Kimmig, P. 2004. *Salmonella* in sesame seed products. *Journal of Food Protection*, **67**, 178–180.
- Buchholz, U., Bernard, H., Werber, D., Böhmer, M. M., *et al.* 2011. German outbreak of *Escherichia coli* O104: H4 associated with sprouts. *New England Journal of Medicine*, **365**, 1763–1770.
- Calhoun, S., Post, L., Warren, B., Thompson, S., and Bontempo, A.R. 2013. Prevalence and concentration of *Salmonella* on raw shelled peanuts in the United States. *Journal of Food Protection*, **76**, 575–579.
- Campbell, B.C., Molyneux, R.J., and Schatzki, T.F. 2003. Current research on reducing pre-and post-harvest aflatoxin contamination of US almond, pistachio, and walnut. *Toxin Reviews*, **22**, 225–266.
- CDC. 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds – United States and Canada, 2003–2004. *Morbidity and Mortality Weekly Report*, **53**, 484–487.
- CDC. 2009. Multistate outbreak of *Salmonella* infections linked to pistachio nuts [Online]. Available at: <http://www.cdc.gov/salmonella/pistachios/> (accessed 14 April 2009).
- CDC. 2011. Multistate outbreak of human *Salmonella* Enteritidis infections linked to Turkish pine nuts [Online]. Available at: <http://www.cdc.gov/salmonella/pinenuts-enteritidis/111711/index.html> (accessed 17 November 2011).
- Chun, H.S., Kim, H.J., Ok, H.E., Hwang, J.-B., and Chung, D.-H. 2007. Determination of aflatoxin levels in nuts and their products consumed in South Korea. *Food Chemistry*, **102**, 385–391.
- CODEX. 2006. Section 2. Codex classification of foods and animal feeds. *Pesticides residues in food*. Food and Agriculture Organization of the United Nations World Health Organization, Rome, Italy.
- Danyluk, M.D., Uesugi, A.R., and Harris, L.J. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *Journal of Food Protection*, **68**, 1613–1622.
- Danyluk, M.D., Jones, T.M., Abd, S.J., Schlitt-Dittrich, F., Jacobs, M., and Harris, A.J. 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. *Journal of Food Protection*, **70**, 820–827.
- Danyluk, M.D., Brandl, M.T., and Harris, L.J. 2008a. Migration of *Salmonella* Enteritidis phage type 30 through almond hulls and shells. *Journal of Food Protection*, **71**, 397–401.
- Danyluk, M.D., Nozawa-Inoue, M., Hristova, K.R., Scow, K.M., Lampinen, B., and Harris, L.J. 2008b. Survival and growth of *Salmonella* Enteritidis PT 30 in almond orchard soils. *Journal of Applied Microbiology*, **104**, 1391–1399.
- Deng, S., Ruan, R., Mok, C.K., Huang, G., Lin, X., and Chen, P. 2007. Inactivation of *Escherichia coli* on almonds using nonthermal plasma. *Journal of Food Science*, **72**, M62–M66.
- Doster, M.A. and Michailides, T.J. 1994a. *Aspergillus* molds and aflatoxins in pistachio nuts in California. *Phytopathology*, **84**, 583–590.
- Doster, M.A. and Michailides, T.J. 1994b. The development of early split pistachio nuts and their contamination by molds, aflatoxins, and insects. *International Symposium on Pistachio*, **419**, 359–364.
- Doster, M.A. and Michailides, T.J. 1999. Relationship between shell discoloration of pistachio nuts and incidence of fungal decay and insect infestation. *Plant Disease*, **83**, 259–264.
- Doster, M.A., Michailides, T.J., and Morgan, D.P. 1996. *Aspergillus* species and mycotoxins in figs from California orchards. *Plant Disease*, **80**, 484–489.
- Du, W.X., Abd, S.J., McCarthy, K.L., and Harris, L.J. (2010) Reduction of *Salmonella* on inoculated almonds exposed to hot oil. *Journal of Food Protection*, **73**, 1238–1246.
- Eaton, D.L. and Gallagher, E.P. 1994. Mechanisms of aflatoxin carcinogenesis. *Annual Review of Pharmacology and Toxicology*, **34**, 135–172.
- EFSA. 2011. Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the Shiga toxin-producing *E. coli* STEC O104:H4 outbreaks in Germany and France. EFSA, Parma,

- Italy. Available at: <http://www.efsa.europa.eu/en/supporting/doc/176e.pdf>.
- Eglezos, S. 2010. The bacteriological quality of retail-level peanut, almond, cashew, hazelnut, Brazil, and mixed nut kernels produced in two Australian nut-processing facilities over a period of 3 years. *Foodborne Pathogens and Disease*, **7**, 863–866.
- Eglezos, S., Huang, B., and Stuttard, E. 2008. A survey of the bacteriological quality of pre-roasted peanut, almond, cashew, hazelnut, and Brazil nut kernels received into three Australian nut-processing facilities over a period of 3 years. *Journal of Food Protection*, **71**, 402–404.
- EU. 2006. Commission Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs.
- FDA. 2009a. Peanut products recall [Online]. Available at: <http://www.fda.gov/Safety/Recalls/MajorProductRecalls/Peanut/default.htm> (accessed 18 June 2009).
- FDA. 2009b. Pistachio product recall [Online]. Available at: <http://www.fda.gov/Safety/Recalls/MajorProductRecalls/Pistachio/default.htm> (accessed 18 June 2009).
- Fernane, F., Sanchis, V., Marín, S., and Ramos, A. 2010. First report on mould and mycotoxin contamination of pistachios sampled in Algeria. *Mycopathologia*, **170**, 423–429.
- Freitas-Silva, O. and Venâncio, A. (2011) Brazil nuts: benefits and risks associated with contamination by fungi and mycotoxins. *Food Research International*, **44**, 1434–1440.
- Gabriel, A.A., Berja, M.C., Estrada, A.M.P., Lopez, M.G.A.A., Nery, J.G.B., and Villafior, E.J.B. 2007. Microbiology of retail mung bean sprouts vended in public markets of National Capital Region, Philippines. *Food Control*, **18**, 1307–1313.
- Gao, M., Tang, J., Villa-Rojas, R., Wang, Y., and Wang, S. 2011. Pasteurization process development for controlling *Salmonella* in in-shell almonds using radio frequency energy. *Journal of Food Engineering*, **104**, 299–306.
- Harris, L.J., Uesugi, A.R., Abd, S.J., and McCarthy, K.L. 2012. Survival of *Salmonella* Enteritidis PT 30 on inoculated almond kernels in hot water treatments. *Food Research International*, **45**, 1093–1098.
- Heperkan, D., Aran, N., and Ayfer, M. 1994. Mycoflora and aflatoxin contamination in shelled pistachio nuts. *Journal of the Science of Food and Agriculture*, **66**, 273–278.
- Hepsag, F., Golge, O., and Kabak, B. 2014. Quantitation of aflatoxins in pistachios and groundnuts using HPLC-FLD method. *Food Control*, **38**, 75–81.
- Imperato, R., Campone, L., Piccinelli, A. L., Veneziano, A., and Rastrelli, L. 2011. Survey of aflatoxins and ochratoxin a contamination in food products imported in Italy. *Food Control*, **22**, 1905–1910.
- Irvin, K. 2012. Identifying turkish pine nuts linked to an outbreak of *Salmonella* Enteritidis: using product traceback in conjunction with laboratory data. In: *International Association for Food Protection Annual Meeting*, pp. 1–128.
- Isaacs, S., Aramini, J., Ciebin, B., Farrar, J., et al. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *Journal of Food Protection*, **68**, 191–198.
- Jeong, S., Marks, B.P., Ryser, E.T., and Harte, J.B. 2012. The effect of X-ray irradiation on *Salmonella* inactivation and sensory quality of almonds and walnuts as a function of water activity. *International Journal of Food Microbiology*, **153**, 365–371.
- Jimenez, M., Mateo, R., Querol, A., Huerta, T., and Hernandez, E. 1991. Mycotoxins and mycotoxigenic moulds in nuts and sunflower seeds for human consumption. *Mycopathologia*, **115**, 121–127.
- Juan, C., Zinedine, A., Moltó, J.C., Idrissi, L., and Mañes, J. 2008. Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. *Food Control*, **19**, 849–853.
- Kim, H.J., Lee, D.S., and Paik, H.D. 2004. Characterization of *Bacillus cereus* isolates from raw soybean sprouts. *Journal of Food Protection*, **67**, 1031–1035.
- Kim, H.K., Lee, Y.J., Beuchat, L.R., Yoon, B.J., and Ryu, J.H. 2009. Microbiological examination of vegetable seed sprouts in Korea. *Journal of Food Protection*, **72**, 856–859.

- Kim, S.A., Kim, O.M., and Rhee, M.S. 2013. Changes in microbial contamination levels and prevalence of foodborne pathogens in alfalfa (*Medicago sativa*) and rapeseed (*Brassica napus*) during sprout production in manufacturing plants. *Letters in Applied Microbiology*, **56**, 30–36.
- Kimber, M.A., Kaur, H., Wang, L., Danyluk, M.D., and Harris, L.J. 2012. Survival of *Salmonella*, *Escherichia coli* O157: H7, and *Listeria monocytogenes* on inoculated almonds and pistachios stored at –19, 4, and 24°C. *Journal of Food Protection*, **75**, 1394–1403.
- King, J.C., Blumberg, J., Ingwersen, L., Jenab, M., and Tucker, K.L. 2008. Tree nuts and peanuts as components of a healthy diet. *The Journal of Nutrition*, **138**, 1736S–1740S.
- Kirk, M.D., Little, C.L., Lem, M., Fyfe, M., *et al.* 2004. An outbreak due to peanuts in their shell caused by *Salmonella enterica* serotypes Stanley and Newport – sharing molecular information to solve international outbreaks. *Epidemiology and Infection*, **132**, 571–577.
- Komitopoulou, E. and Peñaloza, W. 2009. Fate of *Salmonella* in dry confectionery raw materials. *Journal of Applied Microbiology*, **106**, 1892–1900.
- Kothary, M.H. and Babu, U.S. 2001. Infective dose of foodborne pathogens in volunteers: a review. *Journal of Food Safety*, **21**, 49–68.
- Kotzekidou, P. 1998. Microbial stability and fate of *Salmonella* Enteritidis in halva, a low-moisture confection. *Journal of Food Protection*, **61**, 181–185.
- Kwak, T.Y., Kim, N.H., and Rhee, M.S. 2011. Response surface methodology-based optimization of decontamination conditions for *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh-cut celery using thermoultrasound and calcium propionate. *International Journal of Food Microbiology*, **150**, 128–135.
- Ledet, M.L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Plym, F.L., and Andersson, Y. 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005–2006 – suspected source: almonds. *Euro Surveillance: European Communicable Disease Bulletin*, **12**, E9–E10.
- Lee, S.Y., Oh, S.W., Chung, H.J., Reyes-De-Corcuera, J.I., Powers, J.R., and Kang, D.H. 2006. Reduction of *Salmonella enterica* serovar Enteritidis on the surface of raw shelled almonds by exposure to steam. *Journal of Food Protection*, **69**, 591–595.
- Little, C.L., Jemmott, W., Surman-Lee, S., Hucklesby, L., and De Pinna, E. 2009. Assessment of the microbiological safety of edible roasted nut kernels on retail sale in England, with a focus on *Salmonella*. *Journal of Food Protection*, **72**, 853–855.
- Little, C.L., Rawal, N., de Pinna, E., and McLauchlin, J. 2010. Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. *Food Microbiology*, **27**, 171–174.
- Loui, C., Grigoryan, G., Huang, H., Riley, L.W., and Lu, S. 2008. Bacterial communities associated with retail alfalfa sprouts. *Journal of Food Protection*, **71**, 200–204.
- Luttfullah, G. and Hussain, A. 2011. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. *Food Control*, **22**, 426–429.
- McGarvey, J.A., Connell, J.H., Stanker, L.H., and Hnasko, R. 2014. Bacterial population structure and dynamics during the development of almond drupes. *Journal of Applied Microbiology*, **116**, 1543–1552.
- Mehrnezhad, M.R. and Panahi, B. 2006. The influence of hull cracking on aflatoxin contamination and insect infestation in pistachio nuts. *Applied Entomology and Phytopathology*, **73**, 105–123.
- Miksch, R.R., Leek, J., Myoda, S., Nguyen, T., Tenney, K., Svidenko, V., Greeson, K., and Samadpour, M. 2013. Prevalence and counts of *Salmonella* and enterohemorrhagic *Escherichia coli* in raw, shelled runner peanuts. *Journal of Food Protection*, **76**, 1668–1675.
- Miller, B.D., Rigdon, C.E., Ball, J., Rounds, J.M., *et al.* 2012. Use of traceback methods to confirm the source of a multistate *Escherichia coli* O157: H7 outbreak due to in-shell hazelnuts. *Journal of Food Protection*, **75**, 320–327.
- Molyneux, R.J., Mahoney, N., Kim, J.H., and Campbell, B.C. 2007. Mycotoxins in edible tree

- nuts. *International Journal of Food Microbiology*, **119**, 72–78.
- NCMCF (National Advisory Committee on Microbiological Criteria for Food). 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *International Journal of Food Microbiology*, **52**, 123–153.
- Niemira, B.A. 2012. Cold plasma reduction of *Salmonella* and *Escherichia coli* O157: H7 on almonds using ambient pressure gases. *Journal of Food Science*, **77**, M171–M175.
- NIID. 1997. Verocytotoxin-producing *Escherichia coli* (enterohemorrhagic *E. coli*) infections, Japan, 1996–June 1997. *Infectious Agents Surveillance Report*, **18**, 153–154.
- Pan, Z., Bingol, G., Brandl, M.T., and McHugh, T.H. 2012. Review of current technologies for reduction of *Salmonella* populations on almonds. *Food and Bioprocess Technology*, **5**, 2046–2057.
- Pao, S., Khalid, M., and Kalantari, A. 2005. Microbial profiles of on-line-procured sprouting seeds and potential hazards associated with enterotoxigenic *Bacillus* spp. in homegrown sprouts. *Journal of Food Protection*, **68**, 1648–1653.
- Pao, S., Kalantari, A., and Huang, G. 2006. Utilizing acidic sprays for eliminating *Salmonella enterica* on raw almonds. *Journal of Food Science*, **71**, M14–M19.
- Parker, C.T., Huynh, S., Quiñones, B., Harris, L.J., and Mandrell, R.E. 2010. Comparison of genotypes of *Salmonella enterica* serovar Enteritidis phage type 30 and 9c strains isolated during three outbreaks associated with raw almonds. *Applied and Environmental Microbiology*, **76**, 3723–3731.
- Perry, E. and Sibbett, G.S. 1998. *Harvesting and Storing Your Home Orchard's Nut Crop: Almonds, Walnuts, Pecans, Pistachios, and Chestnuts*. University of California Division of Agriculture and Natural Resources Publications, Oakland, CA, USA.
- Podolak, R., Enache, E., Stone, W., Black, D.G., and Elliott, P.H. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection*, **73**, 1919–1936.
- Prakash, A., Lim, E., Duong, C., Caporaso, F., and Foley, D. 2010. The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties. *Radiation Physics and Chemistry*, **79**, 502–506.
- Rodrigues, P., Venâncio, A., and Lima, N. 2012. Mycobiota and mycotoxins of almonds and chestnuts with special reference to aflatoxins. *Food Research International*, **48**, 76–90.
- Ros, E. 2010 Health benefits of nut consumption. *Nutrients*, **2**, 652–682.
- Rosengarten Jr, F. 2004. *The Book of Edible Nuts*. Dover Publications, New York, USA.
- Sagoo, S.K., Little, C.L., Ward, L., Gillespie, I.A., and Mitchell, R.T. 2003. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *Journal of Food Protection*, **66**, 403–409.
- Saroj, S.D., Shashidhar, R., Dhokane, V., Hajare, S., Sharma, A., and Bandekar, J.R. 2006. Microbiological evaluation of sprouts marketed in Mumbai, India, and its suburbs. *Journal of Food Protection*, **69**, 2515–2518.
- Schatzki, T.F. 1996. Distribution of aflatoxin in almonds. *Journal of Agricultural and Food Chemistry*, **44**, 3595–3597.
- Seow, J., Ágoston, R., Phua, L., and Yuk, H.-G. 2012. Microbiological quality of fresh vegetables and fruits sold in Singapore. *Food Control*, **25**, 39–44.
- Sheibani Tezerji, Z. 2011. The role of bugs in contamination of pistachio nuts to aflatoxin. In: *I International Symposium on Mycotoxins in Nuts and Dried Fruits*, Vol. 963, pp. 269–274.
- Taormina, P.J., Beuchat, L.R. and Slutsker, L. 1999. Infections associated with eating seed sprouts: an international concern. *Emerging Infectious Diseases*, **5**, 626.
- Torlak, E., Sert, D., and Serin, P. 2013. Fate of *Salmonella* during sesame seeds roasting and storage of tahini. *International Journal of Food Microbiology*, **163**, 214–217.
- Tournas, V.H. 2005. Moulds and yeasts in fresh and minimally processed vegetables, and sprouts. *International Journal of Food Microbiology*, **99**, 71–77.

- Uesugi, A.R. and Harris, L.-J. 2006. Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. *Journal of Food Protection*, **69**, 712–718.
- Unicomb, L., Simmons, G., Merritt, T., Gregory, J., *et al.* 2005. Sesame seed products contaminated with *Salmonella*: three outbreaks associated with tahini. *Epidemiology and Infection*, **133**, 1065–1072.
- USDA (United States Department of Agriculture). 2007. Almond grown in California; outgoing quality control requirements. 7 CFR Part 981 (Docket No. FV06-981-1 FR). Agricultural Marketing Service.
- Viswanathan, P. and Kaur, R. 2001. Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. *International Journal of Hygiene and Environmental Health*, **203**, 205–213.
- Vural, A. and Erkan, M.E. 2008. The research of microbiological quality in some edible nut kinds. *Journal of Food Technology*, **6**, 25–28.
- Waje, C.K., Jun, S.Y., Lee, Y.K., Kim, B.N., Han, D.H., Jo, C., and Kwon, J.H. 2009. Microbial quality assessment and pathogen inactivation by electron beam and gamma irradiation of commercial seed sprouts. *Food Control*, **20**, 200–204.
- Warriner, K. and Smal, B. 2014. Microbiological safety of sprouted seeds: interventions and regulations, Chapter 11. In: *The Produce Contamination Problem*, (eds Matthews, K.R., Sapers, G.M., and Gerba, C.P.), 2nd edn. Academic Press, San Diego, CA, USA.
- Weller, L.D., Daeschel, M.A., Durham, C.A., and Morrissey, M.T. 2013. Effects of water, sodium hypochlorite, peroxyacetic acid, and acidified sodium chlorite on in-shell hazelnuts inoculated with *Salmonella enterica* serovar Panama. *Journal of Food Science*, **78**, M1885–M1891.
- WHO. 2011. Outbreaks of *Escherichia coli* O104:H4 infection: update 30 (22-07-11). Available at: <http://www.euro.who.int/en/health-topics/emergencies/international-health-regulations/news/news/2011/07/outbreaks-of-e.-coli-o104h4-infection-update-30>.
- Willis, C., Little, C.L., Sagoo, S., de Pinna, E., and Threlfall, J. 2009. Assessment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with a focus on *Salmonella* spp. *Food Microbiology*, **26**, 847–852.
- Winfield, M.D. and Groisman, E.A. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology*, **69**, 3687–3694.
- Yang, Y., Meier, F., Ann Lo, J., Yuan, W., Lee Pei Sze, V., Chung, H.J., and Yuk, H.G. 2013. Overview of recent events in the microbiological safety of sprouts and new intervention technologies. *Comprehensive Reviews in Food Science and Food Safety*, **12**, 265–280.

CHAPTER 21

Microbial ecology of eggs: a focus on *Salmonella* and microbial contamination in post-harvest table shell egg production

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21.1 Introduction

Food-borne disease linked to *Salmonella* spp. continues to be problematic in the United States public health sector with extensive economic consequences and continued need for regulatory action (Scallan *et al.*, 2011; Ricke *et al.*, 2013d). Although food-borne pathogen *Salmonella* spp. can originate from a variety of food sources, poultry meat and table eggs remain prominent sources (Suslow *et al.*, 2003; Foley *et al.*, 2008, 2011, 2013; Hanning *et al.*, 2009; Erickson, 2010; Foley *et al.*, 2011, 2013; Carrasco *et al.*, 2012; Finstad *et al.*, 2012; Howard *et al.*, 2012; Koo *et al.*, 2012; Painter *et al.*, 2013). Several *Salmonella* serovars have been identified in outbreaks of human disease with *Salmonella enterica* serovar Typhimurium along with *S. Enteritidis*, *S. Newport*, and *S. Heidelberg* being the ones most likely to occur as the causative isolate (Finstad *et al.*, 2012; Foley *et al.*, 2013).

For table shell eggs and the associated food products formulated with egg components, *S. Enteritidis* has been the serovar that is usually identified as the primary food-borne disease causing serovar (Patrick *et al.*, 2004; Braden *et al.*, 2006; Curtis, 2007; Finstad *et al.*, 2012; Howard *et al.*, 2012; Martelli and Davies, 2012). Historically, *S. Enteritidis* emerged as primarily an egg-related serovar that remained persistent

in egg production cycles and could be identified with major salmonellosis outbreaks associated with *Salmonella* (St Louis *et al.*, 1988; Cogan and Humphrey, 2003; Martelli and Davies, 2012; Ricke *et al.*, 2013a). This may in part be due to the ability of *S. Enteritidis* to gravitate toward the physiological processes that are exclusive to the generation of eggs by laying hens (Guard-Petter, 2001; Ricke, 2003a; Curtis, 2007; Gantois *et al.*, 2009; Howard *et al.*, 2012; Martelli and Davies, 2012; Galiş *et al.*, 2013).

Consequently, considerable effort has been made to document and describe the role that pre-harvest egg production plays in the generation of contaminated eggs and how this impacts commercial egg production. Post-harvest control measures for reducing *S. Enteritidis* contaminated eggs have also been documented to some extent. However, as alternative egg production systems and local egg marketing expands commercially in the US some of these issues will need to be examined with restrictions unique to those systems in mind. Likewise, as more emphasis is placed on sustainability and recycling this will potentially influence food safety of eggs. Finally, as international commercial egg production expands and opens new local markets egg safety may need to also be an issue under these particular production systems and retail circumstances. Therefore,

the current review will focus on particular aspects of egg processing, carton packaging, and handling in retail both in current production practices as well as historical developments and future considerations as retail production practices and associated markets become more variable.

21.2 Historical and current trends in commercial egg production

Since 2011 the average table layer hen numbers in the United States has continued to expand from 281.6 million layers in 2011 to 296.4 million layers as of December 1, 2013 based on United States Department of Agriculture (USDA) estimates (Ibarburu and Bell, 2013). These recent flock numbers are reflective of the tremendous changes in the US egg industry that have occurred over the past century, where increases in the size of flocks have followed technological changes in layer building efficiency with single buildings housing thousands of birds and farm complexes capable of managing hundreds of thousands of birds (Parkhurst and Mountney, 1988; Klippen, 1990; Dunn and Madison, 1992; Bell, 1993; Anderson, 2009). Implementation of technologies such as mechanical egg gathering systems for in-line collection of eggs contributed to this expansion of numbers of birds per flock and house (Bell *et al.*, 2001). Likewise, innovations in egg processing plants for candling, egg washing, and packaging made further expansion possible (Klippen, 1990; Kuney *et al.*, 1995; Hutchison *et al.*, 2003; Musgrove, 2011). Consequently, the layer farms and number of flocks decreased while the number of birds per farm and per flock increased (Bell *et al.*, 2001). This higher egg supply capacity from fewer flocks and farms and development of much more efficient packing plants along with the market impact of uneven regional changes in US human population growth predictably led to industry-wide interregional relocation shifts (Dunn and

Madison, 1992; Bell, 1993; Ryan *et al.*, 1996). Regional differences in the US were detectable not only from a pricing and product selection standpoint but the type of packaging, variety of products, and egg quality (Bell *et al.*, 2001; Koelkebeck *et al.*, 2001).

Similar trends have occurred internationally as countries become more developed and their corresponding agricultural industries begin to expand and adjust to advanced technologies. This led to shifts in international markets, which were extensively discussed elsewhere by Windhorst (2006, 2008a, 2008b, 2009) as a series of market analyses reviews and will not be discussed in detail in the current review. The following is a brief overview of these key conclusions previously made by Windhorst. For roughly three and half decades, global poultry meat and egg production outpaced beef, veal, and swine (Windhorst, 2006). During this time shifts occurred as egg production volume in developing countries surpassed production in developed countries and by 2005 over 60% originated from the Asian continent and nearly half came from three countries, China, India, and Japan, with China producing over 40% and exhibiting tremendous growth during this time (Windhorst, 2006, 2008a, 2009). Just from 1990 to 2007 alone, nearly a doubling in global egg production occurred (Windhorst, 2009). However, the vast majority of these eggs have remained in domestic markets with relatively few of the total eggs produced being exported and imported (Windhorst, 2006). Part of this may be due to the existence in some of these developing countries of small family flocks that are raised for subsistence consumption and local sales (McLeod *et al.*, 2009). The global egg trade that does occur is highly regional with over two-thirds of the exports and imports occurring in Europe with other regional trade zones centered in the American and Asian continents (Windhorst, 2008a, 2008b, 2009).

Since the early to mid 2000s several factors have come into play to once again shift the US and international egg industry not only in terms of how eggs were marketed but how they were

produced on commercial farms. Specialty egg brands that included eggs originating from organic and welfare-managed production systems, nutritionally modified eggs, fertile eggs, and eggs produced from hens fed all vegetable diets emerged in retail markets as viable, albeit more expensive options for customers (Patterson *et al.*, 2001; Jones *et al.*, 2010). Early interest in generating eggs that were considered more compatible with cholesterol reduction diets in humans led to hens fed vegetarian diets and commercially produced eggs containing higher levels of omega 3 fatty acids (Michella and Slauch, 2000). As public health demands grew further, developments in fortifying egg nutritional value by increasing polyunsaturated fatty acid content, vitamins A, D, and E by specific dietary strategies, and feeding regimes of laying hens became an ongoing focal point (Rossi *et al.*, 2013). Since the egg composition is feed dependent, this trend of dietary manipulation to further alter eggs to fit human nutritional and health needs is anticipated to continue as more understanding is achieved on the physiology and metabolism of egg formation (Shapira, 2010).

Animal welfare and subsequent consumer concerns have also impacted both the US as well as the international egg industry. These concerns led the European Union to ban conventional cage housing in 1999 with the idea of replacing them with alternative housing systems by 2012 (De Reu *et al.*, 2008; Mench *et al.*, 2011). These alternative housing systems consisted of either furnished cages that included a nest box, a dust bath, and a perch, or fully non-cage based systems (De Reu *et al.*, 2008). Non-cage systems contain nest boxes and floor litter areas and are referred to either as aviaries, which consist of some sort of platforms and slatted floors at different heights, or, if at one height, are simply called floor housing and when allowing access to outdoors can be considered free range (De Reu *et al.*, 2008). Holt *et al.* (2011) concluded that as these regulations are implemented and alternative housing operations expand in commercial egg production systems, the impact on egg microbiology and pathogen

prevalence will need to be assessed. Historically, these types of systems were in place in the mid-1900s and some of this information could be potentially gleaned from studies conducted at that time. However, Anderson (2009) points out that this would be problematic as much of the management data available from these earlier flock systems would not be applicable because the breeds and other factors were different from those in current operations.

Most of the more recent microbiology studies have been conducted in Europe. Jones *et al.* (2011) and Holt *et al.* (2011) noted that these results were conflicting, with some concluding that conventional cage operations yielded a greater opportunity for *S. Enteritidis* incidence while others suggesting that non-conventional gave higher incidences of *S. Enteritidis*. Numerous confounding factors probably play a role in these different outcomes, including the impact of flock size, type and frequency of sampling, seasonality, layer breed, management differences, and stress response, just to name a few (Curtis, 2007; De Reu *et al.*, 2008, 2009; Anderson *et al.*, 2009; Dewulf *et al.*, 2011; Holt *et al.*, 2011; Jones *et al.*, 2011). Clearly more work needs to be done not only to characterize microbial contamination levels in these operations on a wider scale but also to develop analyses that can identify and account for the multiple factors that may be different among egg layer operations. Once this is accomplished, management practices and intervention strategies can be designed to be precisely applied to particular types of operations for more effective control of *Salmonella* incidence. In addition, more effort may need to be directed toward detailed epidemiology of *Salmonella* isolates in these systems to avoid potential differences in serovars that could also confound control strategies.

The other major factor that is emerging in egg production and processing systems that potentially will impact egg microbiology is assessment and implementation of improved sustainability. Sustainability of agriculture in general has become an issue as energy costs

both for production of cereal grains used as feed stocks and maintaining livestock housing have gone up while public concerns over environmental impact of confined animal operations have increased as well. For global egg production, Windhorst (2007) raised this question as a function of whether grain-fed layer flocks would be economically compromised as the arable land for corn and oil seed crops become more diverted into bioethanol and biodiesel production in different countries including the US. Increased interest in egg production sustainability also derives from other venues. Certainly, as discussed previously, the development and spread of alternative layer housing raises the issue of balancing animal welfare versus economics as a function of sustainable costs. Likewise, the emerging consumer-driven market for organically produced poultry and animal products with the associated restrictions in grain production and management options has raised the question as to the sustainability of such operations for future expansion into more traditional agriculture markets (Ricke *et al.*, 2012b). Organically produced eggs are also on the rise (Van der Sluis, 2007; Anderson, 2009) so it is anticipated that similar questions will be raised for these types of egg production systems as well.

Sustainability of confined animal operations and impact on the environment are closely linked. For egg production systems Xin *et al.* (2011) reviewed and compared environmental impacts between conventional caged housing systems and non-conventional systems and concluded that non-cage houses generally have poorer air quality due to ammonia and dust and are generally less energy, feed, and land efficient. They suggested that research was needed to compare the overall carbon footprint among the different housing systems to design means to reduce the environmental impact using life cycle assessment tools. Life cycle assessments (LCAs) have been utilized to quantitate sustainability in a number of agriculture systems. Essentially LCA analyses involve constructing a balance sheet for all environmental inputs and outputs involved in

the production, transportation, distribution, disposal, and any other steps associated with the entire life cycle of a product (Guinée *et al.*, 2011). Recently, several LCA studies have been made on egg production systems. Pelletier *et al.* (2014) used LCA to demonstrate that the overall environmental footprint for the US egg industry was reduced in 2010 compared to 1960 and that the three primary environmental impacts during this time span were feed efficiency, feed composition, and manure management. Leinonen *et al.* (2012), using LCA to quantify environmental impact per kg eggs produced from cage, barn, free range, and organic United Kingdom (UK) production systems, concluded that organic eggs required the most hens and cage systems the least number of hens, with organic hens consuming the most feed. They also noted that feed production, processing, and transportation elicited the largest impacts on the environmental footprint. Suggestions for further reducing the greenhouse gas environmental footprint in free range chickens have included substituting alternative protein sources for the cereal grains currently used in these diets (Taylor *et al.*, 2014).

In summary, several drivers are in play that could potentially be changing the dynamics of commercial egg operations on an international scale. As more efforts are made to change housing systems to address animal welfare concerns, and local free range and organic production system markets continue to increase, much of what is known about the prevalence of microorganisms in general and in particular *Salmonella* spp. may no longer apply in these modified systems. Likewise, further attempts to reduce the environmental footprint by altering feed composition and the handling of manure and other waste material could also alter the transmission patterns of the organisms. This may in turn have large impacts on egg food safety as international markets and regional production systems continue to shift. Consequently, it will be critical to examine what is already known about the ecology of *Salmonella* in egg production and processing and use this as the starting

point to revisit and in some cases identify potential critical control points that may be unique to these systems. The following sections will discuss *Salmonella* ecology in egg production and processing and examine current intervention strategies.

21.3 Egg production management on the farm and incidence of *Salmonella*

There are numerous opportunities for laying hens or for that matter chickens in commercial production systems in general to come in contact with *Salmonella* serovars. Traditionally, feed and feed ingredients were thought to be an important contributor to being a *Salmonella* spp. contact source for chickens during growth and under certain circumstances, such as breeder flocks, this could have a considerable impact due to the vertical integration nature of poultry production (Davies and Hinton, 2000; Jones and Richardson, 2004; Maciorowski *et al.*, 2004, 2006b, 2007; Ricke, 2005; Jarquin *et al.*, 2009; Davies and Wales, 2010; Ge *et al.*, 2013). Therefore, it is not surprising that considerable effort has been made to not only implement control measures in feeds and feed ingredients (Leeson and Marcotte, 1993; Ricke, 2003b, 2005; Maciorowski *et al.*, 2004, 2007; Wales *et al.*, 2010; Jones, 2011) but also to develop sensitive detection methods for low levels of *Salmonella* potentially present in the feed matrix (Ricke *et al.*, 1998; Maciorowski *et al.*, 2000, 2005, 2006a; Jarquin *et al.*, 2009; Park *et al.*, 2011, 2014; Soria *et al.*, 2011; Ricke *et al.*, 2013c). Couple this with the fact that exposure to *S. Enteritidis* can originate as a function of laying hens coming in contact with a wide range of carriers well known for carrying *S. Enteritidis*, such as insects, mice, rats, and other lesser known carriers in the houses where these hens reside, and it becomes clear that there are numerous and somewhat unpredictable routes of exposure (Henzler and Opitz, 1992; Hinkle and Hickle, 1999; Olsen and Hammack, 2000;

Davies and Breslin, 2001; De Reu *et al.*, 2008; Lapuz *et al.*, 2008; Park *et al.*, 2008; Holt *et al.*, 2011; Galiş *et al.*, 2013; Ricke *et al.*, 2013a).

Before discussion on egg processing can begin, it is important to understand the relationship between the serovar *S. Enteritidis* and why it particularly occurs as a contaminant during all aspects of egg production. Historically, it became clear fairly early on that *S. Enteritidis* possessed unique aspects that led to its being particularly problematic during egg formation in laying hens (Guard-Petter, 2001; Holt, 1999, 2003; Ricke 2003a). One of the more revealing pieces of evidence for this was the discovery that certain laying hen management practices during egg production could enhance the likelihood of infection by *S. Enteritidis*. To achieve additional egg laying cycles for commercial and economical reasons, laying hen egg production can be halted, commonly referred to as molting, to literally shut down the reproductive tract and in turn arrest egg production for a period of time prior to initiating a second egg laying cycle (North and Bell, 1990; Bell, 2003; Berry, 2003; Park *et al.*, 2004c; Ricke *et al.*, 2010, 2013a). The induction of a molt period can be done via altering the diet and is physiologically manifested in a laying hen in a variety of ways including hormonally, metabolically, and immunologically among other detectable host responses (Berry, 2003; Kuenzel, 2003; Park *et al.*, 2004c; Dunkley *et al.*, 2007a, 2007b; Kim *et al.*, 2012; Ricke *et al.*, 2010, 2013a).

Probably the most effective means for inducing a molt in actively egg producing laying hens was to simply remove their feed for a period of time and then after sufficient time had lapsed reintroduce these hens to feed once again (Bell, 2003; Berry, 2003; Yousaf and Chaudhry, 2008). However, this led to the discovery that feed removal and subsequent emptying of the hen's gastrointestinal tract created a localized microenvironment that was conducive to *S. Enteritidis* colonization and systemic invasion (Durant *et al.*, 1999; Holt, 1999, 2003; Ricke, 2003a; Dunkley *et al.*, 2009). This microenvironment change could be linked to enhanced expression of pathogenesis genes in *S. Enteritidis*

that corresponded to increased invasion and diminished gastrointestinal microbiota activity and in some cases microbial population levels as well (Durant *et al.*, 1999; Ricke, 2003a; Dunkley *et al.*, 2007c, 2007d, 2009). While this was being established a flurry of alternative dietary options was proposed and examined that avoided feed removal and instead represented some sort of nutritional modification that would simultaneously ensure a molt response in the hen while retaining relatively normal gastrointestinal tract microenvironment and simultaneously serving as a barrier to *S. Enteritidis* (Corrier *et al.*, 1997; Seo *et al.*, 2001; Berry, 2003; Holt, 2003; Ricke, 2003a; Moore *et al.*, 2004; Park *et al.*, 2004a, 2004b, 2004c; Woodward *et al.*, 2005; McReynolds *et al.*, 2006; Dunkley *et al.*, 2007c, 2007d; Donalson *et al.*, 2008; Ricke *et al.*, 2010, 2013a).

Other means to limit *S. Enteritidis* establishment in laying hens have also been explored. Administration of probiotic cultures that would lead to early establishment of a beneficial gastrointestinal microbiota that would in turn limit *S. Enteritidis* colonization have been explored, usually in young broiler chicks for *S. Typhimurium* control, but some have been shown to be effective against *S. Enteritidis* as well (Corrier *et al.*, 1994a, 1994b, 1995; Nisbet *et al.*, 1993, 1994, 1996a, 1996b; Ricke and Pillai, 1999; Nisbet, 2002; La Ragione and Woodward, 2003; Patterson and Burkholder, 2003; Revollo *et al.*, 2006; Higgins *et al.*, 2007, 2008; Vilà *et al.*, 2009; Hume, 2011; Siragusa and Ricke, 2012). Bacteriophages that can specifically lyse *S. Enteritidis* bacteriophage have also been examined as a means to remove already established populations of this pathogen either in table or fertile eggs or in the gastrointestinal tract (Borie *et al.*, 2008; Robeson *et al.*, 2011; Bardina *et al.*, 2012; Ricke *et al.*, 2012a; Henriques *et al.*, 2013; Spricigo *et al.*, 2013). Finally, there has been more interest generated on the development of live attenuated vaccines that are based on genetic modifications of *S. Enteritidis* to render it less virulent but still capable of inducing a hen to elicit a protective immune response against

future exposure to *S. Enteritidis* (Babu *et al.*, 2003; Dewaele *et al.*, 2012; Revollo and Ferreira, 2012).

In short, several extensive efforts have been made to short circuit the establishment of *S. Enteritidis* in laying hen production systems and many of these approaches have demonstrated some success in decreasing the incidence of *S. Enteritidis* prior to egg collection and processing.

However, numerous opportunities for contamination by *S. Enteritidis* and other *Salmonella* serovars can still occur during egg collection, processing, packaging, and in the final steps leading to retail and home use. The remainder of this review will focus on the potential for *Salmonella* contamination in the post-harvest phases of egg production and processing.

21.4 Egg processing and microbial contamination: general aspects

Table shell eggs continue to be a primary food source for human consumption either directly as an intact shell cooked product, contents removed from egg shell components, prepared in some fashion and cooked, or as an ingredient in a wide range of prepared foods and baked goods. In 2009 alone, Americans consumed 250 eggs per person with total table eggs being produced standing at 78.5 billion, with 24 billion broken for use in egg products (Ricke *et al.*, 2013b). As discussed previously, the US egg industry has evolved tremendously in the past century. As egg processing equipment became more technologically developed the number of employees required to handle and inspect eggs decreased dramatically and the number of eggs that could be processed and shipped for retail increased with the development of equipment capable of washing, candling, sizing, and packaging over 180 000 eggs per hour (Musgrove, 2011). Eggs produced at the farm can enter the egg processing system either in an “in-line” production system, where eggs are directly moved

via conveyor belts from the layer farm where they are produced directly to an egg processing facility, or as an “off-line” production system, where eggs are collected at the farm and subsequently transported to another site for processing (Musgrove, 2011).

The multiple steps involved in egg processing represent numerous opportunities for microbiological contamination of the egg as it progresses through the system from the hen to the retail market. Identification of critical control points and conducting risk assessment studies have been attempted over the years to assign and prioritize where the optimal strategies for limiting exposure to *Salmonella* can be achieved during egg processing. Intuitively, some aspects such as the presence of cracks in egg shells represent a risk for bacterial penetration and contamination (Todd, 1996). Consequently, efforts to minimize crack formation and egg shell damage during processing, packaging, and transportation would be considered desirable. Alteration of the egg shell surface integrity can easily occur to make it more vulnerable. For example, certain egg sanitizers can actually cause physical damage to the egg shell surface, leading to increased penetration of the egg shell by surface microbial contaminants (Kim and Slavik, 1996; Wang and Slavik, 1998).

Egg temperature has also been identified as a critical factor. Hutchinson *et al.* (2004) concluded that egg wash and rinse water temperatures below 34°C led to detectable egg content contamination by *Salmonella*. However, this does not minimize risk from eggs that have become internally contaminated by transovarian infection of *S. Enteritidis* while the egg is being formed in the reproductive tract of the hen (Humphrey, 1994, 1999; Guard-Petter, 2001; Gantois *et al.*, 2009). Clearly, with these contaminated eggs, strategies that minimize growth of *S. Enteritidis* to keep populations low and developing methods to destroy this pathogen in the intact egg would need to be considered. Combining rapid cooling and whole shell egg pasteurization have been identified as effective means of reducing such risks (Whiting and

Buchanan, 1997; Schroeder *et al.*, 2006). When summarizing more recent risk assessment studies, Chemaly and Salvat (2011) reported that rapid cooling would be helpful to reduce the risk but that maintaining a continuous cold chain to limit condensation was critical and under some circumstances rapid chilling could lead to the formation of cracks in the egg shell. However, studies to evaluate all of the factors that influence internal temperatures in eggs are limited and information on all of these factors is somewhat difficult to obtain.

A comprehensive study across several US states was conducted to follow and compare egg surface and internal temperature from oviposition to distribution to delineate some of these factors for addressing risk questions raised by US regulatory agencies regarding egg temperature and time relationships over the course of egg production, processing, and retail distribution (Anderson *et al.*, 2008; Koelkebeck *et al.*, 2008; Patterson *et al.*, 2008). Patterson *et al.* (2008) compared external and internal egg temperatures during production at the layer house and concluded that the two separate measurements correlated very highly with each other and were influenced by state and seasonal differences. A similar seasonal and geographical impact was noted for egg processing operations and, as expected by the authors, temperatures were higher for in-line operations compared to off-line operations since eggs from in-line systems come immediately from the production house to be processed and would be inherently warmer (Koelkebeck *et al.*, 2008). Anderson *et al.* (2008), when following temperature fluctuations of refrigerated delivery trucks, also noted a seasonal fluctuation from the delivery interaction impact on egg temperature during transport for resale or distribution to retailers, with less cooling occurring during short delivery times.

In summary, some obvious control points such as the occurrence of cracked eggs and fluctuations in egg temperature have been identified as potential contributors to microbiological problems associated with eggs during the

production and processing cycle. However, despite the efforts to provide more detailed information on certain aspects of egg production and processing, many issues remain and *Salmonella* outbreaks originating from table shell eggs continue. As discussed previously, factors that contribute to layer hen infection and the corresponding vertical and horizontal spread of *Salmonella* have been well documented by numerous authors (Guard-Petter *et al.*, 2001; Holt, 1999, 2003; Ricke, 2003a; Gantois *et al.*, 2009; Ricke *et al.*, 2013a). Along these lines, interventions that are either currently implemented or proposed have been well documented by many of these same authors. Consequently, these will not be discussed in this review. Instead, the focus will be on *Salmonella* incidence along with the general microbial ecology and potential intervention measures during egg collection, processing, and distribution. These aspects of the egg industry have not necessarily been ignored historically but need to be revisited as the industry shifts more into alternative production systems and as the international trade in eggs accelerates. Likewise, as consumers create market demands for small-scale locally produced eggs in developed countries and developing countries start to collect, transport, and distribute eggs from their small growers to more distant markets the opportunities for contact with *Salmonella* needs to be reconsidered under the environmental conditions imposed by these processing and retail systems. These issues will be addressed in the following sections.

21.5 Microbial contamination during egg collection at the farm to in-line processing

The level of contact of eggs during the egg laying cycle with carriers and vectors of *Salmonella* represent potential sources of contamination and have been well documented (Baron and Jan, 2011). This would be of particular concern with chickens that have outdoors access such as what

occurs in some organic operations and certainly with free range chickens. Consequently, limiting exposure to insects, mice, and other mobile carriers is warranted. Likewise, the environmental exposure to layer hen fecal material during egg laying could be problematic. Henzler *et al.* (1998) identified flocks with the highest level of *S. Enteritidis* contaminated manure as being the greatest public health risk along with the age of flock where birds that were farther into the production cycle were of greater risk. This is supported by Hannah *et al.* (2011) when they compared aerobic bacterial populations from washed and unwashed shell eggs from caged and cage-free laying hens. Hannah *et al.* (2011) concluded that microbial levels were generally lower in eggs collected from laying hens housed in cages with manure removal belts.

Henzler *et al.* (1998) also noted that eggs from contaminated egg handling equipment were more likely to represent a greater risk. Contaminated egg equipment could be a key source of cross-contamination. Murase *et al.* (2001) used pulse-field gel electrophoresis patterns to identify *Salmonella* serovars isolated from environmental samples of individual poultry houses that were attached to an egg processing facility. They concluded that not only were the isolates the same between the poultry houses and the egg processing facility but that the egg belts were most likely to be a contributor to the dissemination of *Salmonella* serovars among houses. They also noted that the same isolate could be continually recovered from a house for over a year. This long-term persistence has consequences not only as an ongoing source of contamination but also indicates that cleaning and disinfection protocols may be somewhat ineffective and need to be altered.

The type and composition of the egg belts may also be a factor. In an attempt to identify environmental areas in Canadian egg production that could be difficult for cleaning and sanitation programs Stocki *et al.* (2007) focused on egg conveyor belts. They pointed out that egg conveyor belts used in battery cage operations

could be a potential attachment surface for persistence of *Salmonella* that also may be somewhat inaccessible to cleaning. In their study when they compared egg conveyor belts composed of either belt material made of vinyl, nylon, hemp, or plastic they observed that the vinyl conveyor belt material that had the least surface area for *Salmonella* colonization also resulted in the lowest recovery of *S. Typhimurium* and *S. Enteritidis* after washing and disinfection, while the hemp-plastic belt material had the greatest surface area and the corresponding highest level of remaining *S. Typhimurium* and *S. Enteritidis*. They concluded that conveyor belts made of woven materials were more susceptible to persistent and recalcitrant *Salmonella* colonization, and conversely belt materials with less surface area such as vinyl were better for effective *Salmonella* removal.

21.6 Microbial contamination during transportation to off-line egg processing facilities

Some of the general issues discussed with egg collection and microbial contamination would be similar for off-line processing but other unique factors have been identified for these operations as well. Knape *et al.* (2002) compared microbial contamination levels in specific time intervals between on-line and off-line processing systems at four sites, namely the egg collection conveyor belt, during detergent application prior to exposure to the sanitizer, immediately after administering the sanitizer, and immediately before packaging. Using aerobic microbial plate counts as their microbial contamination monitor they noted that for the most part microbial populations were greater as the processing shift advanced over time in the off-line egg processing operations compared to the in-line operations. In particular, by the time eggs were ready for packaging off-line aerobic bacterial contamination was greater at all time points versus in-line microbial contamination.

They concluded that this may in part be due to the fact that the eggs arriving at the processing site were fresher for on-line production systems, thus allowing less time for attachment and growth of microbial contaminants as well as less time for the organic material to adhere that might further facilitate microbial contamination and interfere with cleaning of the eggs.

Knape *et al.* (2002) also speculated that storage time may contribute to the higher levels of microbial contamination in off-line egg processing operations by enhancing adherence of organic matter on the shell as well as allowing for bacterial penetration of the egg shell. Musgrove and coworkers examined this in more detail by focusing on the nest run carts, which consist of plastic flats placed on metal carts with unpainted wooden shelves used to transport eggs from the layer houses to the off-line egg processing facilities. As pointed out by Musgrove *et al.* (2012), such carts may become problematic as eggs can become cracked, subsequently leaking on to the wooden shelves, which in turn are used indefinitely and typically are not part of the sanitation regime. This was based on earlier work where Musgrove *et al.* (2004) surveyed 14 non-egg-contact surface multistate commercial egg in-line, off-line, and mixed (both in-line and off-line) processors for general aerobic and *Enterobacteriaceae* bacterial populations at the end of the processing day and before the next processing shift to assess sanitation impact. Of the various sites sampled, nest cart shelves and nest cart wheels yielded some of the highest microbial population counts with statistically non-significant differences between processing times.

In a follow up study, Musgrove *et al.* (2009) identified the isolates of *Enterobacteriaceae* from nest cart shelves to belong to the genera *Citrobacter* spp., *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., *Hafnia* spp., *Kluyvera* spp., *Leclercia* spp., and *Salmonella* spp., respectively, and concluded that the shelves could serve as a reservoir for *Enterobacteriaceae* including food-borne pathogens such as *Salmonella*. They confirmed this when they sampled shelves from

nest carts used in off-line and mixed-line operations and pre-enriched for *Salmonella* isolation and further identification (Musgrove *et al.*, 2012). Shelves from off-line operations were threefold (36% total positives) more contaminated with *Salmonella* spp. with serotypes *S. Cerro*, *S. Heidelberg*, *S. Infantis*, *S. Mbandaka*, and *S. Oranienberg* recovered from the off-line operation and serotypes *S. Anatum*, *S. Mbandaka*, and *S. Typhimurium* recovered from the mixed operation facility.

21.7 Microbial contamination during egg processing

Once eggs are received in the egg processing plant either from on-line or off-line operations, additional opportunities for microbial cross-contamination can occur during egg processing and subsequent packaging for retail shipment. Typically eggs arrive on conveyor belts from the corresponding on-line and off-line production sources, go through an accumulator, followed by an egg washer, subsequently dried, oiled, candled, and electronically checked for cracks, weighed, sorted, and finally packed for retail distribution (Musgrove, 2011). Much of the egg handling during processing utilizes vacuum loaders, electronic surveillance, and weighing systems, enabling many more eggs to be processed and prepared for retail (Musgrove, 2011). However, despite the trend in increased automation, several research studies indicate that microbial and *Salmonella* cross-contamination during egg processing still remain part of the ongoing concern as *Salmonella* outbreaks continue to occur (Davies and Breslin, 2003).

Early work by Jones *et al.* (1995) assessed *Salmonella* frequency from environmental samples, and external and internal egg contents collected at egg layer farms and an on-line processing facility in the US, and concluded that although approximately 1% of the commercial eggs were positive for *Salmonella* serovars externally, internal contamination was relatively infrequent. In a comprehensive UK

field-based farm egg packing plant study, Davies and Breslin (2003) sampled the packing plant floor, grading table, candler, conveyor belts, rollers, and handler tables either during operation or prior to the respective plant's disinfection schedule. They observed that all sites sampled were overall 20 to 30% contaminated with *Salmonella* and that these levels reflected the level of contamination with the corresponding layer house, with eggs undergoing longer distances on roller conveyors prior to reaching the packing plant being less contaminated. To examine the potential for cross-contamination of eggs during processing in these packing plants, they sterilized eggs by boiling for 10 minutes and subsequently sent them through the packing plant 1 to 3 times, followed by culturing them for *Salmonella*. Although they did not detect *Salmonella* from sterile eggs passed through the plant once, by the third time they observed a minimum acquisition contamination rate of 0.3%. They concluded that disinfection only reduced these levels somewhat, which they believed was a reflection of their observation of generally poor cleaning in the egg packing plants resulting in detectable residual contamination.

Jones *et al.* (2003) assessed the effectiveness of sanitation programs in southeastern US egg processing plants by comparing on-line, off-line, and mixed-line shell egg processing plants located in different states either at the end of the operating day or the next morning prior to the start of operations. They used sterile gauze pads to sample sixteen direct and indirect contact sites within these plants, including belts, scales, check detector, vacuum loaders, egg washer tank brushes, washer lids and tank walls, oiler flaps, and packer head belts, among others, for determining the levels of aerobic bacteria and *Enterobacteriaceae*. In general, they could not detect aerobic bacterial population differences between the end of the operating day versus the next morning, which has also been reported in other studies (Musgrove *et al.*, 2004). However, they did observe high levels of bacteria recovered from the washer tank wall, the rewash belt,

and the vacuum loaders with bacterial counts becoming lower as the eggs moved further along into cleaner areas of the processing line. They concluded that there is the potential need for development and application of cleaning chemicals that better take into account the challenges of a commercial operation and that sanitation could potentially be made more effective if separated into cleaning zones based on the particular types of equipment involved in the different components of the egg packing plant operation.

Musgrove *et al.* (2005) conducted an intensive microbial examination of the individual stages of egg processing in mixed-line operations by using egg shell rinses of eggs collected at different processing sites and enumerating total aerobic bacteria, yeast and molds, *E. coli*, and *Enterobacteriaceae* along with pre-enrichment for *Salmonella* isolation. They noted that generally microbial populations from shell rinses were highest at the accumulator and the rewash belt. They also concluded that prevalence for individual groups of bacteria for the most part also generally declined as eggs transitioned from pre-processing to post-processing, while overall *Salmonella* prevalence ranged at approximately 10% with considerable differences between individual processing plants. They also observed that *Salmonella* was isolated at every site in all stages of egg processing at least once when combining all isolations across all visits and plants. In a follow up study, Musgrove *et al.* (2008) used commercial biochemical-based identification assays and identified *Escherichia coli* and *Enterobacter* spp. from most of the plants with lesser frequencies of other *Enterobacteriaceae* genera including *Citrobacter* and *Klebsiella* and non-*Enterobacteriaceae* bacteria including *Pseudomonas*, *Aeromonas*, and *Vibrio*, among others.

When Jones and Musgrove (2008a, 2008b) examined sterile saline rinsates from vacuum loaders, which are used to transfer eggs from incoming egg flats in mixed and off-site egg production facilities, they observed higher aerobic bacterial populations in mixed operations but higher *Enterobacteriaceae* in the off-line facility.

Based on the biochemical test, predominant bacteria included *Enterobacter*, *Klebsiella*, *Escherichia*, *Citrobacter*, and *Serratia*. *Campylobacter* and *Salmonella* were recovered at rates of 1.6 and 3.3%, respectively, while *Listeria* were recovered in 72% of the samples (of which 98.8% were *L. innocua* and 1.2% were *L. monocytogenes*). The authors speculated that vacuum loader cups could serve as a reservoir for *Listeria*. They also concluded that bacterial growth niches may be appearing in these loader cups, thus stressing the need for better cleaning and sanitation approaches for these particular devices. It is not clear where the *Listeria* may be originating from as they seem to be linked with facilities that have off-line capabilities. Whether they originally were attached to incoming egg flat surfaces or were part of the plant facility environment microbiota remains to be determined. Certainly, *Listeria* spp. are well known inhabitants of a wide range of food processing plant environments (Milillo *et al.*, 2012a) but several species have been isolated from poultry origins as well as including pasture flock poultry birds, eggs, and egg washwater (Laird *et al.*, 1991; Farber *et al.*, 1992; Jones *et al.*, 2006; Milillo *et al.*, 2012b). More surveillance research needs to be done to determine the extent of *Listeria* contamination in egg processing plants and the potential risk to public health.

21.8 Egg washwater and sanitation

Several countries such as the United States, Japan, and Canada wash and grade eggs prior to shipping them out for retail distribution, but egg washing is generally not allowed or practiced in the European Union (Hutchinson *et al.*, 2003; Musgrove, 2011; Howard *et al.*, 2012). As described by Hutchinson *et al.* (2003), the egg washing process essentially involves four steps, namely wetting, washing, rinsing, and drying. Egg washing procedures conducted in commercial operations typically

employ a mechanical washing apparatus that mists an alkaline detergent solution across eggs as they are brushed from side to side while moving along the egg conveyor belt, followed by a final rinse usually containing some sort of chlorine or quaternary-based sanitizer (Hutchinson *et al.*, 2003; Northcutt *et al.*, 2005; Howard *et al.*, 2012). Once the eggs have been washed the remaining egg wash and rinse waters are combined, filtered, and recirculated to a wash tank where they can be reheated for repeat usage in the initial wetting and cleaning stages of the egg washer cycle (Hutchinson *et al.*, 2003; Northcutt *et al.*, 2005; Howard *et al.*, 2012). Details on the development, management, and application of egg washwater has been extensively reviewed elsewhere and will only be briefly discussed in the current review (Moats, 1978; Hutchinson *et al.*, 2003; Messens, 2011).

While reusing egg washwater to minimize overall water use is desirable there are several potential issues with this reuse of egg washwater (Hutchinson *et al.*, 2003). Even though the alkaline pH of the egg washwater is designed to not only clean egg shells but also to limit bacterial proliferation, accumulation of organic matter from recirculation can dilute its efficacy (Kinner and Moats, 1981; Howard *et al.*, 2012). To address this, Northcutt *et al.* (2005) profiled the potential fluctuations in the composition of commercial shell egg washwater from three in-line egg processors in the southeastern US by monitoring temperature, pH, chlorine, soluble iron (ferrous), total dissolved solids, total suspended solids, total Kjeldahl nitrogen, and chemical oxygen demand. They compared fresh incoming tap water with water sampled after the first 2 hours of operation from two egg shell washers that used an end-to-end arrangement as a dual washer system in these facilities. In general they observed considerable variation among plants for most of these parameters and concluded that differences occurred in the age of plant and equipment, management of the plant, source of eggs, and number of broken eggs, among other factors. In particular, when

decreases in pH and increases in soluble iron occur, these could be especially problematic as both can lead to increases in bacterial growth and contamination including *Salmonella* spp. if they reach certain levels where they no longer limit bacterial growth and proliferation (Hutchinson *et al.*, 2003; Messens, 2011; Howard *et al.*, 2012).

The fluctuations in chemical composition raise the general issue as to whether egg washing effectively limits microbial contamination. To determine this, Jones *et al.* (2004) profiled microbial populations by collecting eggs before and after egg processing over three consecutive days from a commercial in-line processor with a dual-tank egg washer. They stored eggs over ten weeks of storage and enumerated total aerobic bacteria, pseudomonads, yeast, molds, and *Enterobacteriaceae* from both external egg shell surface rinses and internal egg contents. While they did not observe differences in total aerobic bacteria, yeasts and molds between unwashed and washed egg contents, they did report substantially less external total aerobic bacterial, and yeast and mold populations in washed eggs compared to unwashed eggs while only low levels of *Enterobacteriaceae* and pseudomonads were detected in any of the eggs sampled. It would be of interest to develop more detailed microbial profiles of egg shell microbial populations to determine shifts in specific groups and even individual microorganisms during egg processing. Likewise, the interaction between the microbiota on incoming eggs versus resident egg processing plant microbial populations would be helpful to elucidate their respective impacts on the efficacy of egg washes and sanitizers. As microbial identification technology progresses with next generation sequence capabilities becoming more routine and readily available, extensive characterization of these corresponding egg processing plant and egg shell surface microbiomes may become a reality.

Despite the apparent effectiveness of egg washing, as Jones *et al.* (2004) pointed out from

their earlier studies (Jones *et al.*, 2003), reduced bacterial contamination is not always observed in commercial egg processing operations. This occurs despite the employment of some sort of chemical sanitizer during the egg processing cycle. Sanitizers used in egg processor facilities are generally applied immediately after the alkaline egg wash cleaning step as a rinse solution containing a chlorine concentration of 100 to 200 ppm or a quaternary ammonium-based compound (Hutchinson *et al.*, 2003; Howard *et al.*, 2012). Given the inconsistencies in effective microbial contamination control and issues such as reducing cost, a wide range of sanitizers and other decontamination technologies for potential use in egg processing have been examined over the past several years (Howard *et al.*, 2012). Individual compounds and other strategies have been described extensively elsewhere (Berardinelli *et al.*, 2011; Howard *et al.*, 2012; Galiş *et al.*, 2013) and will only be briefly discussed here. Generally, the sanitizer approaches can be classified as an application of either unique chemical or biological compounds administered in a similar manner to the more traditional sanitizer compounds. These would include agents such as botanical compounds, enzyme catalyzed bactericidal reactions, or electrolyzed water (Kuo *et al.*, 1997b; McKee *et al.*, 1998; Knape *et al.*, 1999, 2001; Russell, 2003; Bialka *et al.*, 2004; Park *et al.*, 2005; Cao *et al.*, 2009; Upadhyaya *et al.*, 2013). Non-chemical approaches to decontamination include subjecting shell eggs or egg processing equipment to some sort of light, gas, or other form of radiation that is bactericidal, such as ultraviolet light, non-thermal atmospheric gas plasma, ozone, or ionizing radiation (Gao *et al.*, 1997; Kuo *et al.*, 1997a, 1997c; Chavez *et al.*, 2002; Rodriguez-Romo and Yousef, 2005; Keklik *et al.*, 2010; Ragni *et al.*, 2010). Although many of these approaches show promise much remains to be done to assess the effectiveness under different processing conditions as well as the need to address cost and economics for scaling up some of these technologies.

21.9 Egg retail and microbial contamination

Once eggs are processed and sorted they are packaged for retail and distributed to retail markets. Starting in the 1970s considerable effort was made to compare different types of egg packaging that was best suited not only for retail market display but protected eggs from damage to the shell during transportation as they traveled to their respective market destinations. Most of the research and development focus was directed toward either expanded polystyrene (EPS) or molded paper pulp (MPP). Early work addressed issues such as the ability of particular egg carton types to cushion eggs sufficiently to minimize crack formation (Mellor and Gardner, 1970, 1975; Mellor *et al.*, 1975; Denton *et al.*, 1981; Lederer, 1983; Nethercote *et al.*, 1974; Seydim and Dawson, 1999). Nethercote *et al.* (1974) concluded that for both polystyrene and molded pulp cartons design was more important than carton material. Much of the later work has been directed toward the response of these types of egg cartons to temperature fluctuations during storage and transport and rapid cooling systems (Thompson *et al.*, 2000).

While egg packaging has evolved to a large extent in the US, potential contamination issues remain with certain segments of the egg retail market. Historically, used cases, fillers, and flats were considered readily available for reuse and this apparently occurred where deficits were present (Eggleton and Carpenter, 1961). However, Eggleton and Carpenter (1961), after surveying the egg industry at that time, recommended that damaged and soiled flats needed to be avoided and controls should be exercised over reuse policies. Board *et al.* (1963) surveyed new, used, and dirty egg flats and observed that they could become heavily contaminated, especially if they had egg albumen or yolk material remaining on them. Banwart (1964) demonstrated that *Salmonella* and other egg contaminant bacteria could attach to these egg flats and

that only autoclaving the flats completely eliminated them.

This issue has re-emerged for certain local markets where retail egg containers can be reused and there is the potential for contamination to occur over time if these are not properly sanitized. There is evidence for this potential risk based on studies conducted on retail egg markets in other countries. Suresh *et al.* (2006) collected samples of egg shells, egg contents, and egg trays in South India retail markets and isolated and identified *Salmonella* spp. from these sources. Based on the recovered levels of *Salmonella* from these various sources they concluded that reused egg trays were a potential risk for exposure to *Salmonella*. In a later study, Singh *et al.* (2010) profiled eggs transported from farms to wholesale and retail markets located in North India in an attempt to characterize and track where *Salmonella* cross-contamination might be occurring. *S. Typhimurium* was the predominant serovar and they observed a higher incidence from eggs collected in the retail markets versus the levels they isolated in the fresh eggs from the egg layer farms. Based on these comparisons, they suggested that surface contamination must have occurred during handling, storage, and transportation of the eggs from the farms to the market. Utrarachkij *et al.* (2012) conducted an in-depth study on the level of *Salmonella* contamination in samples they had collected from eggshells, egg contents, reusable egg trays, and associated environments in Thailand egg farms and markets. After a comparison among these multiple sources they concluded that reusable egg trays used for these eggs could serve as a potential source of horizontal *Salmonella* transmission.

Based on these studies it is clear that little is known about the interaction between the type of retail packaging and the cross-contamination that may occur between it and the table shell egg. Certainly opportunities exist for potential microbial cross-contamination depending on the type of packaging material, particularly if it is reused and not properly cleaned. More comprehensive microbial studies need to be conducted

on different types of packaging materials to identify better the dynamics of microbial diversity and their potential interactions with food-borne pathogens such as *Salmonella* spp. As discussed with egg processing, advanced molecular technologies such as complete microbiome sequencing offer opportunities to characterize these microbial populations much more thoroughly and delineate patterns that may contribute to persistent contamination problems. This is critical as some of these complex microbial populations that can be present on some surfaces may possess capabilities of forming biofilms, which could harbor pathogens and thus render the pathogens somewhat protected from typical sanitation protocols and administration of sanitizing agents. The other development that may prove useful to egg packaging is the development of unique packaging materials that possess antimicrobial characteristics that could serve as a barrier for preventing cross-contamination. There is a precedent for this in the food industry with other food commodities (Suppakul *et al.*, 2003). One could envisage such packaging not only containing antimicrobials but as technologies develop possessing sensor capabilities for monitoring environmental conditions such as temperatures along with precise tracking capabilities and perhaps eventually incorporating direct microbial contamination level indicators.

21.10 Conclusions and future directions

Salmonellosis continues to be the major cause of food-borne disease in the US, with over one million cases occurring annually and costing several billion dollars (Scallan *et al.*, 2011; Foley *et al.*, 2013). Poultry products are still one of the major sources of *Salmonella* in the US (Foley *et al.*, 2011, 2013; Painter *et al.*, 2013). *Salmonella* and microbial contamination continue to be issues for the commercial egg industry, but the sources and cross-contamination routes have changed over time. In addition, different

Salmonella serovars have become more closely identified with eggs, such as *S. Enteritidis*, which appears to have some host affinity for infecting laying hens. This is partly due to the evolution of *Salmonella* serovars over the years and also to some extent the result of changes in egg production management. In addition, the impact of recent salmonellosis egg associated outbreaks have been more prominent from a public health standpoint, not only because of the larger egg operations involved but also because of the more comprehensive and advanced molecular-based traceback technologies that are now available and can be routinely implemented. These trends will remain as the commercial US egg industry continues to evolve from a small flock local market in the last century to the large flock–multiple house and egg processing plant complex production systems common to the current industry. However, as the egg industry has developed into a vertically integrated system, more narrowly focused commercial niche markets have emerged such as speciality or designer eggs along with the ever-increasing consumer demand for organic and free range layer flocks produced eggs. Some of these systems such as free range chickens present additional challenges for managing food safety. Likewise, as the egg industry shifts more internationally and trade between countries expands, food safety issues associated with these eggs may also become more prevalent.

However, much has been learned about the ecology of *Salmonella* in layer flocks and how this influences subsequent egg contamination. While most *Salmonella* penetrate eggs from the exterior shell, *S. Enteritidis* has shown the ability to infect hens and colonize the reproductive tract, resulting in contamination of the internal contents of the egg. Several environmental factors influenced this, but certain management practices were identified as primary culprits for creating a gastrointestinal tract microenvironment that enhanced *S. Enteritidis* invasiveness. Since these factors became known, a number of management strategies have emerged to limit this. Likewise, as eggs are collected from the

farm and either sent directly via conveyor belt to an on-line egg processing plant or transported to an off-line processing plant, more is now known about the microbial ecology of eggs as they enter the plant. Problem areas still occur, in particular during transport to off-line facilities and the use of nest carts. Other environmental areas for microbial contamination include certain types of conveyor belts and vacuum loaders. During egg processing the use of egg washes and sanitizers can help to reduce the bacterial load during egg processing but improvements and more sanitizer options are needed. In particular, it appears that *Salmonella* cross-contamination of external egg surfaces can occur during processing. Finally, opportunities exist for potential contamination at the retail market, depending on the type of packaging material. Clearly, reuse of egg trays as seen in certain parts of the world have been identified as a potential high risk source of *Salmonella* contamination. This is supported by work in the US during the early 1960s, which demonstrated that trays soiled by egg contents that had been reused were easily contaminated by *Salmonella* attachment to these surfaces and the pathogen could only be eliminated by the extreme measure of autoclaving. This may be particularly important in local egg retail markets where egg containers may be reused without proper cleaning. Clearly, more effort to communicate risks to the public may be warranted as well.

There continues to be a need for an improvement in food safety, especially as it is associated with all aspects of egg production, processing, and retail. This will require not only further development of control measures but also detailed identification of critical high-risk areas where microbial contamination is most likely to occur. Consequently, an in-depth profile of the microbial populations involved at various stages of egg production, particularly during transport, arrival at the egg processing plant, and during the various stages of egg washing, oiling, sorting, and packaging, is needed. Advanced molecular approaches such as microbiome analyses offer intriguing possibilities to provide

more details on how environmental factors such as changes in egg washwater chemistry and temperature may influence the microbiota on the egg shell surface. Likewise, functional genomic analysis offers the opportunity to understand how *Salmonella* spp. may respond to these same conditions. Identifying responses at the genomic level may lead to development of unique antimicrobials and sanitizer strategies that can more precisely target *Salmonella* spp. at all phases of egg production. The ultimate goal will be to produce a safer egg with a longer shelf life at the retail market.

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References

- Anderson, K.E. 2009. Overview of natural and organic egg production: looking back to the future. *Journal of Applied Poultry Research*, **18**, 348–354.
- Anderson, K.E., Patterson, P.H., Koelkebeck, K.W., Darre, M.J., *et al.* 2008. Temperature sequence of eggs from oviposition through distribution: transportation – Part 3. *Poultry Science*, **87**, 1195–1201.
- Babu, U., Scott, M., Myers, M.J., Okamura, M., *et al.* 2003. Effects of live attenuated and killed *Salmonella* vaccine on T-lymphocyte mediated immunity in laying hens. *Veterinary Immunology and Immunopathology*, **91**, 39–44.
- Banwart, G.F. 1964. Elimination of *Salmonellae* from inoculated filler flats. *Poultry Science*, **43**, 939–941.
- Bardina, C., Spricigo, D.A., Cortés, P., and Llagostera, M. 2012. Significance of the bacteriophage treatment schedule in reducing *Salmonella* colonization of poultry. *Applied and Environmental Microbiology*, **78**, 6600–6607.
- Baron, F. and Jan, S. 2011. Egg and egg product microbiology, Chapter 14. In: *Improving the Safety and Quality of Eggs and Egg Products*, Vol. 2: *Egg Safety and Nutritional Quality* (eds Van Immerseel, F., Nys, Y., and Bain, M.), pp. 330–350. Woodhead Publishing Limited, Oxford, UK.
- Bell, D. 1993. The egg industry of California and the USA in the 1990s: a survey of systems. *World's Poultry Science Journal*, **49**, 58–64.
- Bell, D.D. 2003. Historical and current molting practices in the U.S. table egg industry. *Poultry Science*, **82**, 965–970.
- Bell, D.D., Patterson, P.H., Koelkebeck, K.W., Anderson, K.E., *et al.* 2001. Egg marketing in national supermarkets: egg quality – Part 1. *Poultry Science*, **80**, 383–389.
- Berardinelli, A., Cevoli, C., Fabbri, A., Guerzoini, M.E., *et al.* 2011. Alternative egg decontamination techniques to washing, Chapter 10. In: *Improving the Safety and Quality of Eggs and Egg Products*, Vol. 2: *Egg Safety and Nutritional Quality* (Van Immerseel, F., Nys, Y., and Bain, M.), pp. 330–350. Woodhead Publishing Limited, Oxford, UK.
- Berry, W.D. 2003. The physiology of induced molting. *Poultry Science*, **82**, 971–980.
- Bialka, K.L., Demirci, A., Knabel, S.J., Patterson, P.H., and Puri, V.M. 2004. Efficacy of electrolyzed oxidizing water for the microbial safety and quality of eggs. *Poultry Science*, **83**, 2071–2078.
- Board, R.G., Ayres, J.C., Kraft, A.A., and Forsythe, R.H. 1963. The microbiological contamination of egg shells and egg packing materials. *Poultry Science*, **42**, 584–595.
- Borie, C., Albala, I., Sánchez, P., Sánchez, M.L., *et al.* 2008. Bacteriophage treatment reduces *Salmonella* colonization of infected chickens. *Avian Diseases*, **52**, 64–67.
- Braden, C.R. 2006. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. *Clinical Infectious Diseases*, **43**, 512–517.
- Cao, W., Zhu, Z.W., Shi, Z.X., Wang, C.Y., and Li, B.M. 2009. Efficiency of slightly acidic electrolyzed water for inactivation of *Salmonella enteritidis* and its contaminated shell eggs. *International Journal of Food Microbiology*, **130**, 88–93.

- Carrasco, E., Morales-Rueda, A., and García-Gimeno, R.M. 2012. Cross-contamination and recontamination by *Salmonella* in foods: a review. *Food Research International*, **45**, 545–556.
- Chavez, C., Knape, K.D., Coufal, C.D., and Carey, J.B. 2002. Reduction of eggshell aerobic plate counts by ultraviolet irradiation. *Poultry Science*, **81**, 1132–1135.
- Chemaly, M. and Salvat, G. 2011. Foodborne disease associated with eggs: microbial hazards and *Salmonella* Enteritidis risk assessment, Chapter 2. In: *Improving the Safety and Quality of Eggs and Egg Products, Vol. 2: Egg Safety and Nutritional Quality* (eds Van Immerseel, F., Nys, Y., and Bain, M.), pp. 34–45. Woodhead Publishing Limited, Oxford, UK.
- Cogan, T.A. and Humphrey, T.J. 2003. The rise and fall of *Salmonella* Enteritidis in the UK. *Journal of Applied Microbiology*, **94** (Suppl.), 114S–119S.
- Corrier, D.E., Nisbet, D.J., Hollister, A.G., Beier, R.C., Scanlan, C.M., Hargis, B.M., and DeLoach, J.R. 1994a. Resistance against *Salmonella enteritidis* cecal colonization in Leghorn chicks by vent lip application of cecal bacteria culture. *Poultry Science*, **73**, 648–652.
- Corrier, D.E., Nisbet, D.J., Scanlan, C.M., Tellez, G., Hargis, B.M., and DeLoach, J.R. 1994b. Inhibition of *Salmonella enteritidis* cecal and organ colonization in Leghorn chicks by a defined culture of cecal bacteria and dietary lactose. *Journal of Food Protection*, **57**, 377–381.
- Corrier, D.E., Nisbet, D.J., Scanlan, C.M., Hollister, A.G., and DeLoach, J.R. 1995. Control of *Salmonella typhimurium* colonization in broiler chicks with a continuous-flow characterized mixed culture of cecal bacteria. *Poultry Science*, **74**, 916–924.
- Corrier, D.E., Nisbet, D.J., Hargis, B.M., Holt, P.S., and DeLoach, J.R. 1997. Provision of lactose to molting hens enhances resistance to *Salmonella enteritidis* colonization. *Journal of Food Protection*, **60**, 10–15.
- Curtis, P. 2007. Microbiological challenges of poultry egg production in the US. *World's Poultry Science Journal*, **63**, 301–307.
- Davies, R. and Breslin, M. 2001. Environmental contamination and detection of *Salmonella enterica* serovar enteritidis in laying flocks. *The Veterinary Record*, **149**, 699–704.
- Davies, R. and Breslin, M. 2003. Investigation of *Salmonella* contamination and disinfection in farm egg-packing plants. *Journal of Applied Microbiology*, **94**, 191–196.
- Davies, R.H. and Hinton, M.H. 2000. *Salmonella* in animal feed. In: *Salmonella in Domestic Animals* (eds Wray, C. and Wray, A.), pp. 285–300. CABI Publishing, Wallingford, UK.
- Davies, R.H. and Wales, A.D. 2010. Investigations into *Salmonella* contamination in poultry feed-mills in the United Kingdom. *Journal of Applied Microbiology*, **109**, 1430–1440.
- Denton, J.H., Mellor, D.B., and Gardner, F.A. 1981. The effect of egg carton and case type on egg shell damage. *Poultry Science*, **60**, 145–150.
- De Reu, K., Messens, W., Heyndrickx, M., Rodenburg, T.B., Uyttendaele, M., and Herman, L. 2008. Bacterial contamination of table eggs and the influence of housing systems. *World's Poultry Science Journal*, **64**, 5–19.
- De Reu, K., Rodenburg, T.B., Grijspeerd, K., Messens, W., et al. 2009. Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: an international on-farm comparison. *Poultry Science*, **88**, 2442–2448.
- Dewaele, I., Rasschaert, G., Wildemaue, C., Van Meirhaeghe, H., et al. 2012. Polyphasic characterization of *Salmonella* Enteritidis isolates on persistently contaminated layer farms during the implementation of a national control program with obligatory vaccination: a longitudinal study. *Poultry Science*, **91**, 2727–2735.
- Dewulf, J., Van Hoorebeke, S., and Van Immerseel, F. 2011. Epidemiology of *Salmonella* infections in laying hens with special emphasis on the influence of the housing system, Chapter 6. In: *Improving the Safety and Quality of Eggs and Egg Products, Vol. 2: Egg Safety and Nutritional Quality* (eds Van Immerseel, F., Nys, Y., and Bain, M.), pp. 107–119. Woodhead Publishing Limited, Oxford, UK.

- Donalson, L.M., McReynolds, J.L., Kim, W.K., Chalova, V.I., *et al.* 2008. The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract fermentation, *Salmonella* Enteritidis infection, and intestinal shedding in laying hens. *Poultry Science*, **87**, 1253–1262.
- Dunkley, C.S., McReynolds, J.L., Dunkley, K.D., Kubena, L.F., Nisbet, D.J., and Ricke, S.C. 2007a. Molting in *Salmonella* Enteritidis-challenged laying hens fed alfalfa crumbles. III. Blood plasma metabolite response. *Poultry Science*, **86**, 2492–2501.
- Dunkley, C.S., McReynolds, J.L., Dunkley, K.D., Njongmeta, L.N., *et al.* 2007b. Molting in *Salmonella* Enteritidis-challenged laying hens fed alfalfa crumbles. IV. Immune and stress protein response. *Poultry Science*, **86**, 2502–2508.
- Dunkley, K.D., McReynolds, J.L., Hume, M.E., Dunkley, C.S., *et al.* 2007c. Molting in *Salmonella* Enteritidis-challenged laying hens fed alfalfa crumbles. I. *Salmonella* Enteritidis colonization and virulence gene *hlyA* response. *Poultry Science*, **86**, 1633–1639.
- Dunkley, K.D., McReynolds, J.L., Hume, M.E., Dunkley, C.S., *et al.* 2007d. Molting in *Salmonella* Enteritidis-challenged laying hens fed alfalfa crumbles. II. Fermentation and microbial ecology response. *Poultry Science*, **86**, 2101–2109.
- Dunkley, K.D., Callaway, T.R., Chalova, V.I., McReynolds, J.L., *et al.* 2009. Foodborne *Salmonella* ecology in the avian gastrointestinal tract. *Anaerobe*, **15** (Special Issue SI), 26–35.
- Dunn, J.W. and Madison, M.E. 1992. Interregional competition for eggs. *Journal of Applied Poultry Research*, **1**, 48–55.
- Durant, J.A., Corrier, D.E., Byrd, J.A., Stanker, L.H., and Ricke, S.C. 1999. Feed deprivation affects crop environment and modulates *Salmonella enteritidis* colonization and invasion of leghorn hens. *Applied and Environmental Microbiology*, **65**, 1919–1923.
- Eggleton, L.Z. and Carpenter, C.D. 1961. The use of reclaimed egg cases in the shell egg and egg products trade. *Poultry Science*, **40**, 720–727.
- Erickson, M.C. 2010. Microbial risks associated with cabbage, carrots, celery, onions, and deli salads made with these produce items. *Comprehensive Reviews on Food Science and Food Safety*, **9**, 602–619.
- Farber, J.M., Daley, E., and Coates, F. 1992. Presence of *Listeria* spp. in whole eggs and wash water samples from Ontario and Quebec. *Food Research International*, **25**, 143–145.
- Finstad, S., O'Bryan, C.A., Marcy, J.A., Crandall, P.G., and Ricke, S.C. 2012. *Salmonella* and broiler production in the United States: relationship to foodborne salmonellosis. *Food Research International*, **45**, 789–794.
- Foley, S.L., Lynne, A.M., and Nayak, R. 2008. *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *Journal of Animal Science*, **86** (E. Suppl.), E149–E162.
- Foley, S., Nayak, R., Hanning, I.B., Johnson, T.L., Han, J., and Ricke, S.C. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Applied and Environmental Microbiology*, **77**, 4273–4279.
- Foley, S.L., Johnson, T.J., Ricke, S.C., Nayak, R., and Danzeisen, J. 2013. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiology and Molecular Biology Reviews*, **77**, 582–607.
- Galiş, A.N., Marcq, C., Marlier, D., Portetelle, D., Van, I., Beckers, Y., and Théwis, A. 2013. Control of *Salmonella* contamination of shell eggs – preharvest and postharvest methods: a review. *Comprehensive Reviews on Food Science and Food Safety*, **12**, 155–182.
- Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T.J., and Van Immerseel, F. 2009. Mechanisms of egg contamination by *Salmonella* Enteritidis. *FEMS Microbiology Reviews*, **33**, 718–738.
- Gao, F., Stewart, L.E., Joseph, S.W., and Carr, L. E. 1997. Effectiveness of ultraviolet light irradiation in reducing the numbers of *Salmonella* on eggs and egg belt conveyor materials. *Applied Engineering Agriculture*, **13**, 355–359.
- Ge, B., LaFon, P.C., Carter, P.J., McDermott, S.D., *et al.* 2013. Retrospective analysis of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus*

- in animal feed ingredients. *Foodborne Pathogens and Disease*, **10**, 684–691.
- Guard-Petter, J. 2001. The chicken, the egg and *Salmonella enteritidis*. *Environmental Microbiology*, **3**, 421–430.
- Guinée, J.B., Heijungs, R., Huppes, G., Zamagni, A., Masoni, P., Buonamici, R.O., Ekvall, T., and Rydberg, T. 2011. Life cycle assessment: past, present, and future. *Environmental Science and Technology*, **45**, 90–96.
- Hannah, J.F., Wilson, J.L., Cox, N.A., Cason, J.A., et al. 2011. Comparison of shell bacteria from unwashed and washed table eggs harvested from caged laying hens and cage-free floor-housed laying hens. *Poultry Science*, **90**, 1586–1593.
- Hanning, I., Nutt, J.D., and Ricke, S.C. 2009. Salmonellosis outbreaks due to fresh produce: sources and potential intervention measures. *Foodborne Pathogens and Disease*, **6**, 635–648.
- Henriques, A., Sereno, R., and Almeida, A. 2013. Reducing *Salmonella* horizontal transmission during egg incubation by phage therapy. *Foodborne Pathogens and Disease*, **10**, 718–722.
- Henzler, D.J. and Opitz, H.-M. 1992. The role of mice in the epizootiology of *Salmonella enteritidis* infection on chicken layer farms. *Avian Diseases*, **36**, 625–631.
- Henzler, D.J., Kradel, D.C., and Sischo, W.M. 1998. Management and environmental risk factors for *Salmonella enteritidis* contamination of eggs. *American Journal of Veterinary Research*, **59**, 824–829.
- Higgins, S.E., Erf, G.F., Higgins, J.P., Henderson, S.N., Wolfenden, A.D., Gaona-Ramirez, G., and Hargis, B.M. 2007. Effect of probiotic treatment in broiler chicks on intestinal macrophage numbers and phagocytosis of *Salmonella* Enteritidis by abdominal exudate cells. *Poultry Science*, **86**, 2315–2321.
- Higgins, S.E., Higgins, J.P., Wolfenden, A.D., Henderson, S.N., Torres-Rodriguez, A., Tellez, G., and Hargis, B. 2008. Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* Enteritidis in neonatal broiler chicks. *Poultry Science*, **87**, 27–31.
- Hinkle, N.C. and Hickle, L.A. 1999. California caged layer pest management evaluation. *Journal of Applied Poultry Research*, **8**, 327–338.
- Holt, P.S. 1999. Impact of induced molting on immunity and *Salmonella enterica* serovar Enteritidis infection in laying hens, Chapter 33. In: *Salmonella enterica* serovar Enteritidis in Humans and Animals – Epidemiology, Pathogenesis, and Control (eds Saeed, A.M., Gast, R.K., Potter, M.E., and Wall, P.G.), pp. 367–375. Iowa State University Press, Ames, IA, USA.
- Holt, P.S. 2003. Molting and *Salmonella enterica* serovar Enteritidis infection: the problem and some solutions. *Poultry Science*, **82**, 1008–1010.
- Holt, P.S., Davies, R.H., Dewulf, J., Gast, R.K., Huwe, J.K., Jones, D.R., Waltman, D., and Willian, K.R. 2011. The impact of different housing systems on egg safety and quality. *Poultry Science*, **90**, 251–262.
- Howard, Z.R., O'Bryan, C.A., Crandall, P.G., and Ricke, S.C. 2012. *Salmonella* Enteritidis in shell eggs: current issues and prospects for control. *Food Research International*, **45**, 755–764.
- Hume, M.E. 2011. Historic perspective: prebiotics, probiotics, and other alternatives to antibiotics. *Poultry Science*, **90**, 2663–2669.
- Humphrey, T.J. 1994. Contamination of egg shell and contents with *Salmonella enteritidis*: a review. *International Journal of Food Microbiology*, **21**, 31–40.
- Humphrey, T.J. 1999. Contamination of eggs and poultry meat with *Salmonella enterica* serovar Enteritidis, Chapter 18. In: *Salmonella enterica* serovar Enteritidis in Humans and Animals – Epidemiology, Pathogenesis, and Control (eds Saeed, A.M., Gast, R.K., Potter, M.E., and Wall, P.G.), pp. 183–192. Iowa State University Press, Ames, IA, USA.
- Hutchison, M.L., Gittins, J., Walker, A., Moore, A., Burton, C., and Sparks, N. 2003. Washing table eggs: a review of the scientific and engineering issues. *World's Poultry Science Journal*, **59**, 233–248.
- Hutchison, M.L., Gittins, J., Walker, A., Sparks, N., Humphrey, T.J., Burton, C., and Moore, A. 2004. An assessment of the microbiological risks involved with egg washing under commercial conditions. *Journal of Food Protection*, **67**, 4–11.

- Ibarburu, M. and Bell, D. 2013. U.S. flock and egg price projections. Report No. 47 Egg Industry Center, Iowa State University, Ames, IA.
- Jarquín, R., Hanning, I., Ahn, S., and Ricke, S.C. 2009. Development of rapid detection and genetic characterization of *Salmonella* in poultry breeder feeds. *Sensors*, **9**, 5308–5323.
- Jones, F.T. 2011. A review of practical *Salmonella* control measures in animal feed. *Journal of Applied Poultry Research*, **20**, 102–113.
- Jones, D.R. and Musgrove, M.T. 2008a. Identification of *Enterobacteriaceae* on vacuum loaders in shell egg processing. *Poultry Science*, **87**, 1678–1681.
- Jones, D.R. and Musgrove, M.T. 2008b. Assessment of microbial contaminants present on vacuum loaders in shell egg processing facilities. *Journal of Food Safety*, **28**, 346–354.
- Jones, F.T. and Richardson, K.E. 2004. *Salmonella* in commercially manufactured feeds. *Poultry Science*, **83**, 384–391.
- Jones, F.T., Rives, D.V., and Carey, J.B. 1995. *Salmonella* contamination in commercial eggs and an egg production facility. *Poultry Science*, **74**, 753–757.
- Jones, D.R., Northcutt, J.K., Musgrove, M.T., Curtis, P.A., Anderson, K.E., Fletcher, D.L., and Cox, N.A. 2003. Survey of shell egg processing plant sanitation programs: effects on egg contact surfaces. *Journal of Food Protection*, **66**, 1486–1489.
- Jones, D.R., Musgrove, M.T., and Northcutt, J.K. 2004. Variations in external and internal microbial populations in shell eggs during extended storage. *Journal of Food Protection*, **67**, 2657–2660.
- Jones, D.R., Musgrove, M.T., Caudill, A.B., and Curtis, P.A. 2006. Frequency of *Salmonella*, *Campylobacter*, *Listeria*, and *Enterobacteriaceae* detection in commercially cool water-washed shell eggs. *Journal of Food Safety*, **26**, 264–274.
- Jones, D.R., Musgrove, M.T., Anderson, K.E., and Thesmar, H.S. 2010. Physical quality and composition of retail shell eggs. *Poultry Science*, **89**, 582–587.
- Jones, D.R., Anderson, K.E., and Musgrove, M.T. 2011. Comparison of environmental and egg microbiology associated with conventional and free-range laying hen management. *Poultry Science*, **90**, 2063–2068.
- Keklik, N.M., Demirci, A., Patterson, P.H., and Puri, V.M. 2010. Pulsed UV light inactivation of *Salmonella* Enteritidis on eggshells and its effects on egg quality. *Journal of Food Protection*, **73**, 1408–1415.
- Kim, J.W. and Slavik, M.F. 1996. Changes in eggshell surface microstructure after washing with cetylpyridinium chloride or trisodium phosphate. *Journal of Food Protection*, **59**, 859–863.
- Kim, W.K., Bloomfield, S.A., Sugiyama, T., and Ricke, S.C. 2012. Concepts and methods for understanding bone metabolism in laying hens. *World's Poultry Science Journal*, **68**, 71–82.
- Kinner, J.A., and Moats, W.A. 1981. Effect of temperature, pH, and detergent on survival of bacteria associated with shell eggs. *Poultry Science*, **60**, 761–767.
- Klippen, K. 1990. Egg production and processing. *Dairy Food and Environmental Sanitation*, **10**, 266–267.
- Knappe, K.D., Carey, J.B., Burgess, R.P., Kwon, Y.M., and Ricke, S.C. 1999. Comparison of chlorine with an iodine based compound on eggshell surface microbial populations in a commercial egg washer. *Journal of Food Safety*, **19**, 185–194.
- Knappe, K.D., Carey, J.B., and Ricke, S.C. 2001. Response of foodborne *Salmonella* spp. marker strains inoculated on egg shell surfaces to disinfectants in a commercial egg washer. *Journal of Environmental Science Health, Part B – Pesticides, Food Contaminants and Agricultural Wastes*, **36**, 219–227.
- Knappe, K.D., Chavez, C., Burgess, R.P., Coufal, C.D., and Carey, J.B. 2002. Comparison of eggshell surface microbial populations for in-line and off-line commercial egg processing facilities. *Poultry Science*, **81**, 695–698.
- Koelkebeck, K.W., Bell, D.D., Carey, J.B., Anderson, K.E., and Darre, M.J. 2001. Egg marketing in national supermarkets: products, packaging, and prices – Part 3. *Poultry Science*, **80**, 396–400.
- Koelkebeck, K.W., Patterson, P.H., Anderson, K.E., Darre, M.J., et al. 2008. Temperature sequence of eggs from oviposition through distribution: processing – Part 2. *Poultry Science*, **87**, 1187–1194.

- Koo, O.K., Sirsat, S.A., Crandall, P.G., and Ricke, S.C. 2012. Physical and chemical control of *Salmonella* in ready-to-eat products. *Agriculture, Food, and Analytical Bacteriology*, **2**, 56–68.
- Kuenzel, W.J. 2003. Neurobiology of molt in avian species. *Poultry Science*, **82**, 981–991.
- Kuney, D.R., Bokahari, S., Bell, D., and Zeidler, G. 1995. Labor costs and the packaging of table eggs 1962–1991. *Journal of Applied Poultry Research*, **4**, 94–99.
- Kuo, F.L., Carey, J.B., and Ricke, S.C. 1997a. UV irradiation of shell eggs: effect on populations of aerobes, molds, and inoculated *Salmonella typhimurium*. *Journal of Food Protection*, **60**, 639–643.
- Kuo, F.L., Kwon, Y.M., Carey, J.B., Hargis, B.M., Krieg, D.P., and Ricke, S.C. 1997b. Reduction of *Salmonella* contamination on chicken egg shells by a peroxidase-catalyzed sanitizer. *Journal of Food Science*, **62**, 873–874, 884.
- Kuo, F.-L., Ricke, S.C., and Carey, J.B. 1997c. Shell egg sanitation – UV radiation and egg to effectively reduce populations of aerobes, yeasts, and molds. *Journal of Food Protection*, **60**, 694–697.
- Laird, J.M., Bartlett, F.M., and McKellar, R.C. 1991. Survival of *Listeria monocytogenes* in eggwash water. *International Journal of Food Microbiology*, **12**, 115–122.
- Lapuz, R., Tani, H., Sasai, K., Shiota, K., Katoh, H., and Baba, E. 2008. The role of roof rats (*Rattus rattus*) in the spread of *Salmonella* Enteritidis and *S. Infantis* contamination in layer farms in eastern Japan. *Epidemiology and Infection*, **136**, 1235–1243.
- La Ragione, R.M. and Woodward, M.J. 2003. Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype Enteritidis and *Clostridium perfringens* in young chickens. *Veterinary Microbiology*, **94**, 245–256.
- Lederer, B.E. 1983. Egg carton field tests. *Poultry Science*, **62**, 990–999.
- Leeson, S. and Marcotte, M. 1993. Irradiation of poultry feed I. Microbial status and bird response. *World's Poultry Science Journal*, **49**, 19–33.
- Leinonen, I., Williams, A.G., Wiseman, J., Guy, J., and Kyriazakis, I. 2012. Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: egg production systems. *Poultry Science*, **91**, 26–40.
- Maciorowski, K.G., Pillai, S.D., and Ricke, S.C. 2000. Efficacy of a commercial polymerase chain reaction-based assay for detection of *Salmonella* spp. in animal feeds. *Journal of Applied Microbiology*, **89**, 710–718.
- Maciorowski, K.G., Jones, F.T., Pillai, S.D., and Ricke, S.C. 2004. Incidence and control of foodborne *Salmonella* spp. in poultry feeds – a review. *World's Poultry Science Journal*, **60**, 446–457.
- Maciorowski, K.G., Pillai, S.D., Jones, F.T., and Ricke, S.C. 2005. Polymerase chain reaction detection of foodborne *Salmonella* spp. in animal feeds. *Critical Reviews of Microbiology*, **31**, 45–53.
- Maciorowski, K.G., Herrera, P., Jones, F.T., Pillai, S.D., and Ricke, S.C. 2006a. Cultural and immunological detection methods for *Salmonella* spp. in animal feeds – a review. *Veterinary Research Communications*, **30**, 127–137.
- Maciorowski, K.G., Herrera, P., Kunderling, M.M., and Ricke, S.C. 2006b. Animal production and contamination by foodborne *Salmonella*. *Journal of Consumer Protection and Food Safety*, **1**, 197–209.
- Maciorowski, K.G., Herrera, P., Jones, F.T., Pillai, S.D., and Ricke, S.C. 2007. Effects of poultry and livestock feed with bacteria and fungi. *Animal Feed Science and Technology*, **133**, 109–136.
- Martelli, F. and Davies, R.H. 2012. *Salmonella* serovars isolated from table eggs: an overview. *Food Research International*, **45**, 745–754.
- McKee, S.R., Kwon, Y.M., Carey, J.B., Sams, A.R., and Ricke, S.C. 1998. Comparison of a peroxidase-catalyzed sanitizer with other egg sanitizers using a laboratory-scale sprayer. *Journal of Food Safety*, **18**, 173–183.
- McLeod, A., Thieme, O., and Mack, S.D. 2009. Structural changes in the poultry sector: will there be smallholder poultry development in 2030? *World's Poultry Science Journal*, **65**, 191–200.
- McReynolds, J.L., Moore, R.W., Kubena, L.F., Byrd, J.A., Woodward, C.L., Nisbet, D.J., and Ricke, S.C. 2006. Effect of various combinations of

- alfalfa and standard layer diet on susceptibility of laying hens to *Salmonella* Enteritidis during forced molt. *Poultry Science*, **85**, 1123–1128.
- Mellor, D.B. and Gardner, F.A. 1970. The effect of egg cartons on interior quality and breakage of shell eggs. *Poultry Science*, **49**, 793–798.
- Mellor, D.B. and Gardner, F.A. 1975. An evaluation of interior quality of shell eggs packed in the carton while still warm. *Poultry Science*, **54**, 461–465.
- Mellor, D.B., Gardner, F.A., and Campos, E.J. 1975. Effect of type of package and storage temperature on interior quality of shell treated shell eggs. *Poultry Science*, **54**, 742–746.
- Mench, J.A., Sumner, D.A., and Rosen-Molina, J.T. 2011. Sustainability of egg production in the United States – the policy and market context. *Poultry Science*, **90**, 229–240.
- Messens, W. 2011. Egg decontamination by washing, Chapter 9. In: *Improving the Safety and Quality of Eggs and Egg Products*, Vol. 2: *Egg Safety and Nutritional Quality* (eds Van Immerseel, F., Nys, Y., and Bain, M.), pp. 163–180. Woodhead Publishing Limited, Oxford, UK.
- Michella, S.M. and Slaugh, B.T. 2000. Producing and marketing a specialty egg. *Poultry Science*, **79**, 975–976.
- Milillo, S.R., Friedly, E.C., Saldivar, J.C., Muthaiyan, A., O'Bryan, C.A., Crandall, P.G., Johnson, M.G., and Ricke, S.C. 2012a. A review of the ecology, genomics and stress response of *Listeria innocua* and *Listeria monocytogenes*. *Critical Reviews in Food Science and Nutrition*, **52**, 712–725.
- Milillo, S.R., Stout, J.C., Hanning, I.B., Clement, A., Fortes, E.D., den Bakker, H.C., Wiedemann, M., and Ricke, S.C. 2012b. Characteristics of *Listeria* from pasture-reared poultry reveals *L. monocytogenes* and hemolytic *L. innocua* in poultry ceca and the pasture environment. *Poultry Science*, **91**, 2158–2163.
- Moats, W.A. 1978. Egg washing – a review. *Journal of Food Protection*, **41**, 919–925.
- Moore, R.W., Park, S.Y., Kubena, L.F., Byrd, J.A., et al. 2004. Comparison of zinc acetate and propionate addition on gastrointestinal tract fermentation and susceptibility of laying hens to *Salmonella* Enteritidis during forced molt. *Poultry Science*, **83**, 1276–1286.
- Murase, T., Senjyu, K., Maeda, T., Tanaka, M., et al. 2001. Monitoring of chicken houses and an attached egg-processing facility in a laying farm for *Salmonella* contamination between 1994 and 1998. *Journal of Food Protection*, **64**, 1912–1916.
- Musgrove, M.T. 2011. Microbiology and safety of table eggs, Chapter 1. In: *Improving the Safety and Quality of Eggs and Egg Products*, Vol. 2: *Egg Safety and Nutritional Quality* (eds Van Immerseel, F., Nys, Y., and Bain, M.), pp. 3–33. Woodhead Publishing Limited, Oxford, UK.
- Musgrove, M.T., Jones, D.R., Northcutt, J.K., Curtis, P.A., Anderson, K.E., Fletcher, D.L., and Cox, N.A. 2004. Survey of shell egg processing plant sanitation programs: effects on non-egg-contact surfaces. *Journal of Food Protection*, **67**, 2801–2804.
- Musgrove, M.T., Jones, D.R., Northcutt, J.K., Harrison, M.A., and Cox, N.A. 2005. Impact of commercial processing on the microbiology of shell eggs. *Journal of Food Protection*, **68**, 2367–2375.
- Musgrove, M.T., Northcutt, J.K., Jones, D.R., Cox, N.A., and Harrison, M.A. 2008. Enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. *Poultry Science*, **87**, 1211–1218.
- Musgrove, M.T., Jones, D.R., Shaw, J.D., Sheppard, M., and Harrison, M.A. 2009. Enterobacteriaceae and related organisms isolated from nest run cart shelves in commercial shell egg processing facilities. *Poultry Science*, **88**, 2113–2117.
- Musgrove, M.T., Shaw, J.D., and Harrison, M.A. 2012. *Salmonella* collected from nest run cart shelves in commercial shell egg processing facilities. *Poultry Science*, **91**, 2386–2389.
- Nethercote, C.H., Boisvenu, C.N., and Fletcher, D.A. 1974. Egg carton tests. *Poultry Science*, **53**, 311–325.
- Nisbet, D.J. 2002. Defined competitive exclusion cultures in the prevention of enteropathogen colonization in poultry and swine. *Antonie van Leeuwenhoek*, **81**, 481–486.

- Nisbet, D.J., Corrier, D.E., Scanlan, C.M., Hollister, A.G., Beier, R.C., and DeLoach, J.R. 1993. Effect of a defined continuous-flow derived bacterial culture and dietary lactose on *Salmonella typhimurium* colonization in broiler chickens. *Avian Diseases*, **37**, 1017–1025.
- Nisbet, D.J., Ricke, S.C., Scanlan, C.M., Corrier, D.E., Hollister, A.G., and DeLoach, J.R. 1994. Inoculation of broiler chicks with a continuous-flow derived bacterial culture facilitates early native cecal bacterial colonization and increases resistance to *Salmonella typhimurium*. *Journal of Food Protection*, **57**, 12–15.
- Nisbet, D.J., Corrier, D.E., Ricke, S.C., Hume, M.E., Byrd II, J.A., and DeLoach, J.R. 1996a. Cecal propionic acid as a biological indicator of the early establishment of a microbial ecosystem inhibitory to *Salmonella* in chicks. *Anaerobe*, **2**, 345–350.
- Nisbet, D.J., Corrier, D.E., Ricke, S.C., Hume, M.E., Byrd II, J.A., and DeLoach, J.R. 1996b. Maintenance of the biological efficacy in chicks of a cecal competitive-exclusion culture against *Salmonella* by continuous-flow fermentation. *Journal of Food Protection*, **59**, 1279–1283.
- North, M.O. and Bell, D.D. 1990. *Commercial Chicken Production Manual*, 4th edn. Chapman & Hall, New York, USA.
- Northcutt, J.K., Musgrove, M.T., and Jones, D.R. 2005. Chemical analyses of commercial shell egg wash water. *Journal of Applied Poultry Research*, **14**, 289–295.
- Olsen, A.R. and Hammack, T.S. 2000. Isolation of *Salmonella* spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aeneascens* (Wiedemann) (Diptera: Muscidae), at caged-layer houses. *Journal of Food Protection*, **63**, 958–960.
- Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F., Angulo, F.J., and Griffin, P.M. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases*, **19**, 407–415.
- Park, S.Y., Birkhold, S.G., Kubena, L.F., Nisbet, D.J., and Ricke, S.C. 2004a. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. *Biological Trace Element Research*, **101**, 147–163.
- Park, S.Y., Birkhold, S.G., Kubena, L.F., Nisbet, D.J., and Ricke, S.C. 2004b. Effects of high zinc diets using zinc propionate on molt induction, organs, and postmolt egg production and quality in laying hens. *Poultry Science*, **83**, 24–33.
- Park, S.Y., Kim, W.K., Birkhold, S.G., Kubena, L.F., Nisbet, D.J., and Ricke, S.C. 2004c. Induced moulting issues and alternative dietary strategies for the egg industry in the United States. *World's Poultry Science Journal*, **60**, 196–209.
- Park, C.-M., Hung, Y.-C., Lin, C.-S., and Brackett, R.E. 2005. Efficacy of electrolyzed water in inactivating *Salmonella enteritidis* and *Listeria monocytogenes* on shell eggs. *Journal of Food Protection*, **68**, 986–990.
- Park, S.Y., Woodward, C.L., Kubena, L.F., Nisbet, D.J., Birkhold, S.G., and Ricke, S.C. 2008. Environmental dissemination of foodborne *Salmonella* in preharvest poultry production: reservoirs, critical factors and research strategies. *Critical Reviews on Environmental Science and Technology*, **38**, 73–111.
- Park, S.H., Jarquin, R., Hanning, I., Almeida, G., and Ricke, S.C. 2011. Detection of *Salmonella* spp. survival and virulence in poultry feed by targeting the *hlyA* gene. *Journal of Applied Microbiology*, **111**, 426–432.
- Park, S.H., Aydin, M., Khatiwara, A., Dolan, M.C., Gilmore, D.F., Bouldin, J.L., Ahn, S., and Ricke, S.C. 2014. Application of current and emerging rapid detection methodologies for identification of *Salmonella* spp. in poultry and poultry products. *Food Microbiology*, **38**, 250–262.
- Parkhurst, C.R. and Mountney, G.J. 1988. *Poultry and Meat Production*. Chapman & Hall, New York, USA.
- Patrick, M.E., Adcock, P.M., Gomez, T.M., Altekruze, S.F., Holland, B.H., and Tauxe, R.V. 2004. *Salmonella* Enteritidis infections, United States, 1985–1999. *Emerging Infectious Diseases*, **10**, 1–7.
- Patterson, J.A. and Burkholder, K.M. 2003. Application of prebiotics and probiotics in poultry production. *Poultry Science*, **82**, 627–631.

- Patterson, P.H., Koelkebeck, K.W., Bell, D.D., Carey, J.B., Anderson, K.E., and Darre, M.J. 2001. Egg marketing in national supermarkets: Specialty eggs – Part 2. *Poultry Science*, **80**, 390–395.
- Patterson, P.H., Koelkebeck, K.W., Anderson, K.E., Darre, M.J., *et al.* 2008. Temperature sequence of eggs from oviposition through distribution: production – Part 1. *Poultry Science*, **87**, 1182–1186.
- Pelletier, N., Ibarburu, M., and Xin, H. 2014. Comparison of the environmental footprint of the egg industry in the United States in 1960 and 2010. *Poultry Science*, **93**, 241–255.
- Ragni, L., Berardinelli, A., Vannini, L., Montanari, C., Sirri, F., Guerzoni, M.E., and Guarnieri, A. 2010. Non-thermal atmospheric gas plasma device for surface decontamination of shell eggs. *Journal Food Engineering*, **100**, 125–132.
- Revolledo, L. and Ferreira, A.J.P. 2012. Current perspectives in avian salmonellosis: vaccines and immune mechanisms of protection. *Journal of Applied Poultry Research*, **21**, 418–431.
- Revolledo, L., Ferreira, A.J.P., and Mead, G.C. 2006. Prospects in *Salmonella* control: competitive exclusion, probiotics, and enhancement of avian intestinal immunity. *Journal of Applied Poultry Research*, **15**, 341–351.
- Ricke, S.C. 2003a. The gastrointestinal tract ecology of *Salmonella* Enteritidis colonization in molting hens. *Poultry Science*, **82**, 1003–1007.
- Ricke, S.C. 2003b. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science*, **82**, 632–639.
- Ricke, S.C. 2005. Ensuring the safety of poultry feed, Chapter 7. In: *Food Safety Control in the Poultry Industry* (ed. Mead, G.C.), pp. 174–194. Woodhead Publishing, Cambridge, UK.
- Ricke, S.C. and Pillai, S.D. 1999. Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. *Critical Reviews of Microbiology*, **25**, 19–38.
- Ricke, S.C., Pillai, S.D., Norton, R.A., Maciorowski, K.G., and Jones, F.T. 1998. Applicability of rapid methods for detection of *Salmonella* spp. in poultry feeds: a review. *Journal of Rapid Methods in Automatic Microbiology*, **6**, 239–258.
- Ricke, S.C., Dunkley, C.S., McReynolds, J.L., Dunkley, K.D., and Nisbet, D.J. 2010. Molting in laying hens and *Salmonella* infection. In: *Dynamics in Animal Nutrition* (eds van der Aar, P.J. and J. Doppenberg, J.), pp. 135–146. Wageningen Academic Publishers, Wageningen, The Netherlands.
- Ricke, S.C., Hererra, P., and Biswas, D. 2012a. Bacteriophages for potential food safety applications in organic meat production, Chapter 23. In: *Organic Meat Production and Processing* (eds Ricke, S.C., Van Loo, E.J., Johnson, M.G., and C.A. O'Bryan, C.A.), pp. 407–424. Wiley Scientific/IFT, New York, USA.
- Ricke, S.C., Van Loo, E.J., Johnson, M.G., and O'Bryan, C.A. (eds) 2012b. *Organic Meat Production and Processing*, 444 pp. Wiley Scientific/IFT, New York, USA.
- Ricke, S.C., Dunkley, C.S., and Durant, J.A. 2013a. A review on development of novel strategies for controlling *Salmonella* Enteritidis colonization in laying hens: fiber-based molt diets. *Poultry Science*, **92**, 502–525.
- Ricke, S.C. Jones, D.R., and Gast, R.K. 2013b. Eggs and egg products, Chapter 46. In: *Compendium of Methods for the Microbiological Examinations of Foods* (eds Doores, S., Salfinger, Y., and Tortorello, M.L.), 5th edn, pp. 1–11. American Public Health Association, Washington, DC, USA.
- Ricke, S.C., Khatiwara, A., and Kwon, Y.M. 2013c. Application of microarray analysis of foodborne *Salmonella* in poultry production: a review. *Poultry Science*, **92**, 2243–2250.
- Ricke, S.C., Koo, O.-K., Foley, S., and Nayak, R. 2013d. *Salmonella*, Chapter 7. In: *Guide to Foodborne Pathogens* (eds Labbé, R. and García, S.), 2nd edn, pp. 112–137. Wiley-Blackwell, Oxford, UK.
- Robeson, J., Valencia, M., Retamales, J., and Borie, C. 2011. Stability inside hen eggs of a *Salmonella enterica* serovar Enteritidis bacteriophage. *Electronic Journal of Biotechnology*, **14**, 1–4.
- Rodriguez-Romo, L.A. and Yousef, A.E. 2005. Inactivation of *Salmonella enterica* serovar Enteritidis on shell eggs by ozone and UV radiation. *Journal of Food Protection*, **68**, 711–717.
- Rossi, M., Nys, Y., Anton, M., Bain, M., De Ketelaere, B., *et al.* 2013. Developments in

- understanding and assessment of egg and egg product quality over the last century. *World's Poultry Science Journal*, **69**, 414–429.
- Russell, S.M. 2003. The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs. *Poultry Science*, **82**, 158–162.
- Ryan, L.L., Carey, J.B., and Birkhold, S.G. 1996. Economic impact of the broiler and egg industries in Texas. *Journal of Applied Poultry Research*, **8**, 447–451.
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., and Griffen, P.M. 2011. Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases*, **17**, 7–15.
- Schroeder, C.M., Latimer, H.J., Schlosser, W.D., Golden, N.J., Marks, H.M., Coleman, M.E., Hogue, A.T., Ebel, E.D., Quiring, N.M., Kadry, A.-R.M., and Kauss, J. 2006. Overview and summary of the food safety and inspection service risk assessment for *Salmonella* Enteritidis in shell eggs, 2005. *Foodborne Pathogens and Disease*, **3**, 403–412.
- Seo, K.H., Holt, P.S., and Gast, R.K. 2001. Comparison of *Salmonella* Enteritidis infection in hens molted via long-term feed withdrawal versus full-fed wheat middling. *Journal of Food Protection*, **64**, 1917–1921.
- Seydim, A.C. and Dawson, P.L. 1999. Packaging effects on shell egg breakage rates during simulated transportation. *Poultry Science*, **78**, 148–151.
- Shapira, N. 2010. Every egg may have a targeted purpose: toward a differential approach to egg according to composition and functional effect. *World's Poultry Science Journal*, **66**, 271–284.
- Singh, S., Yadav, A.S., Singh, S.M., and Bharti, P. 2010. Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Research International*, **43**, 2027–2030.
- Siragusa, G.R. and Ricke, S.C. 2012. Probiotics as pathogen control agents for organic meat production, Chapter 20. In: *Organic Meat Production and Processing* (eds Ricke, S.C., Van Loo, E.J., Johnson, M.G., and O'Bryan, C.A.), pp. 331–349. Wiley Scientific/IFT, New York, USA.
- Soria, M.C., Soria, M.A., Bueno, D.J., and Colazo, J.L. 2011. A comparative study of culture methods and polymerase chain reaction assay for *Salmonella* detection in poultry feed. *Poultry Science*, **90**, 2606–2618.
- Spricigo, D.A., Bardina, C., Cortés, P., and Llagostera, M. 2013. Use of a bacteriophage cocktail to control *Salmonella* in food and the food industry. *International Journal of Food Microbiology*, **165**, 169–174.
- St Louis, M.E., Morse, D.L., Potter, M.E., DeMelfi, T.M., Guzewich, J.J., Tauxe, R.V., and Blake, P.A. 1988. The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections – new implications for the control of salmonellosis. *Journal of American Medical Association*, **259**, 2103–2107.
- Stocki, S.L., Annett, C.B., Sibley, C.D., McLaws, M., Checkley, S.L., Singh, N., Surette, M.G., and A.P. White 2007. Persistence of *Salmonella* on egg conveyor belts dependent on the belt type but not on the rdar morphotype. *Poultry Science*, **86**, 2375–2383.
- Suppakul, P., Miltz, J., Sonneveld, K., and Bigger, S.W. 2003. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*, **68**, 408–420.
- Suresh, T., Hatha, A.A.M., Sreenivasan, D., Sangeetha, N., and Lashmanaperumalsamy, P. 2006. Prevalence and antimicrobial resistance of *Salmonella* enteritidis and other salmonellas in the eggs and egg-storing trays from retail markets of Coimbatore, South India. *Food Microbiology*, **23**, 294–299.
- Suslow, T.V., Oria, M.P., Beuchat, L.R., Garrett, E.H., Parish, M.E., Harris, L.J., Farber, J.N., and Busta, F.F. 2003. Production practices as risk factors in microbial food safety of fresh and fresh-cut produce. *Comparative Reviews in Food Science and Food Safety*, **2** (Suppl.), 38–77.
- Taylor, R.C., Omed, H., and Edwards-Jones, G. 2014. The greenhouse emissions footprint of free-range eggs. *Poultry Science*, **93**, 231–237.
- Thompson, J.E., Knutson, J., Ernst, R.A., Kuney, D., Riemann, H., Himathongkham, S., and Zeidler, G. 2000. Rapid cooling of shell eggs. *Journal of Applied Poultry Research*, **9**, 258–268.

- Todd, E.C.D. 1996) Risk assessment of use of cracked eggs in Canada. *International Journal of Food Microbiology*, **30**, 125–143.
- Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Baskaran, S.A., Mooyottu, S., Darre, M.J., and Venkitanarayanan, K. 2013. Rapid inactivation of *Salmonella* Enteritidis on shell eggs by plant-derived antimicrobials. *Poultry Science*, **92**, 3228–3235.
- Utrarachkij, F., Pornraungwong, S., Siripanichgon, K., Nakajima, C., Suzuki, Y., and Suthienkul, O. 2012. Possible horizontal transmission of *Salmonella* via reusable egg trays in Thailand. *International Journal of Food Microbiology*, **154**, 73–78.
- Van der Sluis, W. 2007. More organic poultry and eggs in the U.S. *World Poultry*, **23**, 26–28.
- Vilà, B., Fontgibell, A., Badiola, I., Esteve-Garcia, E., Jiménez, G., Castillo, M., and Brufau, J. 2009. Reduction of *Salmonella enterica* var. Enteritidis colonization and invasion by *Bacillus cereus* var. *toyoi* inclusion in poultry feeds. *Poultry Science*, **88**, 975–979.
- Wales, A.D., Allen, V.M., and Davies, R.H. 2010. Chemical treatment of animal feed and water for the control of *Salmonella*. *Foodborne Pathogens and Diseases*, **7**, 3–15.
- Wang, H. and Slavik, M.F. 1998. Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times. *Journal of Food Protection*, **61**, 276–279.
- Whiting, R.C. and Buchanan, R.L. 1997. Development of a risk assessment model for *Salmonella enteritidis* in pasteurized liquid shell eggs. *International Journal of Food Microbiology*, **36**, 111–125.
- Windhorst, H.-W. 2006. Changes in poultry production and trade worldwide. *World's Poultry Science Journal*, **62**, 585–562.
- Windhorst, H.-W. 2007. Bio-energy production – a threat to the global egg industry? *World's Poultry Science Journal*, **63**, 365–379.
- Windhorst, H.-W. 2008a. Asia's changing role in the global egg industry – an analysis of past, present and foreseeable future dynamics. *World's Poultry Science Journal*, **64**, 533–552.
- Windhorst, H.-W. 2008b. A projection of the regional development of egg production until 2015. *World's Poultry Science Journal*, **64**, 356–376.
- Windhorst, H.-W. 2009. Recent patterns of egg production and trade: a status report on a regional basis. *World's Poultry Science Journal*, **65**, 685–708.
- Woodward, C.L., Kwon, Y.M., Kubena, L.F., Byrd, J.A., Moore, R.W., Nisbet, D.J., and Ricke, S.C. 2005. Reduction of *Salmonella enterica* serovar Enteritidis colonization and invasion by an alfalfa diet during molt in leghorn hens. *Poultry Science*, **84**, 185–193.
- Xin, H., Gates, R.S., Green, A.R., Mitloehner, F.M., Moore Jr, P.A., and Wathes, C.M. 2011. Environmental impacts and sustainability of egg production systems. *Poultry Science*, **90**, 263–277.
- Yousaf, M. and Chaudhry, A.S. 2008. History, changing scenarios and future strategies to induce moulting in laying hens. *World's Poultry Science Journal*, **64**, 65–75.

CHAPTER 22

Microbial ecology of beef carcasses and beef products

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22.1 Introduction

The muscle tissues of carcasses from healthy animals are sterile until meat surfaces are exposed during dressing operations. The bacteria transferred from the hide to the meat are largely non-pathogenic spoilage bacteria. However, small numbers of enteric pathogenic bacteria may also be present. Verotoxigenic *Escherichia coli* (VTEC), particularly of the serotype O157:H7, has attracted much attention since the report of the first outbreak of food-borne illness associated with the organism in 1982, attributed to consumption of contaminated hamburgers (Riley *et al.*, 1983). *E. coli* O157:H7 infection can lead to hemolytic uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and acute renal failure (Banatvala *et al.*, 2001). During 1992 and 1994, a multistate outbreak of *E. coli* O157:H7 infection associated with consumption of undercooked hamburgers from a fast food restaurant chain caused more than 500 illnesses and 4 deaths (CDC, 1993). These events led the US Department of Agriculture/Food Safety and Inspection Service (USDA/FSIS) to declare the organism *E. coli* O157:H7 an adulterant in ground beef in 1994 and to promulgate regulations requiring the establishment of hazard analysis critical control point (HACCP) systems at all federally inspected meat packing plants in 1996 (USDA, 1996). Thereafter, HACCP programs were mandated in meat packing plants in

Australia, the European Union, Canada, and major trading partners of the United States (Fearne *et al.*, 2004). Beef packing facilities in the US and Canada have also implemented various decontamination interventions for carcasses as part of their HACCP plan (Figure 22.1). The implementation of HACCP has resulted in significant improvement in the microbiological condition of beef irrespective of whether decontamination is used (Khatri and Collins, 2007; Wilhelm *et al.*, 2011; Yang *et al.*, 2012). Thus, in this chapter, the effects on the microbial flora of beef of the beef production process as well as of the antimicrobial interventions currently in use in commercial beef packing plants will be discussed.

22.2 Carcass production process

The production of beef at slaughtering plants involves four major stages: the slaughtering of animals, carcass dressing, carcass chilling, and carcass breaking (Romans *et al.*, 2000). In most plants, the processes are performed in that order. However, in some plants carcasses are deboned before they are cooled to chiller temperatures, i.e., hot boning, and in such cases the carcass breaking is performed before completion of the cooling process (Gill, 2005).

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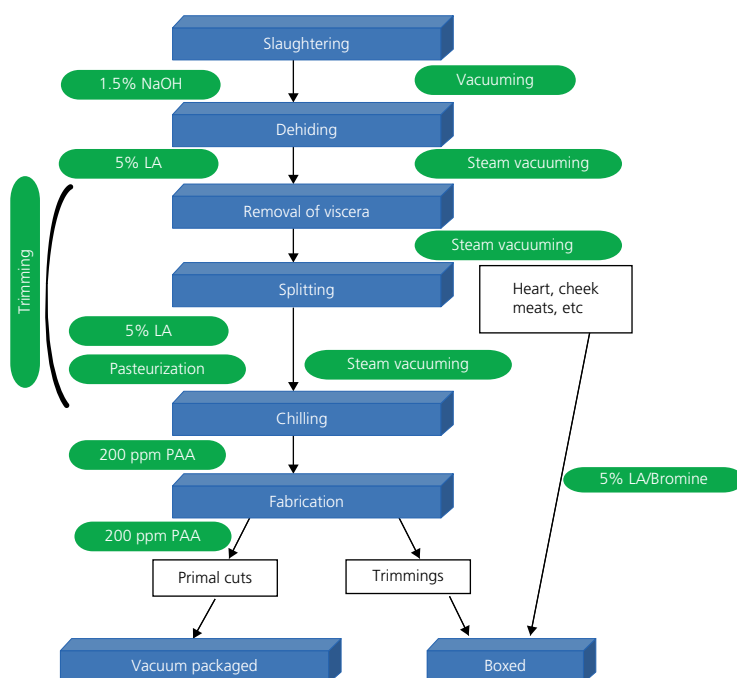


Figure 22.1 Major stages of the beef production process and antimicrobial interventions implemented at some large beef packing plants. LA, lactic acid; PAA, peroxyacetic acid.

22.2.1 Slaughtering

Slaughtering of cattle usually consists of stunning and sticking and bleeding of the animal (Gill, 2005). In some plants, electrical stimulation of the carcasses is performed to accelerate the development of rigor, and thus avoid toughening of meat during chilling. The carcass can be contaminated from the stunning or sticking wounds. However, a study of Mackey and Derrick (1979) demonstrated that bacteria could be recovered from muscle only when instruments like pithing rods were artificially contaminated with large numbers of bacteria. Thus, contamination of meat with bacteria during slaughtering is unusual and the muscle tissues of animals remain sterile until meat surfaces are contaminated during carcass dressing. Even so, damage to the brain during slaughtering should be minimized to prevent contamination of the muscle tissues with prions.

22.2.2 Carcass dressing process

22.2.2.1 Skinning operations

Skinning/dehiding of beef carcasses is often performed with the carcass being hung from a rail by a shackle attached to one hind leg (Gill *et al.*, 1998). The hide is often opened in the crotch areas of the hindquarters of the carcass and gradually freed by manually skinning and/or automatic hide pullers. Contamination of meat along the pattern opening lines is unavoidable as the cuts are made from the outer to the inner surfaces, but can be minimized by reducing the number and extents of opening cuts, particularly in heavily contaminated areas of the hide. After the opening cuts, contamination can be controlled by inserting the knife under the hide and cutting from the inside to the outside. The anus is often freed after skinning of the hindquarters and before the skinning of the carcass is completed, by cutting around the anus. A length of the rectum is

pulled from the body cavity and the anus is enclosed in a plastic bag and then pushed back into the body cavity. Hooves are removed during the skinning process. During skinning, bacteria can be transferred to the meat from the hide through various routes: by direct contact between the outer surfaces of the hide and the meat if part of the hide that has been freed rolls or folds inwards at a cut edge; from blades of knives when they cut through the hide during the opening cuts; from equipment, clothes, or hands of workers, if they contact both the hide and the meat; or from particles or water droplets shaken from the hide during skinning operations (Gill, 2005). Thus, the hygienic condition of skinned carcasses is largely dictated by the microbiological condition of the hides and the skinning operations.

Various studies have investigated the microbiological condition of hides, by enumeration of three groups of bacteria: aerobes, enteric bacteria (*Enterobacteriaceae* or coliforms are used), and *E. coli* (an indicator of faecal contamination). Aerobes on hides have been reported at numbers between 10^4 and 10^{10} cfu/cm² in various studies from several European countries, the US, and Canada (Antic *et al.*, 2010; Bacon *et al.*, 2000; Blagojevic *et al.*, 2011, 2012; Serraino *et al.*, 2012; Yang *et al.*, 2015; Zweifel *et al.*, 2014). The numbers of *Enterobacteriaceae* on hides in the European studies were mostly between 3 and 5 log units less than the corresponding numbers of aerobes, whereas the difference between the numbers of coliforms, a subgroup of *Enterobacteriaceae*, and aerobes on hides in the North American studies was mostly less than 2 log units. The coliforms recovered from the latter cattle were often mostly *E. coli*. The microbiological condition of cattle hides can be affected by many factors, including climate, geographic location, and methods of transportation (Sofos, 1994). The different ratios of enteric bacteria to aerobes between the European and the North American studies are likely a result from the difference in animal husbandry practices in those areas. In the three North American studies, the beef cattle were

from feedlots and such cattle frequently carry variable amounts of tag, i.e., manure, bedding, and soil (Van Donkersgoed *et al.*, 1997). In contrast, pasture raised cattle in other studies likely carried bacteria mostly of soil origin (Sofos, 1994). The higher ratio of enteric bacteria to aerobes on the hides of cattle from feedlots is then expected.

It has been widely assumed that “visually dirtier hides” will generate “dirtier carcasses” (Bell, 1997). However, reported relationships between the microbiological condition of the skinned carcass and the degree of hide cleanliness are not wholly consistent, from no correlation (Antic *et al.*, 2010; Van Donkersgoed *et al.*, 1997) to somewhat correlated (Blagojevic *et al.*, 2012; McEvoy *et al.*, 2000) or positively correlated (Serraino *et al.*, 2012). Hauge and co-workers (2012) investigated the hygienic condition of skinned carcasses derived from cattle of three hide cleanliness categories: category 0: visually clean hide with minor quantities of faecal material or mud adhering; category 1: moderately dirty; category 2: very dirty. The category 2 cattle were skinned and trimmed more carefully. The findings of that study were that the category 2 cattle yielded carcasses with fewer aerobes than did the category 1 cattle. These findings suggest that skinning operations have a greater impact on the microbiological condition of the skinned carcasses than the condition of the hides if the process is well controlled. It has been suggested that the ratio between carcass and hide microflora be used as an indicator for abattoir process hygiene (Blagojevic *et al.*, 2011).

The microbiological condition of carcasses after hide removal has been examined in a number of studies from Canada, Norway, and Ireland (Gill and Landers, 2003; Hauge *et al.*, 2012; Kennedy *et al.*, 2014). In those studies, hides were not treated with any antimicrobial interventions. Kennedy and co-workers assessed an upward and downward dehiding technique in a commercial beef processing plant in Ireland and found that both dehiding processes yielded skinned carcasses carrying aerobes at 1.2 log cfu/cm².

In addition to aerobes, the other two studies also enumerated coliforms and *E. coli* (Gill and Landers, 2003) or *E. coli* (Hauge *et al.*, 2012). The study of Gill and Landers in which the dressing process at four beef packing plants was assessed reported that the aerobes on skinned carcasses were 3.1, 1.6, 1.9, and 2.9 log cfu/cm², respectively, and the corresponding numbers of coliforms, which were mostly *E. coli*, were 1.9, 1.0, 2.6, and 2.8/100 cm², respectively. In the study of Hauge and co-workers, the numbers of aerobes and *E. coli* on skinned carcasses were between 4.1 and 5.0 log cfu/cm² and 1.0 and 1.7 log cfu/100 cm², respectively. These various studies show that the microflora on skinned carcasses varies largely, as a result of skinning operations.

22.2.2.2 Evisceration operations

After carcasses have been skinned, the head is usually removed and during the process of head removal, the oesophagus is often clipped to block the flow of ingesta from the paunch (Gill, 2005). The viscera are removed after the sternum is split. The exact order of evisceration operations at different beef plants may differ considerably (Gill *et al.*, 1996; McEvoy *et al.*, 2004). After the viscera are removed, carcasses are split through the backbone into carcass sides and the spinal cord is completely removed, due to concerns about bovine spongiform encephalopathy, as the causative agent for that can also affect humans (Bolton *et al.*, 2001). The meat can be contaminated with enteric bacteria if the evisceration operations are not well controlled. For instances, the rump area can be contaminated if the rectum is nicked or faecal material leaks from the anus; the brisket can be contaminated by spillage of guts contents if the paunch or intestine is ruptured (Gill *et al.*, 1996). As a result, the numbers of aerobes on eviscerated carcasses will not change drastically, but the numbers of enteric bacteria may remain the same or increase variably depending on how well the evisceration process is controlled (McEvoy *et al.*, 2004; Yang *et al.*, 2012).

22.2.3 Carcass decontaminating treatments that are currently used in commercial beef packing plants

Treatments that have potential to be used to control the microbial contamination of beef carcasses have been very extensively investigated (Carlson *et al.*, 2008; Gill, 2009; Koochmaraie *et al.*, 2005; Loretz *et al.*, 2011). Most researched decontaminating treatments are effective for removing bacteria from or inactivating bacteria on meat surfaces under experimental circumstances. However, the efficacies of those treatments in commercial practice cannot be assumed because of differences under laboratory and commercial conditions of factors such as the types of surfaces on which the bacteria are deposited, the numbers and physiological conditions of the bacteria on surfaces, and the manner in which the treatments are applied, etc. (Gill, 2009).

22.2.3.1 Dehairing or washing before skinning

Treatment of hide-on carcasses to reduce the numbers of bacteria on hides before skinning of beef carcasses has long been seen as a possible means of enhancing control over microbiological contamination of the carcass, by disrupting the transfer of bacteria from the hide to the meat. Dehairing can be achieved by treatment of hide-on carcasses with two applications of 10% sodium sulfide (Na₂S) and neutralizing residual Na₂S with two applications of 3% hydrogen peroxide (H₂O₂), with the carcasses being washed with water after each application of a solution (Nou *et al.*, 2003; Schnell *et al.*, 1995). Both studies investigated whether chemical dehairing could improve the microbiological condition of skinned carcasses in commercial plants and found that the treatment was ineffective for reducing numbers of aerobes and *Enterobacteriaceae* on hides. However, in the study of Nou and co-workers the numbers of those bacteria were 2 log units less on skinned carcasses derived from the animals that had

been dehaired than those that had not been. The treatment also reduced the prevalence of *E. coli* O157:H7 on skinned carcasses by 49%. Even so, chemical dehairing of cattle hide has not been widely adopted in commercial practice due to issues dealing with sodium sulfide generated and processing of hydrolyzed hair and trimming off tissues at the stick wound that has been exposed to the chemicals.

Despite the fact that washing hides after slaughtering with various antimicrobial solutions, including acetic acid, lactic acid, sodium hydroxide, hypobromous acid, and cetylpyridinium chloride, can substantially reduce the numbers of bacteria on hides (Loretz *et al.*, 2011; Schmidt *et al.*, 2012), washing hide-on carcasses with a sodium hydroxide solution appears to be the only treatment for hide-on beef carcasses that is used routinely in commercial practice (Bosilevac *et al.*, 2005; Yang *et al.*, 2012, 2015). After the cattle are stunned and exsanguinated, and before any other operation, the carcasses are sprayed with a solution of 1.5% sodium hydroxide at 55 or 65°C, while scrubbed with rotating brushes, and are rinsed with an NaOCl solution containing 1 ppm free chlorine. Excessive liquid is removed from the hide along the lines where opening cuts will be made by application of a vacuum cleaning head. Bosilevac and co-workers found that the treatment reduced the numbers of aerobes and *Enterobacteriaceae* on hides by 2.1 and 3.4 log cfu/100 cm², respectively. Aerobes and *Enterobacteriaceae* on skinned carcasses were both reduced by 0.8 log cfu/100 cm², and the prevalence of *E. coli* O157 on skinned carcasses was reduced from 17 to 2% when the cabinet was in use. The study of Yang and co-workers investigated the effect of the treatment on cattle hides of various conditions as cattle hides in some regions of Canada are normally dusty and dry in fall, wet and muddy in spring, and heavily tagged in winter. That study found that the decontaminating treatment reduced the numbers of aerobes on

hides only in fall, by 1.4 log cfu/1000 cm², but reduced the log total numbers of coliforms, which were mostly *E. coli* in winter, spring, and fall, by from >3 log to >7 log cfu/25 000 cm². It should be noted that the log total numbers rather than the mean log was used for coliforms and *E. coli* in the study of Yang and co-workers because, for analysis of microbiological data obtained from meat, when bacteria are recovered from ≥80% of the samples mean log or log mean is often used for statistical analysis and log total numbers of bacteria recovered from all samples are used otherwise (Kilsby and Pugh, 1981). Taken together, these various findings show that the hide-on carcass treatment may not result in consistent reductions of aerobes on hides, but can invariably reduce the numbers of bacteria of enteric origin on hides and skinned carcasses. Thus, the disruption of the usual transfer of enteric bacteria from the hide to the meat will improve the safety of beef carcasses.

22.2.3.2 Removal of visible contamination by trimming and vacuum cleaning

Removing visible contamination from carcasses by cutting away the contaminated tissues is carried out at all plants (Gill, 2009). At some plants, visibly contaminated areas are treated by vacuum cleaning with cleaning heads that contain outlets from which hot water or steam can be sprayed. All such treatments are effective for removal of visible contamination and bacteria that are associated with it (Kochevar *et al.*, 1997; Reagan *et al.*, 1996), but cannot reliably improve the overall microbiological condition of carcasses due to the limited surface area on which the treatment is applied and the lack of a necessary relationship between visible contamination and microbiological contamination; i.e., surfaces carrying no visible contamination can harbor large numbers of bacteria, while surfaces having visible contamination may carry few (Gill, 2004).

22.2.3.3 Washing carcasses with water

Washing carcass sides at the end of the carcass-dressing process has long been in practice at beef packing plants for removal of visible contaminants such as soil, hair, or debris (Loretz *et al.*, 2011). In recent years, washing carcasses after skinning but before evisceration has been introduced to some plants in North America (Gill, 2009). A study of Algino and co-workers (Algino *et al.*, 2007) in which the effects of spraying carcass sides using a hand-held device with low-pressure water at temperatures $>65^{\circ}\text{C}$ or with high-pressure water at 50°C for a duration $\geq 70\text{ s}$ were investigated found that either treatment reduced the numbers of *Enterobacteriaceae*, coliforms, and *E. coli* on carcasses all by about 1 log cfu/cm². Those reductions were likely caused by the physical removal of bacteria rather than heat inactivation because of the rapid cooling of hot water during the travel from the nozzle to the carcass (Davey, 1989). In two studies in which the effects of automatic washing of carcass sides with water at temperatures $<40^{\circ}\text{C}$ for $<40\text{ s}$ were examined, washing did not affect the numbers of aerobes, coliforms, or *E. coli* (Bell, 1997; Jericho *et al.*, 1995). One study in which the effects on the microbiological condition of dressed carcasses of the process at a large Canadian beef packing plant were investigated reported that washing uneviscerated carcasses did not affect the numbers of aerobes, but reduced the numbers of coliforms and *E. coli* by about 1 log unit (Yang *et al.*, 2012). In contrast, washing carcass sides did not affect the numbers of coliforms and *E. coli*, but increased the numbers of aerobes, probably as a result of redistribution. It should be noted, however, that the numbers of all three groups of bacteria in the study of Yang and co-workers were very low. These various studies suggest that when carcasses are heavily contaminated during skinning or subsequent operations, washing for relatively long times will substantially reduce the numbers of bacteria on carcasses and will be largely ineffective otherwise.

22.2.3.4 Spraying carcasses with antimicrobial solutions

The effects on bacterial numbers of spraying beef with various antimicrobial solutions, including chlorine, sodium hypochlorite, acidified sodium hypochlorite and cetylpyridinium chloride; acetic, citric, lactic, peroxyacetic, and other short-chain organic acids; and ozone, trisodium phosphate, nisin, and lactoferrin, have been very extensively investigated (Gill, 2009; Koohmaraie *et al.*, 2005; Loretz *et al.*, 2011). As with hide decontaminating interventions, solutions of most of those antimicrobials are effective for reducing the numbers of bacteria on meat. However, only lactic, acetic, and peroxyacetic acid (PAA) solutions have been routinely applied to carcasses at substantial numbers of North American beef packing plants (Algino *et al.*, 2007; Gill, 2009; Gill and Badoni, 2004; Yang *et al.*, 2012). PAA is often applied to chilled carcass sides as a post-chilling treatment (Gill and Badoni, 2004; King *et al.*, 2005). In the study of King and co-workers, the treatment of chilled beef carcasses with up to 600 ppm PAA was ineffective for reducing the numbers of *E. coli* or *Salmonella*. Gill and Badoni found that spraying meat with 200 ppm PAA reduced the numbers of aerobes, coliforms, and *E. coli* by about 1 log unit on product from one of the two plants investigated, but was entirely ineffective for meat from the other plant. The authors suggested that the decontaminating effect was due to the washing part of the treatment, rather than any effect of the PAA solution. The limited effect of PAA as a meat decontaminant is not surprising because PAA, a powerful oxidizing agent, will react with the mass of non-bacterial organic material at meat surfaces.

Lactic acid is the most commonly used organic acid for decontaminating beef carcasses at many North American beef packing plants (Bosilevac *et al.*, 2006). When spraying beef carcasses with lactic acid was first introduced in commercial beef packing plants, a solution of 2% lactic acid was applied automatically as a misting spray to uneviscerated carcasses

immediately after they were washed with cold water or carcasses sides immediately after they were pasteurized with hot water or steam (Gill, 2009). Such treatments were largely ineffective for reducing the numbers of aerobes, coliforms, and *E. coli*, likely due to the diluting effect of the wet carcasses/carcass sides (Arthur *et al.*, 2004; Gill and Landers, 2003). When the carcasses were not washed prior to the application of 2% lactic acid solution, reductions of up to 1.6 log units for aerobes and *E. coli* were observed (Bosilevac *et al.*, 2006; Gill and Badoni, 2004). Reductions of aerobes by 3.3 log units were achieved when a solution of 4–5% lactic acid was applied to chilled carcass sides (Castillo *et al.*, 2001). Consequently, spraying uneviscerated carcasses and carcass sides with 5% lactic acid has been implemented in commercial practice in recent years (Yang *et al.*, 2012). The treatment reduced the log total number of aerobes from 25 carcasses by 1.4 log units and the levels of coliforms and *E. coli* to below the detection limit of 1 cfu/100 cm². The treatment for carcass sides, however, was ineffective. The effectiveness of lactic acid treatment, like any other treatments with antimicrobial solutions, depends on the amount and concentration of the solution, the microbial load of the meat, the condition of the carcasses, and contact of the bacteria with the decontaminating solution. It is then conceivable that treatment with antimicrobial solutions will not be effective for removing bacteria in fissures that the solutions could not reach, and thus no such treatment can be expected to eliminate all bacteria from the meat. On the other hand, treatment of carcasses with lactic acid solution will inevitably give rise to alteration in the composition of the microbial flora on carcasses, likely on the final product, because bacteria differ substantially in their susceptibilities to lactic acid (Alakomi *et al.*, 2000; Cotter and Hill, 2003).

22.2.3.5 Pasteurization of carcass sides

At most large North American and some Australian beef packing plants, beef carcass sides are routinely pasteurized with hot water or

steam after they are washed at the end of the carcass dressing process. The decontamination effects of pasteurization are achieved by rapid heat inactivation of bacterial cellular components and thus are determined by the efficiency of the heat transfer from the medium (water or steam) to the meat. Steam pasteurization used in commercial beef plants consists of three phases: removing the water, pasteurizing, and surface cooling (Nutsch *et al.*, 1998). Carcasses that are to be pasteurized with steam have to be dry; otherwise, contaminated surfaces will not be heated to pasteurization temperatures. Steam pasteurizing treatments of whole carcass sides that have been reported in the literature have involved the use of steam at temperatures of 75–105 °C with treatment times of 6–11 s (Corantin *et al.*, 2005; Gill and Bryant, 1997b; Minihan *et al.*, 2003; Nutsch *et al.*, 1997, 1998). In most studies aerobes were reduced by between 1 and 1.5 log units and in some studies the numbers of *E. coli* were reduced by >2 log units, with the organism being detected in very few samples, at a level of detection of 1 cfu/100 cm². One study investigating the microbiological effect of steam pasteurization when carcasses were exposed to steam at various temperatures for 11 s demonstrated that the maximum reductions, with elimination of detectable *E. coli* at a level of detection of 0.4 cfu/cm², were obtained when the steam temperature was ≥85 °C (Retzlaff *et al.*, 2005).

Sprayed hot water will cool rapidly with increasing distance from the spray nozzle due to the large surface area-to-volume ratio for water in the form of droplets (Davey, 1989). Thus, for hot water spray to be effective, water has to be distributed to all parts of the carcass surface while the water remains at a temperature sufficient for inactivation of bacteria. A deluging system that delivers water in sheets is more effective for bacterial reduction than cabinets that spray water in droplets (EFSA, 2010). In some plants in North America, the hot water used for carcass decontamination is recirculated. However, the European Union permits

only potable water because of concerns over accumulation of bacterial spores (EFSA, 2010). One study investigating the accumulation of spores in the recirculated hot waters used at two Canadian plants found that most spores recovered from recirculated waters were of species of *Bacillus* or *Paenibacillus* and that the maximum numbers of spores recovered from any samples were 1 cfu/ml, much lower than those in milk, 10 cfu/ml (Yang *et al.*, 2011). Thus, the use of recirculated hot waters for pasteurization of beef carcasses is unlikely to pose risks to consumers. The antimicrobial efficacy and the effects on appearance of carcasses of pasteurization of beef carcasses with hot water have been investigated in several studies (Bosilevac *et al.*, 2006; Gill and Bryant, 2000; Gill *et al.*, 1999a). The study of Bosilevac and co-workers demonstrated washing uneviscerated carcasses with hot water at 74°C for 5.5 s reduced the numbers of aerobes and *Enterobacteriaceae* by 2.7 log cfu/100 cm² and the prevalence of *E. coli* O157:H7 by 81%. The latter two studies found that treating carcass sides with hot water at 85°C for 10 s reduced the numbers of aerobes and log total numbers of coliforms and *E. coli* by about 1.5 log units and 2 log units, respectively, but did not cause unacceptable damage to the appearance of the product. By far, pasteurization with hot water or steam is the most consistent and effective treatment for decontaminating beef carcasses.

22.2.4 Chilling

After dressing, carcass sides are cooled to adequately low temperatures to avoid proliferation of bacteria, particularly pathogens (Stopforth and Sofos, 2005). Regulatory authorities in the US and the European Union and Canada require that a temperature of 10 or 7°C, respectively, be reached at the center of the deep hip before further processing. In addition to the microbial growth, chilling conditions affect the appearance, tenderness, weight loss, and drip as well (Savell *et al.*, 2005). Dry chilling involving rapid movement of air at low temperatures was traditionally used and is still in

use at small abattoirs in many countries. Various studies have reported reductions in bacterial counts during dry chilling processes (Algino *et al.*, 2007; Youssef *et al.*, 2013; Zweifel *et al.*, 2014). Although bacteria differ substantially in their susceptibilities to the effects of drying, Gram-negative bacteria are in general more sensitive to drying than Gram-positive organisms. Thus, greater reductions in numbers of coliforms and *E. coli* than in the numbers of total aerobes can be expected. The study of Youssef and co-workers, conducted at a federally inspected small plant in Canada, demonstrated that the numbers of aerobes and the log total numbers of coliforms and *E. coli* were reduced by 1 and 2 log units, respectively. Similar effects of dry chilling on the numbers of coliforms and *E. coli* were reported in the study of Algino and co-workers in which 10 federally inspected small plants in the US were involved. The study of Zweifel and co-workers, conducted at two medium-sized abattoirs in Switzerland, found that dry chilling reduced the numbers of *Enterobacteriaceae* on carcass sides by ≥ 1.5 log units at one plant where the carcasses were visually dry after cooling, but was entirely ineffective at the other plant. The inconsistent efficacy of dry chilling at those two plants was probably a result of the degree of desiccation to which the carcasses were dried. In dry chilling, the dry air promotes evaporation of water from and desiccation of the carcass surface, with consequent reduction of the carcass weight (Savell *et al.*, 2005). Therefore, most large packing plants have adopted spray chilling in which carcasses are sprayed intermittently with water or solutions of antimicrobials during the initial stages of chilling to increase chilling rates and to reduce weight loss. The effects of spraying chilling on the numbers of bacteria on carcasses vary considerably, from increases by up to 2 log units (Gill *et al.*, 2003; Ruby *et al.*, 2007) to no changes (Arthur *et al.*, 2004; Gill and Bryant, 1997a; Yang *et al.*, 2012) or to decreases by up to 2 log units (Gill and Bryant, 1997a). No explanation for this discrepancy is apparent.

22.2.5 Microbiological conditions of carcass sides

The mean log numbers of aerobes and *E. coli* on carcasses were between 2 and 3 log cfu/cm² and 1 log cfu/cm², respectively, with *E. coli* recovered from most carcasses in North American plants before decontaminating of carcasses became the norm (Gill *et al.*, 1996; Sofos *et al.*, 1999). As a result of the implementation of novel treatments for decontaminating carcasses, the microbiological conditions of dressed beef carcass sides have greatly improved in recent years (Table 22.1). The numbers of aerobes on dressed carcasses were about 1 log cfu/100 cm² (Arthur *et al.*, 2004; Ruby *et al.*, 2007) or about 2 log cfu/100 cm² (Gill *et al.*, 2003; Gill and Landers, 2003; Yang *et al.*, 2012). *E. coli* was not recovered from most carcasses, at a

level of detection of 1 cfu/100 cm² or 1 cfu/1000 cm² in the cases of the Canadian studies. The microbiological conditions of dressed carcass sides at plants where no decontaminating treatments are applied have also improved, albeit to a lesser extent. Even so, plants that do not use decontaminating treatments often cool carcasses by dry chilling, which can result in reduction in numbers of aerobes and *E. coli* by up to 1 and 2 log units, respectively (Youssef *et al.*, 2013; Zweifel *et al.*, 2014). The microbiological conditions of chilled carcasses are similar to those of dressed carcass sides where decontamination treatments are routinely used (Table 22.2), with effectively decontaminated sides mostly carrying aerobes at numbers ≤ 2 log cfu/100 cm² and very few *E. coli*. Well controlled dressing processes alone,

Table 22.1 Microbiological profile of beef carcasses at the end of the dressing line.

Reference	Location/ number of abattoirs	Treatments ^a	Aerobes		<i>E. coli</i>	
			% neg ^b	$\bar{x}$ ^c	N ^d	% neg
Gill and Landers, 2003	Canada/1	1a-2-3a	0	3.3	n.d. ^e	100
	Canada/1	1a-3a-4	8	1.9	n.d.	100
	Canada/1	1a-3b-4	4	2.6	n.d.	100
	Canada/1	5a	0	3.7	2.9	68
Gill <i>et al.</i> , 2003	Canada/1	1a-4	12	1.7	0.3	96
Yang <i>et al.</i> , 2012	Canada/1	1b-5b-3a	36	2.4 ^f	n.d.	100
Ruby <i>et al.</i> , 2007	USA/3	1b-3b-5b	— ^g	0.9–1.3	—	—
Bacon <i>et al.</i> , 2000	USA/4	1a-3b-5a	0–5	3.9–7.1	2.8–4.4	0–58
	USA/1	1a-3b	0	3.8	2.4	70
	USA/3	3b-5a	0	5.3–7.0	2.6–2.7	55–88
Arthur <i>et al.</i> , 2004	USA/2	1a-3b-2-3a	—	1.2/1.3	—	—
Zweifel <i>et al.</i> , 2014	Switzerland/2	0	—	3.7/4.0	2.6/3.1	90/97
Hauge <i>et al.</i> , 2012	Norway/1	0	—	5.1–5.6	1.7–2.0	—
Youssef <i>et al.</i> , 2013	Canada/1	0	0	3.6	1.9	60

^a0, no treatment other than washing was applied; 1a, washing and spraying unviscerated carcasses with 2% lactic or acetic acid; 1b, spraying unviscerated carcasses with 5% lactic acid; 2, spraying sides with 200 ppm peroxyacetic acid; 3a, pasteurizing sides with steam; 3b, pasteurizing sides with hot water; 4, pasteurizing sides with steam followed by spraying with 2% lactic or acetic acid; 5a, spraying sides with 2% lactic acid; 5b, spraying sides with 5% lactic acid.

^bPercentage of aerobe or *E. coli* negative samples.

^cMean value in cfu/100 cm² for the log counts of aerobes.

^dlog of the total number of *E. coli* recovered from a total surface area of 2500 cm².

^eNone detected.

^fMean log value was not available because aerobes were recovered from less than 80% of the samples. This value was calculated from the log of the total number of aerobes of equivalent area.

^gNot reported in the reference.

Table 22.2 Microbiological conditions of chilled beef carcass sides.

Reference	Decontamination	Number of sides/plants	Aerobes (log cfu/100 cm ²)	<i>E. coli</i>	
				Neg% ^d	DL ^e
Arthur <i>et al.</i> , 2004	Y ^a	576/2	1.4/1.4	— ^f	—
Bacon <i>et al.</i> , 2000	Y ^a	320/8	2.3–5.3	98	0.9 log cfu/100 cm ²
Ruby <i>et al.</i> , 2007	Y ^b	3140/3	1.0–2.0	—	—
Yang <i>et al.</i> , 2012	Y ^a	100/1	2.1	98	1 cfu/1000 cm ²
Sumner <i>et al.</i> , 2004, 2011	N ^c	64577	2.9	95	0.08 cfu/cm ²
Zweifel <i>et al.</i> , 2014	N	100/2	3.0/4.0	—	—
Youssef <i>et al.</i> , 2013	N	25/1	2.5	96	1 cfu/100 cm ²

^aMultiple decontaminating interventions were applied to carcasses before but not after chilling.

^bMultiple decontaminating interventions were applied to carcasses before chilling and chilled carcasses were sprayed with 5% lactic acid.

^cCarcasses were not treated with any antimicrobial solutions.

^dPercentage of *E. coli* negative samples.

^eDetection limit.

^fNot reported in the reference.

however, can also yield carcasses with aerobes at < 3 log cfu/100 cm² and very few *E. coli* (Sumner *et al.*, 2004, 2011; Youssef *et al.*, 2013).

22.3 Carcass breaking

After cooling, carcass sides are fabricated into primal and subprimal cuts and trimmings, which are packaged and shipped elsewhere for further fabrication or processing (Scanga, 2005). In most plants, the facility for fabrication of a carcass is segregated from the facility for slaughtering and dressing to minimize cross-contamination. The fabrication processes between plants vary widely in technology and facility design. In general, the carcass sides are divided into quarters, i.e., hindquarters and forequarters, before or after some portions are removed from the sides. The different portions are dropped to separate conveyor belts, from which they are taken to cutting boards for the removal of bones. Meat pieces obtained during deboning that do not meet any meat cut labeling specification are collected by a series of conveyors as trimmings. In large packing plants, the removal of bones and fabrication of cuts are performed by multiple workers and, as such,

portions are repeatedly taken from the conveyor, handled by a worker, and put back on to the conveyor until the fabrication is completed. During the fabrication process, bacteria can be introduced to the meat from hands of workers, personal equipment, and the meat contact surfaces of conveying equipment, if not properly cleaned (Gill, 2005; Yang *et al.*, 2012; Youssef *et al.*, 2013). In addition, persisting detritus can be transferred to the meat contacting surfaces from other areas and consequently contaminates the product during operation of the equipment. Decontaminating interventions are not currently applied to any significant extent in the carcass breaking process in commercial practice. The conveyor belts and cuts were sprayed with solutions of 200 PAA through cabinets that were fitted at the end of cut fabrication belts in a Canadian large beef packing plant (Yang *et al.*, 2012). However, the numbers of aerobes, coliforms, and *E. coli* recovered from cuts and belts that had been sprayed with PAA were similar to those recovered from cuts and belts that had not been sprayed. Thus, the microbiological conditions of cuts and trimmings largely depend upon the microbiological condition of carcasses and equipment and the extent to which the meat is handled.

22.3.1 Microbiological conditions of beef cuts

The microbiological conditions of 10 types of primal cuts ($n=1022$) produced at two processing plants in the US were monitored for a period of 6 months (Stopforth *et al.*, 2006). The numbers of aerobes ranged from 4.0 log cfu/g for butts and rib eye rolls to 6.2 log cfu/g on club ends, the numbers of coliforms ranged from 1.1 log cfu/g on bottom sirloin butt to 1.8 log cfu/g on top sirloin butt, and the numbers of *E. coli* ranged from 0.8 log cfu/g on most of the cuts to 1.0 log cfu/g on rib eye rolls, butts, and miscellaneous cuts. Also in the US, clod and striploins were reported carrying aerobes at 2.3 log cfu/cm² (Ware *et al.*, 2001). In Canada, the microbiological conditions of beef primal cuts were determined in two studies (Yang *et al.*, 2012; Youssef *et al.*, 2014c). The numbers of aerobes were between 0.2 and 2.5 log cfu/cm². The levels of coliforms and *E. coli* were found at <0.5 and -0.1 log cfu/100 cm², respectively (Yang *et al.*, 2012). In Switzerland, beef primals ($n=200$) sampled at a European Union approved establishment had aerobes at 2.4 log cfu/cm², with 13% of the sampled being positive for *Enterobacteriaceae* (Zweifel *et al.*, 2005). A more comprehensive study was conducted for Australian beef in which samples were collected from primal cuts fabricated at 29 establishments with combined throughput accounting for 80% of Australian beef production (Phillips *et al.*, 2012). The findings of that survey were that the numbers of aerobes recovered from striploins ($n=572$) and outsides ($n=572$) were 1.3 and 1.5 log cfu/cm², respectively, and that *E. coli* were recovered from 11 and 25% of striploins and outsides, respectively, at mean counts of ≤ -0.3 log cfu/cm² on positive samples. The prevalence of coliforms was about 20% higher than that of *E. coli* for both types of cut.

In North America, a large fraction of cuts is mechanically tenderized to improve the tenderness of beef, by incising the meat with multiple thin blades or needles (Clyma, 2013; George-Evins *et al.*, 2000; Maddock, 2008). Mechanical tenderization is carried out at

packing and processing plants, at the meat processing facilities of retail stores, and at restaurants. Mechanical tenderization will inevitably result in some of the bacteria on the surfaces of the meat and meat contacting surfaces of the tenderizer being carried into previously sterile deep tissues (Youssef *et al.*, 2014c). Studies of translocation of surface-inoculated *E. coli* O157:H7 found that the organism was primarily transferred to the topmost 1 cm of the cuts from which the numbers of *E. coli* O157:H7 recovered were positively correlated with the numbers on the surfaces (Huang and Sheen, 2011; Luchansky *et al.*, 2008). However, the extent of translocation of bacteria from the surfaces of naturally contaminated beef cuts to the deep tissues is not as predictable as those with artificially inoculated cuts (Gill and McGinnis, 2004, 2005; Gill *et al.*, 2005; Youssef *et al.*, 2014c). Those latter studies found that the relationship between numbers of bacteria on the surface and in the deep tissues was weak at best when the numbers of bacteria on the surfaces were low.

The minimum temperature at which meat can be stored indefinitely without freezing is $-1.5 \pm 0.5^\circ\text{C}$ (Gill *et al.*, 1988). Even so, some spoilage bacteria can grow at temperatures as low as or below -3°C . Consequently, meat that is stored at chiller temperatures will inevitably be spoiled by the activities of meat microflora, irrespective of the storage temperature. A storage life of 10 days can be attained when meat is stored in air at 0°C , with the spoilage flora dominated by species of *Pseudomonas*, particularly *P. fragi* (Stanbridge and Davies, 1998). Pseudomonads are strictly aerobic and preferentially utilize glucose for growth (Gill and Newton, 1977). The utilization of other substrates is prevented by catabolite repression when glucose is available (Nychas *et al.*, 1988). When the numbers of pseudomonads on meat surfaces approach the carrying capacity of 10^8 log cfu/cm², glucose diffused to the surface of meat becomes insufficient to match the demand of bacterial growth. Consequently, pseudomonads begin to utilize

other fermentable substrates including nitrogenous compounds, from which malodorous substances will be formed. Signs of spoilage then become apparent. Over the years, much work has been undertaken to extend the storage life of meat as well as to meet various consumer demands. Consequently, various preservative packaging methods for meat have been developed, namely vacuum packs, high oxygen modified atmosphere packs, low oxygen modified atmosphere packs, and controlled atmosphere packs (Gill and Gill, 2005). The storage life and microbial ecology of beef stored under various conditions have been extensively investigated and comprehensively reviewed (Gill and Gill, 2005, 2009; Gill, 1996; Stanbridge and Davies, 1998). As beef is primarily distributed from slaughtering plants in the form of vacuum packaged primal cuts (Delmore, 2009), discussion in this section will focus mainly on the microbial ecology of vacuum packaged beef in relation to the improved microbiological condition of beef cuts.

When meat is vacuum packaged with a film of low gas permeability, the oxygen concentration on the surface of the meat is too low to support substantial growth of pseudomonads. Under these circumstances, the spoilage microflora is generally dominated by lactic acid bacteria (Stanbridge and Davies, 1998). Lactic acid bacteria primarily utilize glucose and the growth ceases when glucose is exhausted, which usually occurs as the numbers of lactic acid bacteria are between 7 and 8 log cfu/cm². Spoilage, manifested by mildly offensive, dairy/cheesy odours, will develop only some time after maximum numbers have been attained by lactic acid bacteria. At the optimal storage temperature, i.e., -1.5°C, vacuum packaged beef has been expected to have a storage life of 90 days (Bell and Garout, 1994; MLA, 2004). Various studies found that the initial flora on vacuum packaged beef included large fractions of lactic acid bacteria (Brightwell *et al.*, 2009; Fontana *et al.*, 2006; Jones, 2004; Nissen *et al.*, 1996; Sakala *et al.*, 2002; Yost and Nattress, 2002) and that the spoilage microflora was ultimately composed

predominantly of species of *Lactobacillus* and *Leuconostoc* (Blixt and Borch, 2002; Brightwell *et al.*, 2009; Ercolini *et al.*, 2011; Fontana *et al.*, 2006; Jones, 2004; Nissen *et al.*, 1996; Pennacchia *et al.*, 2011; Sakala *et al.*, 2002; Yost and Nattress, 2002). In some instances, carnobacteria predominated in the spoilage microflora until the microflora attained maximum numbers, but were displaced by lactobacilli and/or leuconostocs when storage was extended (Jones, 2004; Pennacchia *et al.*, 2011; Sakala *et al.*, 2002). The initial dominance of carnobacteria is likely to be the result of the growth rate advantage of carnobacteria over other bacteria under vacuum packaging conditions because during growth of a spoilage microflora to maximum numbers, the microflora will be progressively enriched for the organisms that grow most rapidly under the storage conditions (Gill, 1996). When the initial numbers of spoilage organisms are small, the most rapidly growing species will greatly predominate in the spoilage flora when maximum numbers are attained. As discussed before, the improved practices and implementation of effective decontaminating treatments have resulted in significant changes in both numbers and composition of the microflora on beef carcasses. This will inevitably affect the microbial ecology of vacuum packaged cuts derived from such beef. Indeed, storage life of vacuum packaged beef of up to 160 days has been recently reported (Small *et al.*, 2012; Youssef *et al.*, 2014a). Contrary to the previously established maximum numbers of 7–8 log cfu/cm² attained by lactic acid bacteria on vacuum packaged beef, the maximum numbers of lactic acid bacteria on those cuts of very long storage life were only about 5 and 6 log cfu/cm². It is conceivable that it would take longer for spoilage odour to be perceptible from smaller numbers of lactic acid bacteria. Large differences in the numbers of aerobes and lactic acid bacteria recovered from individual cuts selected for sampling before storage or during storage were observed in both studies. The study of Youssef and co-workers also determined the initial

microflora and the development of it during storage at -1.5 and 2°C . The initial microflora was largely composed of obligate aerobes of soil or water origin, with a very small fraction (5%) of lactic acid bacteria. Bacteria recovered from cuts stored for 160 days were mainly *Carnobacterium* spp. that grew on an acetate-containing agar generally selective for lactic acid bacteria other than *Carnobacterium*. *C. divergens* and *C. maltaromaticum* were recovered from cuts stored at 2°C but *C. maltaromaticum* was the only species of *Carnobacterium* recovered from cuts stored at -1.5°C . No lactic acid bacteria of genera that usually predominate in the spoilage microflora of vacuum package beef at late storage times were recovered. In addition, the numbers of *Enterobacteriaceae*, about $2 \log \text{cfu/cm}^2$ when both lactic acid bacteria and aerobes attained maximum numbers of about $6 \log \text{cfu/cm}^2$ after 140 days of storage at -1.5°C , were 4 log units less than those for vacuum packaged beef in earlier studies (Blixt and Borch, 2002; Lee and Yoon, 2001). Members of *Enterobacteriaceae* such as *Serratia* and *Hafnia* are potent spoilage organisms and can cause putrid spoilage of vacuum packaged beef (Samelis, 2006). Small numbers of these organisms in the final spoilage microflora, either resulting from inhibition by lactic acid bacteria or small initial numbers, could also explain the very long storage life. Another recent study in which beef cuts from carcasses that had been decontaminated by multiple interventions also found that the initial microflora of vacuum packaged beef consisted mainly of obligate aerobes of soil or water origin and included very small fractions of lactic acid bacteria (Youssef *et al.*, 2014b). These various findings suggest when spoilage bacteria on beef are few, and probably of limited diversity, development of spoilage microflora on the vacuum packaged product may differ in important respects from the development of spoilage microflora generally reported for beef contaminated with relatively large fractions and diverse populations of spoilage flora at the time of vacuum packaging.

Despite the long storage life that is currently attainable, vacuum packaged beef can be spoiled by swelling of packs, i.e., blown pack spoilage (BPS). BPS was first described for vacuum packaged beef imported to the UK from southern Africa (Dainty *et al.*, 1989; Helps *et al.*, 1999). Since then it has been reported for chilled beef, lamb, and venison from North and South America, northern Europe, and New Zealand (Adam *et al.*, 2010). There are two types of BPS, one characterized by gross swelling of packs well before the end of the expected storage life, i.e., early onset BPS (Byrne *et al.*, 2009), and the other by slackening of packs after prolonged storage (Brightwell *et al.*, 2007). Pack swelling after prolonged storage could be caused by species of psychrotrophic *Enterobacteriaceae* (Brightwell *et al.*, 2007) or heterofermentive lactic acid bacteria (Chaves *et al.*, 2012; Yang *et al.*, 2013). A range of psychrotrophic and psychrotolerant clostridia have been recovered from blown pack spoiled chilled meats (Adam *et al.*, 2010). Even so, *Clostridium estertheticum* is apparently by far the most common cause of early onset BPS (Cavill *et al.*, 2011) due, at least in part, to the fact that the organism can produce large amounts of hydrogen and CO_2 gases from lactate, which is abundant in meat (Yang *et al.*, 2009). *C. estertheticum* is a spore-forming, obligate anaerobic organism that grows optimally at $8\text{--}10^{\circ}\text{C}$ and preferentially utilizes glucose when it grows in meat juice (Dainty *et al.*, 1989; Spring *et al.*, 2003; Yang *et al.*, 2009). Meat can be contaminated with spores of *C. estertheticum* directly or indirectly from animal hides (Moschonas *et al.*, 2009). Packs of vacuum packaged beef can develop BPS even when as few as ≤ 30 spores of *C. estertheticum* are present at the time of vacuum packaging (Clemens *et al.*, 2010; Yang *et al.*, 2013). Controlling BPS is rather difficult as spores can survive the decontaminating treatments routinely applied at meat packing plants (Setlow, 2007). The reason for the phenomenon of BPS in more recent years remains obscure. It is arguable, but unlikely, that it is caused by fewer lactic acid bacteria in the initial flora of vacuum packaged beef to

compete for glucose because substrate on meat surfaces will be limiting only after maximum numbers have been attained (Gill, 1976).

22.3.2 Trimmings and offal meats

In Australia, national surveys of the microbiological condition of beef carcasses and trimmings have been conducted at intervals of five years or so since 1993 (Phillips *et al.*, 2001, 2006; Vanderlinde *et al.*, 1998). In the 2004 survey the numbers of aerobes on carcasses and trimmings were 1.3 log cfu/cm² and 1.3 log cfu/g, respectively, more than 1 log unit less than those in the two previous surveys that had been conducted before the implementation of HACCP in Australia. The prevalence of *E. coli* positive samples also decreased from 17 to 1.8%. However, the level of aerobes on trimmings was elevated to 2.2 log cfu/g in the national survey conducted in 2011 (Phillips *et al.*, 2012), the reason for which, suggested by the authors, was the severe flooding in the livestock production and processing areas in Australia during the period when samples were collected. National survey data on microbiological conditions of beef trimmings from other countries are not readily available. However, researchers of the USDA evaluated beef trimmings from Australia, New Zealand, Uruguay, and the US found that the numbers of aerobes were 1.6, 2.2, 2.8, and 2.5 log cfu/g, respectively, and the prevalence of *E. coli* was 2, 1, 23, and 27%, respectively, on trimmings from those countries (Bosilevac *et al.*, 2007). A Canadian study reported that trimmings produced at a large plant had aerobes at -0.01 log cfu/cm² and *E. coli* at 0.3 log cfu/2500 cm² (Yang *et al.*, 2012).

The meat from the head is likely to be heavily contaminated with bacteria when they are removed from the carcasses because the mouths of cattle harbor large numbers of bacteria (Grau, 1986). The generally poor microbiological condition of other tissues is, however, likely caused by proliferation of bacteria introduced during removal as a result of insufficient cooling (Gill, 1998). Gill and co-workers evaluated the microbiological conditions of head meats at two

packing plants and found that the numbers of aerobes on tongues, cheeks, and lips at the time of packaging were 1.7 and 2.6, 3.3 and 1.9, and 2.3 and 1.8 log cfu/cm², respectively (Gill *et al.*, 1999b). The numbers of coliforms that were mostly *E. coli* varied widely, from <0.5 log cfu/100 cm² to 2.4 log cfu/100 cm². A more heavily contaminated condition, with the numbers of aerobes up to 4.6, 5.0, and 5.5 log cfu/g on cheeks, lips, and tongues, respectively, was reported for such products from two other large packing plants (Delmore *et al.*, 2000). The efficacy of a variety of treatments, including solutions of oxidizing chemicals, organic acids, and trisodium phosphate and hot water and steam were evaluated for the decontamination of edible offal meats (Delmore *et al.*, 2000; Kalchayanand *et al.*, 2008; Schmidt *et al.*, 2014). Despite the substantial reductions attainable by several treatments, documented implementation of decontaminating treatments of edible offal meats at packing plants appears to be lacking. Spraying the head with solutions of bromine or 5% lactic acid and the heart with 5% lactic acid are currently used at some plants (personal communication). However, the effects of these treatments in commercial practice are largely lacking.

References

- Adam, K.H., Flint, S.H., and Brightwell, G. 2010. Psychrophilic and psychrotrophic clostridia: sporulation and germination processes and their role in the spoilage of chilled, vacuum-packaged beef, lamb and venison. *International Journal of Food Science and Technology*, **45**, 1539–1544.
- Alakomi, H.L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., and Helander, I. M. 2000. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology*, **66**, 2001–2005.
- Algino, R.J., Ingham, S.C., and Zhu, J. 2007. Survey of antimicrobial effects of beef carcass intervention treatments in very small state-inspected

- slaughter plants. *Journal of Food Science*, **72**, M173–M179.
- Antic, D., Blagojevic, B., Ducic, M., Nastasijevic, I., Mitrovic, R., and Buncic, S. 2010. Distribution of microflora on cattle hides and its transmission to meat via direct contact. *Food Control*, **21**, 1025–1029.
- Arthur, T.M., Bosilevac, J.M., Nou, X., Shackelford, S.D., et al. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *Journal of Food Protection*, **67**, 658–665.
- Arthur, T.M., Bosilevac, J.M., Brichta-Harhay, D.M., Guerini, M.N., et al. 2007. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. *Journal of Food Protection*, **70**, 280–286.
- Bacon, R.T., Belk, K.E., Sofos, J.N., Clayton, R.P., Reagan, J.O., and Smith, G.C. 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection*, **63**, 1080–1086.
- Banatvala, N., Griffin, P.M., Greene, K.D., Barrett, T.J., Bibb, W.F., Green, J.H., and Wells, J.G. 2001. The United States national prospective hemolytic uremic syndrome study: microbiologic, serologic, clinical, and epidemiologic findings. *Journal of Infectious Diseases*, **183**, 1063–1070.
- Bell, R.G. 1997. Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, **82**, 292–300.
- Bell, R.G., and Garout, A.M. 1994. The effective product life of vacuum-packaged beef imported into Saudi Arabia by sea, as assessed by chemical, microbiological and organoleptic criteria. *Meat Science*, **36**, 381–396.
- Blagojevic, B., Antic, D., Ducic, M., and Buncic, S. 2011. Ratio between carcass- and skin-microflora as an abattoir process hygiene indicator. *Food Control*, **22**, 186–190.
- Blagojevic, B., Antic, D., Ducic, M., and Buncic, S. 2012. Visual cleanliness scores of cattle at slaughter and microbial loads on the hides and the carcasses. *Veterinary Record*, **170**, 563.
- Blixt, Y. and Borch, E. 2002. Comparison of shelf life of vacuum-packed pork and beef. *Meat Science*, **60**, 371–378.
- Bolton, D.J., Doherty, A.M., and Sheridan, J.J. 2001. Beef HACCP: intervention and non-intervention systems. *International Journal of Food Microbiology*, **66**, 119–129.
- Bosilevac, J.M., Nou, X., Osborn, M.S., Allen, D.M., and Koohmaraie, M. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *Journal of Food Protection*, **68**, 265–272.
- Bosilevac, J.M., Nou, X., Barkocy-Gallagher, G.A., Arthur, T.M., and Koohmaraie, M. 2006. Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and *Enterobacteriaceae* and reduce the prevalence of *Escherichia coli* O157:H7 on preevisceration beef carcasses. *Journal of Food Protection*, **69**, 1808–1813.
- Bosilevac, J.M., Guerini, M.N., Brichta-Harhay, D.M., Arthur, T.M., and Koohmaraie, M. 2007. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. *Journal of Food Protection*, **70**, 440–449.
- Brightwell, G., Clemens, R., Ulrich, S., and Boerema, J. 2007. Possible involvement of psychrotolerant *Enterobacteriaceae* in blown pack spoilage of vacuum-packaged raw meats. *International Journal of Food Microbiology*, **119**, 334–339.
- Brightwell, G., Clemens, R., Adam, K., Ulrich, S., and Boerema, J. 2009. Comparison of culture-dependent and independent techniques for characterisation of the microflora of peroxyacetic acid treated, vacuum-packaged beef. *Food Microbiology*, **26**, 283–288.
- Byrne, B., Monaghan, A.M., Lyng, J.G., Sheridan, J.J., and Bolton, D.J. 2009. A case of “blown pack” meat linked to *Clostridium estertheticum* in Ireland. *Journal of Food Safety*, **29**, 629–635.

- Carlson, B.A., Ruby, J., Smith, G.C., Sofos, J.N., *et al.* 2008. Comparison of antimicrobial efficacy of multiple beef hide decontamination strategies to reduce levels of *Escherichia coli* O157:H7 and *Salmonella*. *Journal of Food Protection*, **71**, 2223–2227.
- Castillo, A., Lucia, L.M., Mercado, I., and Acuff, G.R. 2001. In-plant evaluation of a lactic acid treatment for reduction of bacteria on chilled beef carcasses. *Journal of Food Protection*, **64**, 738–740.
- Cavill, L., Renteria-Monterrubio, A.L., Helps, C.R., and Corry, J.E.L. 2011. Detection of cold-tolerant clostridia other than *Clostridium estertheticum* in raw vacuum-packed chill-stored meat. *Food Microbiology*, **28**, 957–963.
- CDC. 1993. Update: Multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers – Western United States, 1992–1993. In: *Morbid Mortal Weekly Report*, vol. 42, pp. 258–263. Centers for Disease Control and Prevention, Atlanta, GA.
- Chaves, R.D., Silva, A.R., Sant’Ana, A.S., Campana, F.B., and Massaguer, P.R. 2012. Gas-producing and spoilage potential of *Enterobacteriaceae* and lactic acid bacteria isolated from chilled vacuum-packaged beef. *International Journal of Food Science and Technology*, **47**, 1750–1756.
- Clemens, R.M., Adam, K.H., and Brightwell, G. 2010. Contamination levels of *Clostridium estertheticum* spores that result in gaseous spoilage of vacuum-packaged chilled beef and lamb meat. *Letters in Applied Microbiology*, **50**, 591–596.
- Clyma, K. 2013. Washing away risks. *Meat Poultry*, **April**, 38–41.
- Corantin, H., Quessy, S., Gaucher, M., Lessard, L., Leblanc, D., and Houde, A. 2005. Effectiveness of steam pasteurization in controlling microbiological hazards of cull cow carcasses in a commercial plant. *Canadian Journal of Veterinary Research*, **69**, 200–207.
- Cotter, P.D. and Hill, C. 2003. Surviving the acid test: responses of Gram-positive bacteria to low pH. *Microbiology and Molecular Biology Reviews*, **67**, 429–453.
- Dainty, R.H., Edwards, R.A., and Hibbard, C.M. 1989. Spoilage of vacuum packed beef by a *Clostridium* sp. *Journal of the Science of Food and Agriculture*, **49**, 473–486.
- Davey, K.R. 1989. Theoretical analysis of two hot water cabinet systems for decontamination of sides of beef. *International Journal of Food Science and Technology*, **24**, 291–304.
- Delmore, R.J. 2009. Beef shelf-life. Accessed: October 10, 2013. <http://www.beefresearch.org/CMDocs/BeefResearch/Beef%20Shelf-life.pdf>.
- Delmore, R.J., Sofos, J.N., Schmidt, G.R., Belk, K.E., Lloyd, W.R., and Smith, G.C. 2000. Interventions to reduce microbiological contamination of beef variety meats. *Journal of Food Protection*, **63**, 44–50.
- EFSA. 2010. Scientific opinion on the safety and efficacy of using recycled hot water as a decontamination technique for meat carcasses. *EFSA Journal*, **8**, 1827–1895.
- Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., *et al.* 2011. Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. *Applied and Environmental Microbiology*, **77**, 7372–7381.
- Fearne, A., Garcia, M., Henson, S., Collins, R., Khatri, Y., McEvoy, J., Nganje, W., and le Roux, G. 2004. *Global HACCP implementation in meat producing countries: final report*. Food Standards Agency, London, UK.
- Fontana, C., Cocconcelli, P.S., and Vignolo, G. 2006. Direct molecular approach to monitoring bacterial colonization on vacuum-packaged beef. *Applied and Environmental Microbiology*, **72**, 5618–5622.
- George-Evins, C.D., Unruh, J.A., Marsden, J.L., & Kastner, C.L. 2000. A survey on the use of blade tenderizers by beef fabrication plants. Accessed: July 05, 2014. <http://krex.k-state.edu/dspace/bitstream/handle/2097/4686/cattle00pg119-120.pdf?sequence=1>.
- Gill, C.O. 1976. Substrate limitation of bacterial growth at meat surfaces. *Journal of Applied Bacteriology*, **41**, 401–410.
- Gill, C.O. 1996. Extending the storage life of raw chilled meats. *Meat Science*, **43** (Suppl. 1(0)), 99–109.

- Gill, C. 1998. Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In: *The Microbiology of Meat and Poultry* (eds. Davies, A.R. and Board, R.G.), pp. 118–157. Blackie Academic & Professional, London, UK.
- Gill, C.O. 2004. Visible contamination on animals and carcasses and the microbiological condition of meat. *Journal of Food Protection*, **67**, 413–419.
- Gill, C.O. 2005. Sources of microbial contamination at slaughtering plants. In: *Improving the Safety of Fresh Meat* (ed. Sofos, J.N.), pp. 461–502. Woodhead Publishing Limited, Cambridge, UK.
- Gill, C.O. 2009. Effects on the microbiological condition of product of decontaminating treatments routinely applied to carcasses at beef packing plants. *Journal of Food Protection*, **72**, 1790–1801.
- Gill, C.O. and Badoni, M. 2004. Effects of peroxy-acetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. *International Journal of Food Microbiology*, **91**, 43–50.
- Gill, C.O. and Bryant, J. 1997a. Assessment of the hygienic performances of two beef carcass cooling processes from product temperature history data or enumeration of bacteria on carcass surfaces. *Food Microbiology*, **14**, 593–602.
- Gill, C.O. and Bryant, J. 1997b. Decontamination of carcasses by vacuum-hot water cleaning and steam pasteurizing during routine operations at a beef packing plant. *Meat Science*, **47**, 267–276.
- Gill, C.O. and Bryant, J. 2000. The effects on product of a hot water pasteurizing treatment applied routinely in a commercial beef carcass dressing process. *Food Microbiology*, **17**, 495–504.
- Gill, A.O. and Gill, C.O. 2005. Preservative packaging for fresh meats, poultry and fin fish. In: *Innovations in food packaging* (ed. Han, J.H.), pp. 204–220. Elsevier Ltd, London, UK.
- Gill, A. and Gill, C.O. 2009. Packaging and the shelf life of fresh red and poultry meats. In: *Food Packaging and Shelf Life* (ed. Robertson, G.L.), pp. 259–277. CRC Press, Boca Raton, FL, USA.
- Gill, C.O. and Landers, C. 2003. Microbiological effects of carcass decontaminating treatments at four beef packing plants. *Meat Science*, **65**, 1005–1011.
- Gill, C.O. and McGinnis, J.C. 2004. Microbiological conditions of mechanically tenderized beef cuts prepared at four retail stores. *International Journal of Food Microbiology*, **95**, 95–102.
- Gill, C.O. and Newton, K.G. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. *Journal of Applied Bacteriology*, **43**, 189–195.
- Gill, C.O., Phillips, D.M., and Harrison, J.C.L. 1988. Product temperature criteria for shipment of chilled meats to distant markets. In: *Refrigeration for Food and People*, pp. 40–47. International Institute of Refrigeration, Paris, France.
- Gill, C.O., McGinnis, J. C., and Badoni, M. 1996. Assessment of the hygienic characteristics of a beef carcass dressing process. *Journal of Food Protection*, **59**, 136–140.
- Gill, C.O., McGinnis, J.C., and Bryant, J. 1998. Microbial contamination of meat during the skinning of beef carcass hindquarters at three slaughtering plants. *International Journal of Food Microbiology*, **42**, 175–184.
- Gill, C.O., Bryant, J., and Bedard, D. 1999a. The effects of hot water pasteurizing treatments on the appearances and microbiological conditions of beef carcass sides. *Food Microbiology*, **16**, 281–289.
- Gill, C.O., McGinnis, J.C., and Jones, T. 1999b. Assessment of the microbiological conditions of tails, tongues, and head meats at two beef-packing plants. *Journal of Food Protection*, **62**, 674–677.
- Gill, C.O., Bryant, J., and Landers, C. 2003. Identification of critical control points for control of microbiological contamination in processes leading to the production of ground beef at a packing plant. *Food Microbiology*, **20**, 641–650.
- Gill, C.O., McGinnis, J.C., Rahn, K., Young, D., Lee, N., and Barbut, S. 2005. Microbiological condition of beef mechanically tenderized at a packing plant. *Meat Science*, **69**, 811–816.
- Grau, F.H. 1986. Microbial ecology of meat and poultry. In: *Advances in Meat Research* (eds.

- Pearson, A.M. and Duston, T.R.), pp. 1–48. AVI Publishing Co, Westport, USA.
- Hauge, S.J., Nafstad, O., Røtterud, O.-J., and Nesbakken, T. 2012. The hygienic impact of categorisation of cattle by hide cleanliness in the abattoir. *Food Control*, **27**, 100–107.
- Helps, C.R., Harbour, D.A., and Corry, J.E.L. 1999. PCR-based 16S ribosomal DNA detection technique for *Clostridium estertheticum* causing spoilage in vacuum-packed chill-stored beef. *International Journal of Food Microbiology*, **52**, 57–65.
- Huang, L. and Sheen, S. 2011. Quantitative analysis of vertical translocation and lateral cross-contamination of *Escherichia coli* O157:H7 during mechanical tenderization of beef. *Journal of Food Safety*, **31**, 108–114.
- Jericho, K., Bradley, J., and Kozub, G. 1995. Microbiological evaluation of carcasses before and after washing in a beef slaughter plant. *Journal of the American Veterinary Medical Association*, **206**, 452–455.
- Jones, R.J. 2004. Observations on the succession dynamics of lactic acid bacteria populations in chill-stored vacuum-packaged beef. *International Journal of Food Microbiology*, **90**, 273–282.
- Kalchayanand, N., Arthur, T.M., Bosilevac, J.M., Brichta-Harhay, D.M., Guerini, M.N., Wheeler, T.L., and Koohmaraie, M. 2008. Evaluation of various antimicrobial interventions for the reduction of *Escherichia coli* O157:H7 on bovine heads during processing. *Journal of Food Protection*, **71**, 621–624.
- Kennedy, T.G., Giotis, E.S., and McKeivitt, A.I. 2014. Microbial assessment of an upward and downward dehiding technique in a commercial beef processing plant. *Meat Science*, **97**, 486–489.
- Khatri, Y. and Collins, R. 2007. Impact and status of HACCP in the Australian meat industry. *British Food Journal*, **109**, 343–354.
- Kilsby, D.C. and Pugh, M.E. 1981. The relevance of the distribution of micro-organisms within batches of food to the control of microbiological hazards from foods. *Journal of Applied Bacteriology*, **51**, 345–354.
- King, D.A., Lucia, L.M., Castillo, A., Acuff, G.R., Harris, K.B., and Savell, J.W. 2005. Evaluation of peroxyacetic acid as a post-chilling intervention for control of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. *Meat Science*, **69**, 401–407.
- Kochevar, S.L., Sofos, J.N., Bolin, R.R., Reagan, J.O., and Smith, G.C. 1997. Steam vacuuming as a pre-evisceration intervention to decontaminate beef carcasses. *Journal of Food Protection*, **60**, 107–113.
- Koohmaraie, M., Arthur, T.M., Bosilevac, J.M., Guerini, M., Shackelford, S.D., and Wheeler, T.L. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Science*, **71**, 79–91.
- Lee, K.-T. and Yoon, C.-S. 2001. Quality changes and shelf life of imported vacuum-packaged beef chunks during storage at 0°C. *Meat Science*, **59**, 71–77.
- Loretz, M., Stephan, R., and Zweifel, C. 2011. Antibacterial activity of decontamination treatments for cattle hides and beef carcasses. *Food Control*, **22**, 347–359.
- Luchansky, J.B., Phebus, R.K., Thippareddi, H., and Call, A.E. 2008. Translocation of surface-inoculated *Escherichia coli* O157:H7 into beef subprimals following blade tenderization. *Journal of Food Protection*, **71**, 2190–2197.
- Mackey, B.M. and Derrick, C.M. 1979. Contamination of the deep tissues of carcasses by bacteria present on the slaughter instruments or in the gut. *Journal of Applied Bacteriology*, **46**, 355–366.
- Maddock, R. 2008. Mechanical tenderization of beef. Accessed: July 05, 2014. http://www.beefresearch.org/CMDocs/BeefResearch/PE_Fact_Sheets/Mechanical_Tenderization_of_Beef.pdf.
- McEvoy, J.M., Doherty, A.M., Finnerty, M., Sheridan, J.J., McGuire, L., Blair, I.S., McDowell, D.A., and Harrington, D. 2000. The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*, **30**, 390–395.
- McEvoy, J.M., Sheridan, J.J., Blair, I.S., and McDowell, D.A. 2004. Microbial contamination on beef in relation to hygiene assessment based on criteria used in EU Decision 2001/471/EC. *International Journal of Food Microbiology*, **92**, 217–225.

- Minihan, D., Whyte, P., O'Mahony, M., and Collins, J.D. 2003. The effect of commercial steam pasteurization on the levels of *Enterobacteriaceae* and *Escherichia coli* on naturally contaminated beef carcasses. *Journal of Veterinary Medicine, Series B*, **50**, 352–356.
- MLA. 2004. Vacuum packaging primal cuts. In: *Meat and Livestock Australia*. North Sydney, Australia.
- Moschonas, G., Bolton, D.J., Sheridan, J.J., and McDowell, D.A. 2009. Isolation and sources of "blown pack" spoilage clostridia in beef abattoirs. *Journal of Applied Microbiology*, **107**, 616–624.
- Nissen, H., Sørheim, O., and Dainty, R. 1996. Effects of vacuum, modified atmospheres and storage temperature on the microbial flora of packaged beef. *Food Microbiology*, **13**, 183–191.
- Nou, X., Rivera-Betancourt, M., Bosilevac, J.M., Wheeler, T.L., Shackelford, S.D., Gwartney, B.L., Reagan, J.O., and Koohmaraie, M. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *Journal of Food Protection*, **66**, 2005–2009.
- Nutsch, A.L., Phebus, R.K., Riemann, M.J., Schafer, D.E., Boyer, J.E., Wilson, R.C., Leising, J.D., and Kastner, C.L. 1997. Evaluation of a steam pasteurization process in a commercial beef processing facility. *Journal of Food Protection*, **60**, 485–492.
- Nutsch, A.L., Phebus, R.K., Riemann, M.J., Kotrola, J.S., Wilson, R.C., Boyer, J.E., and Brown, T.L. 1998. Steam pasteurization of commercially slaughtered beef carcasses: evaluation of bacterial populations at five anatomical locations. *Journal of Food Protection*, **61**, 571–577.
- Nychas, G.J., Dillon, V.M., and Board, R.G. 1988. Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. *Biotechnology and Applied Biochemistry*, **10**, 203–231.
- Pennacchia, C., Ercolini, D., and Villani, F. 2011. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiology*, **28**, 84–93.
- Phillips, D., Sumner, J., Alexander, J.E., and Dutton, K.M. 2001. Microbiological quality of Australian beef. *Journal of Food Protection*, **64**, 692–696.
- Phillips, D., Jordan, D., Morris, S., Jenson, I., and Sumner, J. 2006. A national survey of the microbiological quality of beef carcasses and frozen boneless beef in Australia. *Journal of Food Protection*, **69**, 1113–1117.
- Phillips, D., Bridger, K., Jenson, I., and Sumner, J. 2012. An Australian national survey of the microbiological quality of frozen boneless beef and beef primal cuts. *Journal of Food Protection*, **75**, 1862–1866.
- Reagan, J.O., Acuff, G.R., Buege, D.R., Buyck, M.J., et al. 1996. Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. *Journal of Food Protection*, **59**, 751–756.
- Retzlaff, D., Phebus, R., Kastner, C., and Marsden, J. 2005. Establishment of minimum operational parameters for a high-volume static chamber steam pasteurization system (SPS 400-SC™) for beef carcasses to support HACCP programs. *Foodborne Pathogens and Disease*, **2**, 146–151.
- Riley, L.W., Remis, R.S., Helgersson, S.D., McGee, H.B., et al. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine*, **308**, 681–685.
- Romans, J.R., Costello, W.J., Carlson, W.C., Greaser, M.L., and Jones, K.W. 2000. *The Meat We Eat*, 14th edn. Prentice Hall.
- Ruby, J.R., Zhu, J., and Ingham, S.C. 2007. Using indicator bacteria and *Salmonella* test results from three large-scale beef abattoirs over an 18-month period to evaluate intervention system efficacy and plan carcass testing for *Salmonella*. *Journal of Food Protection*, **70**, 2732–2740.
- Sakala, R.M., Hayashidani, H., Kato, Y., Hirata, T., et al. 2002. Change in the composition of the microflora on vacuum-packaged beef during chiller storage. *International Journal of Food Microbiology*, **74**, 87–99.
- Samelis, J. 2006. Managing microbial spoilage in the meat industry. In: *Food Spoilage Micro-*

- organisms* (ed. Blackburn, C.W.), pp. 213–286. Woodhead Publishing, Cambridge, UK.
- Scavell, J.W., Mueller, S.L., and Baird, B.E. 2005. The chilling of carcasses. *Meat Science*, **70**, 449–459.
- Scanga, J.A. 2005. Slaughter and fabrication/boning processes and procedures. In: *Improving the Safety of Fresh Meat* (ed. Sofos, J.N.), pp. 260–271. Woodhead Publishing Limited, Cambridge, UK.
- Schmidt, J.W., Wang, R., Kalchayanand, N., Wheeler, T.L., and Koohmaraie, M. 2012. Efficacy of hypobromous acid as a hide-on carcass antimicrobial intervention. *Journal of Food Protection*, **75**, 955–958.
- Schmidt, J.W., Bosilevac, J.M., Kalchayanand, N., Wang, R., Wheeler, T.L., and Koohmaraie, M. 2014. Immersion in antimicrobial solutions reduces *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* on beef cheek meat. *Journal of Food Protection*, **77**, 538–548.
- Schnell, T.D., Sofos, J.N., Littlefield, V.G., Morgan, J.B., Gorman, B.M., Clayton, R.P., and Smith, G.C. 1995. Effects of postexsanguination dehairing on the microbial load and visual cleanliness of beef carcasses. *Journal of Food Protection*, **58**, 1297–1302.
- Serraino, A., Bardasi, L., Riu, R., Pizzamiglio, V., Liuzzo, G., Galletti, G., Giacometti, F., and Meriardi, G. 2012. Visual evaluation of cattle cleanliness and correlation to carcass microbial contamination during slaughtering. *Meat Science*, **90**, 502–506.
- Setlow, P. 2007. I will survive: DNA protection in bacterial spores. *Trends in Microbiology*, **15**, 172–180.
- Small, A.H., Jenson, I., Kiermeier, A., and Sumner, J. 2012. Vacuum-packed beef primals with extremely long shelf life have unusual microbiological counts. *Journal of Food Protection*, **75**, 1524–1527.
- Sofos, J.N. 1994. Microbial growth and its control in meat, poultry and fish. In: *Quality Attributes and Their Measurement in Meat, Poultry and Fish Products* (eds Pearson, A.M. and Dutson, T.R.), pp. 359–403. Blackie Academic & Professional, Glasgow, UK.
- Sofos, J.N., Kochevar, S.L., Reagan, J.O., and Smith, G.C. 1999. Extent of beef carcass contamination with *Escherichia coli* and probabilities of passing U.S. regulatory criteria. *Journal of Food Protection*, **62**, 234–238.
- Spring, S., Merkhoffer, B., Weiss, N., Kroppenstedt, R.M., Hippe, H., and Stackebrandt, E. 2003. Characterization of novel psychrophilic clostridia from an Antarctic microbial mat: description of *Clostridium frigoris* sp. nov., *Clostridium lacusfryxellense* sp. nov., *Clostridium bowmanii* sp. nov. and *Clostridium psychrophilum* sp. nov. and reclassification of *Clostridium laramiense* as *Clostridium estertheticum* subsp. *laramiense* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 1019–1029.
- Stanbridge, L.H. and Davies, A.R. 1998. The microbiology of chill stored meat. In: *Microbiology of Meat and Poultry* (eds Davies, A. and Board, R.), pp. 174–219. Blackie Academic & Professional, London, UK.
- Stopforth, J.D., and Sofos, J.N. 2005. Carcass chilling. In: *Improving the Safety of Fresh Meat* (ed. Sofos, J.N.), pp. 364–387. Woodhead Publishing, Cambridge, UK.
- Stopforth, J.D., Lopes, M., Shultz, J.E., Miksch, R.R., and Samadpour, M. 2006. Microbiological status of fresh beef cuts. *Journal of Food Protection*, **69**, 1456–1459.
- Sumner, J., Raven, G., and Givney, R. 2004. Have changes to meat and poultry food safety regulation in Australia affected the prevalence of *Salmonella* or of salmonellosis? *International Journal of Food Microbiology*, **92**, 199–205.
- Sumner, J., Kiermeier, A., and Jenson, I. 2011. Verification of hygiene in Australian manufacturing beef processing – Focus on *Escherichia coli* O157. *Food Protection Trends*, **31**, 514–520.
- USDA. 1996. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule. Accessed: July 01, 2014. <http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/93-016F.pdf>.
- Vanderlinde, P.B., Shay, B., and Murray, J. 1998. Microbiological quality of Australian beef carcass meat and frozen bulk packed beef. *Journal of Food Protection*, **61**, 437–443.

- Van Donkersgoed, J., Jericho, K.W.F., Grogan, H., and Thorlakson, B. 1997. Preslaughter hide status of cattle and the microbiology of carcasses. *Journal of Food Protection*, **60**, 1502–1508.
- Ware, L., Kain, M., Sofos, J.N., Belk, K.E., Reagan, J.O., and Smith, G.C. (2001). Influence of sampling procedure, handling and storage on the microbiological status of fresh beef. *Dairy Food and Environment Sanitation*, **21**, 14–19.
- Wilhelm, B., Rajić, A., Greig, J.D., Waddell, L., and Harris, J. 2011. The effect of hazard analysis critical control point programs on microbial contamination of carcasses in abattoirs: a systematic review of published data. *Foodborne Pathogens and Disease*, **8**, 949–960.
- Yang, X., Balamurugan, S., and Gill, C.O. 2009. Substrate utilization by *Clostridium estertheticum* cultivated in meat juice medium. *International Journal of Food Microbiology*, **128**, 501–505.
- Yang, X., Rajagopal, S., and Gill, C. 2011. Accumulation of spores in hot waters recirculated in beef carcass pasteurizers. *Internet Journal of Food Safety*, **13**, 321–324.
- Yang, X., Badoni, M., Youssef, M.K., and Gill, C.O. 2012. Enhanced control of microbiological contamination of product at a large beef packing plant. *Journal of Food Protection*, **75**, 144–149.
- Yang, X., Wang, H., and Badoni, M. 2013. Effects of meat pH and the initial numbers of spores of *Clostridium estertheticum* on the development of blown pack spoilage of vacuum-packaged beef. *International Journal of Food Science and Technology*, **49**, 1619–1625.
- Yang, X., Badoni, M., Tran, F., and Gill, C.O. 2015. Seasonal variation in the effects on dressed carcass contamination of a commercial on-line decontaminating treatment of hide-on beef carcasses. *Journal of Food Protection*, **78**, 256–265.
- Yost, C.K. and Nattress, F.M. 2002. Molecular typing techniques to characterize the development of a lactic acid bacteria community on vacuum-packaged beef. *International Journal of Food Microbiology*, **72**, 97–105.
- Youssef, M.K., Badoni, M., Yang, X., and Gill, C.O. 2013. Sources of *Escherichia coli* deposited on beef during breaking of carcasses carrying few *E. coli* at two packing plants. *Food Control*, **31**, 166–171.
- Youssef, M.K., Gill, C.O., Tran, F., and Yang, X. 2014a. Unusual compositions of microflora of vacuum packaged beef primal cuts of very long storage life. *Journal of Food Protection*, **77**, 2161–2167.
- Youssef, M.K., Gill, C.O., and Yang, X. 2014b. Storage life at 2°C or –1.5°C of vacuum packaged boneless and bone-in cuts from decontaminated beef carcasses. *Journal of the Science of Food and Agriculture*, **94**, 3118–3124.
- Youssef, M.K., Yang, X., and Gill, C.O. 2014c. The relationship between numbers of bacteria on surfaces and in deep tissues of mechanically tenderized beef. *Food Control*, **46**, 502–507.
- Zweifel, C., Capek, M., and Stephan, R. 2014. Microbiological contamination of cattle carcasses at different stages of slaughter in two abattoirs. *Meat Science*, **98**, 198–202.
- Zweifel, C., Zychowska, M., and Stephan, R. 2005. Regular checks in processing plants: data collection on the microbiological status of meat cuts before delivery. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene*, **96**, 127–140.

CHAPTER 23

Microbial ecology of pork meat and pork products

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23.1 Introduction

Because of its chemical composition and its physical traits, meat is an excellent culture medium for most of the pollutant microorganisms.

In the living animal, the muscles (living muscular tissues) are protected against the microorganisms by the mechanisms that nature disposes for this purpose: skin, antimicrobial substances in the mucose of the natural openings (e.g., lysozyme and lactoferrin in saliva, tears, and nasal secretions), and the mucosal barrier immune system, which, aided by the cellular immune function, produce secretory immunoglobulins that protect the organism from invasion by microorganisms.

The living animal contains a wide variety of microorganisms, mainly in the mucous membranes of the natural openings: the mouth, nostrils, eyelids, external genitalia, and anus. Microorganisms are especially abundant in the gastrointestinal tract, with the stomach of monogastric animals being an exception due to the presence of acids, although it is not completely sterile. The microbial content is highest in the large intestine, particularly in the final sections (caecum, colon, and rectum); the microbial load of the pig caecum is approximately 10^{10} – 10^{11} culturable bacteria/g (Leser *et al.*, 2000).

However, most of these microorganisms are strict anaerobes and cannot survive in the external atmosphere; thus they pose no danger of meat contamination.

In contrast to the internal and external body surfaces, the internal organs and tissues of the living animal exhibit an almost complete absence of microorganisms. In some cases, the liver and gallbladder may harbor some germs, a situation that is equally exceptional in other tissues and in the blood. Living animals also have internal defence mechanisms (such as the lymphatic system) that are able to quickly detect and remove these microorganisms.

Therefore, the organs and tissues of healthy animals (including the muscle tissue) are sterile or they have negligible microbial loads in healthy animals. However, in some circumstances, certain pathogen microorganisms can overcome the body's defences and can invade intact organs or even the bloodstream, causing an infection. Meat, organs, and excreta from a sick animal may contain the causative microorganism of the disease; when this microorganism is also a human pathogen, it can cause the disease in humans through contact with or the consumption of contaminated organs or tissues.

In general, when an animal is slaughtered and the carcass is obtained, the natural

protective mechanisms in the live animal become ineffective and the tissues (meat) are invaded by a wide variety of microorganisms from different sources: the environment, handlers, instruments used during the slaughter and dressing of the animals, equipment, etc. Furthermore, due to the high microbial load of the intestines and skin, minimal contamination from these sources caused by incorrect gutting or skinning practices may provoke an important increase in the microbial load of the obtained meat.

The microorganisms that contaminate meat take from it the necessary nutrients for growth and also eliminate the metabolite from their activity into the environment. These actions result in a progressive alteration of the sensorial characteristics of the meat (color, smell, and texture), which in later stages makes the meat unfit for consumption.

To avoid the undesirable action of the spoiling microorganisms and to increase the shelf life of the meat, food technology has created different preservation procedures based on two different strategies: (i) the establishment of environmental conditions incompatible with the microbial growth, e.g., reducing the water activity (a_w), pH, or redox potential (Eh) values, or the environmental temperature; and (ii) the destruction of the spoilage microorganisms by an appropriate treatment (heat treatment, high hydrostatic pressure, etc.). These treatments should be accompanied by airtight packaging to avoid recontamination.

Because of the different environmental features that arise from the application of the different preservation methods, and because of the different microbial groups' varying sensitivity to these preservation systems, the microbial species present and their proportions vary notably in the different pork products. In this chapter, the microbial ecology of pork meat and of the pork products resulting from the different preservation methods are exposed and addressed in the depth that is allowed by the length of a book chapter.

23.2 Pork meat as a substrate for microbial growth: chemical and physical characteristics

The growth of contaminant biota in food substrates, and in pork in particular, depends on the environmental conditions that are well described in the literature (ICMSF, 1980). It is habitual to distinguish internal (intrinsic) factors related to the composition and conditions of the substrate from external (extrinsic) factors, namely environmental characteristics, that are independent of the substrate.

23.2.1 Intrinsic factors

23.2.1.1 Nutrients

On average, pork contains 69.4% water, 20.4% protein, 8.9% lipids, and 1% minerals in addition to vitamins acting as nutrients (USDA, 2014). Therefore, pork offers excellent conditions for the growth of most microorganisms. The higher vitamin B1 content in pork (900 $\mu\text{g}/100\text{ g}$) compared with beef (75 $\mu\text{g}/100\text{ g}$) (USDA, 2014) has been invoked to explain pork's shorter shelf life.

23.2.1.2 Structure

Microbial growth is not homogeneous in all pig carcass areas. The connective tissues (tendons, fascia, and aponeuroses) constitute natural barriers that impede the penetrations of microorganisms into the depth of the muscles; the same occurs for the fat surrounding the muscles. For these reasons, microbial growth occurs particularly rapidly in transversely cut muscle masses that are unprotected.

23.2.1.3 Water activity (a_w)

Water activity (a_w) is a measure of the amount of water that is available for the microorganisms and usable for growth. Values of a_w for fresh pork are in the 0.985–0.995 range, which is optimal for microbial growth. It is influenced by the environmental relative humidity. Meat tends to dehydrate on its surface under normal storage conditions. The drop in a_w values on the meat's surface inhibits microbial growth, especially that of the psychrotrophic flora. A contrasting

and detrimental effect would result in the accumulation (condensation) of water on the meat's surface.

23.2.1.4 pH

Normal pork reaches an ultimate pH of 5.6–5.7 within 3 to 5 hours after slaughter. Suboptimal values do not have a significant effect on the microbial growth. Pale, soft, exudative (PSE) pork undergoes a very quick pH decrease immediately after slaughter, reaching values of approximately 5.2; for this reason, PSE meat is slightly better preserved than other meats, but it has deficient characteristics (color and water holding capacity (WHC)) for processing. Dark, firm, dry (DFD) pork (pH values above 6.0–6.2) is more easily altered because it has pH values that are closer to neutral, which are optimal for most of the spoiling bacteria.

23.2.1.5 Eh

Eh values notably vary in the different pork tissues and products (e.g., whole raw liver, –200 mV; whole raw muscle, –150 mV; minced pork, +225 mV). Eh values are positive (from +150 to +200 mV) in the tissues of the living animal, but they drop immediately after slaughter, most likely because of O₂ consumption by the enzyme activities acting in the muscle. In contrast, cutting and chopping operations favor access to atmospheric oxygen and increase the Eh values. Eh values determine the growth of aerobic or anaerobic microorganisms (e.g., the growth of *Clostridia* on horse muscles does not occur until the Eh value has fallen from +150 mV to –50 mV (Lawrie and Ledward, 2006). Storage time diminishes the O₂ concentration due to microbial consumption and favors the growth of anaerobic microorganisms.

23.2.1.6 Other compounds

CO₂ and acids have some importance. CO₂ is formed as a consequence of the metabolism of some microorganisms and also as a product of the tissue respiration; CO₂ has an inhibitory effect on the aerobic bacteria. Acids, particularly lactic acid, produced during meat maturation

also have an inhibitory effect on the spoilage microorganisms.

The relevance of meat's intrinsic factors to sensorial shelf life has been widely demonstrated (see Blixt and Borch, 2002).

23.2.2 Extrinsic factors

23.2.2.1 Temperature

Microorganisms can grow and multiply within the temperature range of –15 to +75 °C. However, each microbial genus or even species limits its activity to a restricted range within this wide interval. Psychrotrophic bacteria are the main microorganisms responsible for the spoilage of pork, given typical storage temperatures.

23.2.2.2 Other factors

Some factors depend on the interaction of the microorganisms between them; these are related to the particular behavior of the different microbial species during growth. These interactions can be manifested in different ways, either inhibiting (antagonism) or stimulating (synergy) microbial growth.

23.3 Microbial ecology of fresh pork meat: sources of contamination and microbial groups

It is debatable whether meat contamination occurs during slaughter (Prändl *et al.*, 1988). As different authors pointed out (Gill, 1979; Zickrick *et al.*, 1986) it is very difficult to show viable microorganisms in the depth of muscles when slaughtering operates correctly.

The sources of pork contamination after slaughtering are diverse. Microorganisms come firstly from skin and accumulated dirt in the hooves, which often contain faecal debris. Microorganisms from the skin are, above all, psychrotrophs. Microorganisms from skin also come from deposited dust particles that provide soil flora, mainly sporulated bacteria. Dirt from the hooves in particular provides microorganisms of intestinal origin (*Enterobacteriaceae*,

enterococci, and lactic acid bacteria (LAB)). Microorganisms of intestinal origin can also access the carcass as a result of defective evisceration, which causes the breakdown of intestinal loops and the output of intestinal content that contaminates meat. This occurrence is often the source of major pathogens frequently present in pork, such as *Yersinia enterocolitica*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, etc.

Traditional scalding operations consisting of immersion in water baths can increase the microbial load of the skin surface due to the intense contamination of the scalding water. Given the water temperature (60–62°C), the deposition of sporulated aerobic bacteria from this source is mainly expected; however, pathogens such as *Yersinia enterocolitica* have recently been isolated from scalding water in abattoirs (Bonardi *et al.*, 2013). Modern scalding operations based on hot water showers or on the passage of pigs through steam-saturated tunnels avoid microbial contamination during scalding, and these methods can destroy the more heat-sensitive bacteria present on pig skin. Subsequent dehairing operations seem to increase the microbial load on the carcass surface (Pearce *et al.*, 2004). In any case, the skin as a source of microbial contamination is less relevant in pork than in cattle (Antic *et al.*, 2010) given the high microbial load of cattle hides and the possible transmission to meat. Carcass-washing operations can also increase the microbial load on the surface if the water is contaminated. The microbial load can vary among carcasses submitted to the same slaughtering process or even in the same abattoir or quartering room on different processing days. Zweifel *et al.* (2008), for example, reported that in different small-scale Swiss abattoirs, the total viable counts on the surfaces of pig carcasses ranged from 2.4 to 4.2 log₁₀ colony-forming units (cfu)/cm². The back was the most contaminated area (average counts of 3.22 log₁₀ cfu/cm²), followed by the neck (3.05 log₁₀ cfu/cm²), belly (2.86 log₁₀ cfu/cm²), and the ham (2.74 log₁₀ cfu/cm²). *Enterobacteriaceae* were detected in

23.9% of the carcasses but in low counts; the maximum *Enterobacteriaceae* counts of 3.81 log₁₀ cfu/cm² were detected in the neck area. Regarding the presence of pathogens, information in the literature is certainly abundant. In a recent study, Choi *et al.* (2013) reported that 77 of the 100 pig carcasses studied were positive for non-pathogenic *Escherichia coli* and/or pathogenic bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., or *Staphylococcus aureus*) in at least more than one sampling site. *B. cereus* was the most frequently detected pathogen (found in 48 of the 100 carcasses), followed by *L. monocytogenes* (27/100), *S. aureus* (8/100), and *Salmonella* spp. (1/100); *E. coli* O157:H7 and *Y. enterocolitica* were not detected in any of the 100 carcasses sampled. Bouvet *et al.* (2002) reported the presence of verotoxin-producing *E. coli* (VTEC) in 12% of the carcasses in French cutting plants. Bonardi *et al.* (2013) isolated *Salmonella enterica* from 10.9% of 451 carcasses studied and *Y. enterocolitica* from 2.4%. These same authors, after isolating and typing the strains isolated from the carcasses and also from faecal samples and tonsils, reported that apparently carcass contamination by *S. enterica* and *Y. enterocolitica* is more likely attributable to cross-contamination than to self-contamination. Regarding *Clostridium difficile* and methicillin-resistant *S. aureus*, a very low proportion (0–15%) of contaminated carcasses were found within the pork processing line (Hawken *et al.*, 2013).

Later, carcasses and meat cuts can be contaminated by handlers (hands and clothes). Hands can transfer microorganisms of nasal or faecal origin to meat as a result of handlers' poor personal hygiene; additionally, dirty clothes are important sources of contamination. In both cases, enterobacteria and psychrotrophs are the main microbial contaminants involved. The equipment used in the quartering rooms (knives, saws, tables, baskets, etc.) as well as the contaminated surfaces (floors and walls) are also a not negligible source of microorganisms; air can also be a source of contamination (Byrne *et al.*, 2008). Contamination from

the environment in quartering rooms usually consists of psychrotrophic microorganisms. Pork contamination and microbial growth continues next in retail outlets and local markets.

As a global result of all these contaminations, the microbial load (viable counts) on the surface of pork cuts usually ranges from 3 to 5 log₁₀ cfu/cm²; among these, coliforms and psychrotrophs usually represent more than 1–2 log₁₀ cfu/cm². Augustin and Minvielle (2008) reported mean counts ranging from 0.6 to 2.2 log₁₀ cfu/cm² for *Enterobacteriaceae* and from 1.1 to 4.4 log₁₀ cfu/cm² for *Pseudomonas* in pork meat cuts from cutting plants; the observed counts depended on the year of processing, the type of meat cut, and mainly the cutting plant. Concerning pathogens, Choi *et al.* (2013) again reported that *L. monocytogenes* and *B. cereus* were the most prevalent microorganisms in fresh pork loins in the processing lines of the slaughterhouses, retail outlets, and local markets; *E. coli* O157:H7 and *Yersinia enterocolitica* were also not detected in pork in markets. These authors did not report a high correlation between the frequency of pathogens in end-products at local markets and the initial contamination of the carcasses at the slaughterhouses, which indicates the importance of the contamination during the meat distribution chain. Bouvet *et al.* (2002) reported that meat contamination with verotoxin-producing *E. coli* (VTEC) increased between carcasses (12%) and primary cuts (19%) and then decreased between primary and secondary cuts (5%).

23.4 Microbial ecology of chilled pork meat

Storage at low temperatures, slightly higher than 0°C, in aerobic conditions is the most commonly used method for preserving meat, and this method minimally modifies its organoleptic characteristics. Although different temperatures near to 0°C (or even slightly lower than this value) are used in the storage chambers, studies are currently performed at 4°C.

As indicated in the previous section, the microbiota of the pork cuts immediately after quartering and cutting is highly variable depending on the sources of contamination, but consists mainly of mesophilic (psychrotrophic) bacteria belonging to the *Staphylococcaceae*, *Micrococcaceae* and *Enterobacteriaceae* families, and to the genera *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Lactobacillus*, and *Pseudomonas*; coryneforms and *Brochothrix thermosphacta* are also present in important proportions (Dainty *et al.*, 1983). During chill-storage under aerobic conditions, as a result of the selective action of the low temperatures, the initial mesophilic flora progressively change, mainly to psychrophilic Gram-negative rods that belong primarily to the *Acinetobacter*, *Pseudomonas*, and *Psychrobacter* genera (Dainty and Mackey, 1992). After storage at low temperatures, the *Pseudomonas* genus can account for up to 90% of the spoiling bacteria (Dainty and Mackey, 1992); *Pseudomonas fragi* is the main species present, followed by *P. lundensis* and *P. fluorescens* (Shaw and Latty, 1982; Blickstad and Molin, 1983; Molin and Terström, 1986; Dainty and Mackey, 1992; Molin, 2000).

This evolution in the microbiota from fresh to chilled pork, initially demonstrated using classical culture-dependent methods, was later corroborated using molecular approaches (Olson *et al.*, 2003; Li *et al.*, 2006). Olson *et al.* (2003), using methods of amplification, cloning, and sequencing of 16S rRNA, indicated that in fresh pork the isolated clones mainly resembled the *Acinetobacter*, *Staphylococcus*, and *Macroccoccus* genera; after 4 days of storage at 4°C, the isolated clones mainly belonged to the putative genera *Pseudomonas* (mainly *P. lundensis*), *Aeromonas* (being *A. veronii* dominant), and *Acinetobacter*. Using PCR-denaturing gradient gel electrophoresis (DGGE) techniques, Li *et al.* (2006) observed that *Pseudomonas* spp. and *Brochothrix thermosphacta* were the dominant bacteria during chill-storage (4°C); *Aeromonas* spp. increased up to the 4th day and then slightly decreased on the 7th day of storage. These authors

reported that the bacterial diversity of chilled pork decreased with the storage time.

The use of molecular culture-independent methods provides more accurate knowledge and descriptions of the microbial diversity in foods. Such methods have been used to better define the changes in microbial species present during chill-storage and to describe the variation in the genotypic characteristics of the strains within a concrete species. In this sense, Papadopoulou *et al.* (2012) observed a significant diversity among isolates of *Brochothrix thermosphacta* isolated from minced pork and found that the strains isolated at the initial stage differed from those present at the end of the storage at chill temperatures.

23.5 Microbial ecology of vacuum/modified atmosphere packaged pork meat

Because of the predominantly aerobic condition of spoilage bacteria, storage in free-oxygen or low-oxygen concentration atmospheres is widely used to increase the shelf life of pork. Low-O₂ modified atmosphere packaging (MAP) is often used for pork preservation, but vacuum packaging (VP) is the most simple, effective, and widely used method. In the low-O₂ MAP, an anoxic atmosphere with N₂ and CO₂ is used; while N₂ is an inert gas that does not react with meat pigments and is not absorbed by the meat, CO₂ interacts with the meat slightly and changes its properties, particularly its color.

In these preservation systems, the pork is firstly placed in trays and then sealed with a film with a well defined permeability to the gasses, after the application of a vacuum or flushing with the appropriate desired gas mixture (see Zhou *et al.*, 2010, for details).

The microbial flora present in pork preserved using these methods is obviously dependent on the specific nature of the atmosphere used and its gaseous composition, but, in general, MAP and VP favor the

evolution of the initial psychrotrophs population (mainly *Pseudomonas*) to a dominance of facultative anaerobes, mainly LAB and *B. thermosphacta* (Lee *et al.*, 1985; Nychas and Skandamis, 2005; Samelis, 2006; Schillinger *et al.*, 2006). It is well known that high concentrations of CO₂ (up to 10%) inhibit the growth of *Pseudomonas* in red meat; this inhibition is particularly effective in the case of *P. fragi* (Gill and Tan, 1980; Stambridge and Davies, 1998). In any case, the storage temperature also affects the type of dominant bacteria in MAP pork; McMullen and Stiles (1993) reported that at 10°C the prevalent microflora consisted of *Aeromonas*, *Enterobacteriaceae*, and LAB, while at 4.4 and -1°C, *Aeromonas*, *B. thermosphacta*, and LAB dominated. In a recent study, Nieminen *et al.* (2011), using molecular culture-independent methods, again demonstrated the dominance of LAB and *B. thermosphacta* in MAP minced pork meat. According to these authors, *Leuconostoc* (*L. gasicomitatum* and *L. gelidium*) and *Carnobacterium* (*C. divergens* and *C. maltaromaticum*) dominated in the LAB population, followed by *Lactobacillus algidus*, *Lactococcus* spp., and *Weissella* spp. *Hafnia alvei* dominated the *Enterobacteriaceae* community in both MAP (Säde *et al.*, 2013) and VP (Borch *et al.*, 1996) pork.

Regarding VP pork, Jiang *et al.* (2010) used PCR-DGGE methods and observed that LAB increased gradually during chilled storage at 4°C, while *Carnobacterium* sp./*C. divergens*, *Lactobacillus sakei*, and *Lactococcus* sp./*Lc. piscium* became the dominant bacteria at the end of the storage period. Holley *et al.* (2004) reported that the microbial flora of VP fresh pork also seemed to depend on the meat's microbial load before packaging and on the hygienic practices in the plants; LAB (and specifically *Carnobacterium*) dominated in all cases, but the meat produced in low-hygiene plants exhibited large counts of *Enterobacteriaceae*, *Aeromonas* spp., and *Shewanella* spp. The use of an appropriate plant-cleaning program yielded few *Enterobacteriaceae*, *Aeromonas*, and *Shewanella*.

23.6 Microbial ecology of marinated pork meat

From ancient times, marinades have been used to tenderize meat and to improve its organoleptic properties (Young and Lyon, 1997). The shelf life of fresh meat can often be extended by packaging products under vacuum or modified-atmosphere conditions, in addition to chill storage (Branen, 1983; Stanbridge and Davies, 1998; Tsigarida *et al.*, 2000). Today, marinade recipes vary considerably among countries, and sometimes salting, adding phosphates and some spices, is considered as marinating. In northern Europe, industrial marination is a process in which meat products are injected with a brine containing salt and phosphates before they are packed in a flavoring sauce containing salt, sugar, organic acids, and herbs or spices; in Mediterranean countries, where wine production is a tradition, the marinade is made with wine (usually red), salt, garlic, and other facultative seasonings. In Korea, different soy sauce-based marinades are used to produce “*Bulgodi*” or “*Galbi*”; they contain soy sauce, containing onion, garlic, sesame oil, and other seasonings, as well as hot-pepper paste along with garlic, onions, pears, starch syrup, and other seasonings. Recently, the availability of commercial marinades and ready-to-cook marinated meat has increased worldwide in response to a continuous increment of consumer demand (Schirmer *et al.*, 2009; Choi *et al.*, 2009; Ahn *et al.*, 2012; Linares *et al.*, 2013).

A few studies have examined the ecology of marinated pork meat and have underlined the product’s stability for approximately the first 10 days. The initial load depends on the microbial quality of the meat. Schirmer *et al.* (2009) established that LAB were the principal microorganisms involved, starting at 10^5 – 10^6 cfu/g, but they remained stable for approximately 10 days. The same trend was observed for the aerobes. After that time, all of the microbial groups increased, reaching values of 10^8 – 10^9 cfu/g. The anaerobic bacterial count was approximately 10^2 cfu/g at day 0 but increased exponentially

for 13 days. Coliforms ranged from 10^2 and 10^4 cfu/g, whereas *E. coli* were always below the detection limit (Ahn *et al.*, 2012).

The development of microbial flora in marinated and vacuum-packed pork shifts from aerobic, Gram-negative *Pseudomonas* to Gram-positive LAB. Regarding pathogens, *Bacillus cereus*, *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* were never detected in the survey by Ahn *et al.* (2012); however, *S. aureus* was detected in 22.2% of the tested samples, a percentage that is higher than that found by other authors (Pichner *et al.*, 2014). However, the presence of *Salmonella*, and particularly *S. Typhimurium*, was usually reported in 2 to 6% of pork meat samples (Prendergast *et al.*, 2008; Friedrich *et al.*, 2010; Hauser *et al.*, 2010; Meyer *et al.*, 2010; Rabsch *et al.*, 2013). *L. monocytogenes* was estimated in the range of 1 to 36% of samples (Yeh *et al.*, 2005; Netschajew *et al.*, 2009). Most of the isolates belonged to serotype 1/2a and 1/2c (Hong *et al.*, 2007; Hellstrom *et al.*, 2010). Other authors affirmed that the use of garlic, along with a starter culture of indigenous *L. sakei* in wine-based marinades, contributed to the control of both *L. monocytogenes* and *Salmonella* spp. (Linares *et al.*, 2013).

According to Pichner *et al.* (2014), *B. cereus* was isolated from 64 marinated pork steak samples (21%) at a concentration $<10^3$ cfu/g.

At the expiry point, LAB, especially *Lactobacillus* spp., *Leuconostoc* spp., and *Carnobacteria*, dominated in the late storage phases, and the production site had an effect on the development of the bacterial flora and their final composition in the product (Susiluoto *et al.*, 2003; Bjorkroth *et al.*, 2005; Koort *et al.*, 2005). Strains of *Lactobacillus algidus*, *L. sakei*, *L. curvatus*, *Carnobacterium divergens*, *C. maltaromaticum*, *Leuconostoc mesenteroides*, and *Leuc. carnosum* were isolated. As Schirmer *et al.* (2009) observed, *L. algidus* may be an important, but underestimated, spoilage organism because of the sour and intense off-odors associated with its presence.

23.7 Microbial ecology of cured and fermented/ripened pork meats

During the production of fermented sausages, which dates back to the Roman time in the Mediterranean regions (Comi *et al.*, 2005; Hammes *et al.*, 2008), all of the above-mentioned hurdles (the reduction of water activity and pH, smoking, chilling, and the use of additives) are used, but also the antagonistic compounds produced by the fermenting microflora contribute in the progressive reduction of the undesirable microorganisms. During the production of cured meat products (e.g., dried and cured ham) the most effective hurdle involved in the production process is the decrement of a_w and lactic acid fermentation did not occur.

Fermentation of sausages is a well established and well known microbial process; the first ecological studies date back to the 1960s (Lerche and Reuter, 1960; Reuter, 1972; Lücke, 1974). During fermentation complex biochemical and physical reactions occur, resulting in a significant change of the initial characteristics, and the production of aromatic compounds, which significantly defines the sensorial characteristics of the final product.

The microflora that develop are often closely related to the ripening technique and ingredient utilized (Lebert *et al.*, 2007; Talon *et al.*, 2007). Sausages with a short ripening time have more lactobacilli right from the early stages of fermentation and at the end of the ripening time an acid flavor with little aroma predominates in salami. In contrast, sausages with longer maturing times contain higher numbers of coagulase-negative cocci (CNC) (Iacumin *et al.*, 2006a).

The LAB counts of raw meat can vary from 10^2 – 10^3 cfu/g to 10^3 – 10^5 cfu/g, depending on the meat's quality (Rebecchi *et al.*, 1998; Cocolin *et al.*, 2001a, 2001b; Chevallier *et al.*, 2006; Talon *et al.*, 2007). LABs can rapidly dominate the fermentation, reaching values of 10^7 – 10^9 cfu/g (Comi *et al.*, 2005; García-Fontán *et al.*, 2007a, 2007b; Fonseca *et al.*, 2013) in both the

core and the external layers of the sausage (Coppola *et al.*, 2000). In some other cases, LABs continue to increase throughout the entire process, but similar final values were detected at the end of the process.

LABs decrease pH via lactic acid production and produce bacteriocins against pathogenic and spoilage microorganisms (Vogel *et al.*, 1993; Hugas *et al.*, 1995; Aymerich *et al.*, 2006; Bonomo *et al.*, 2008); contribute to the flavor, color, and texture development; and have a photolytic effect on muscle sarcoplasmatic proteins (Hammes *et al.*, 1990; Hammes and Knauf, 1994; Fontana *et al.*, 2005; Fadda *et al.*, 1998, 1999a, 1999b, 2002). Moreover, LABs are responsible for the “tangy” flavor of sausages and for the small amounts of acetic acid, ethanol, acetoin, pyruvic acid, and carbon dioxide (Demeyer, 1982; Bacus, 1986). Among LABs, *Lactobacillus sakei*, *L. curvatus*, and *L. plantarum* are the most commonly isolated species (Hammes *et al.*, 1990; Aymerich *et al.*, 2003; Papamanoli *et al.*, 2003; Lebert *et al.*, 2007). *L. sakei* is considered as the dominant species and can account for 42% (Italian and Greek sausages), 76–89% (Spanish sausages), and even up to 100% of the isolates (French sausages) (Coppola *et al.*, 2000; Papamanoli *et al.*, 2003; Ammor *et al.*, 2005; Comi *et al.*, 2005; Greco *et al.*, 2005; Talon *et al.*, 2007). *L. curvatus* is generally the second species found, although some authors have stated that in some Greek and Italian sausages, it can share the fermentation with or even dominate *L. sakei* (Rantsiou *et al.*, 2005). *L. plantarum* was the third most commonly isolated species in all cases except for one Greek traditional fermented sausage, in which it was the dominant LAB species (37.2%) (Drosinos *et al.*, 2005). *L. paracasei* subsp. *paracasei*, *L. alimentarius*, *L. casei*, *L. rhamnosus*, *L. brevis*, *L. farciminis*, *L. paraplantarum*, *L. pentosus*, *L. salivarius*, *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Ln. pseudomesenteroides*, *Ln. carnosus*, *Lactococcus lactis* subsp. *lactis*, *Lc. garviae*, *Pediococcus acidilactici*, *P. pentosaceus*, and *Enterococcus faecium* were also detected at

a lower concentration and often only at specific times of fermentation.

Coagulase-negative catalase-positive cocci (CNCPC) constitute the second most common microbiota in sausage fermentation. Usually, the initial load is approximately 10^3 – 10^4 cfu/g, but CNCPC can then grow throughout the production process or only during fermentation or ripening, depending on the applied production technology, reaching values of 10^6 – 10^8 cfu/g (Iacumin *et al.*, 2006a; Fonseca *et al.*, 2013). CNCPCs are responsible for the decomposition of peroxide (Andres, 1977) and the development and stability of the characteristic red color, as a result of the nitrate reductase activity. Nitrites, which are also produced, can limit lipid oxidation. Moreover, the proteolytic and lipolytic activities of CNCPC influence both texture and flavor development via the release of such compounds as peptides, amino acids, aldehydes, amines, and free fatty acids, which are important flavor compounds or precursors of flavor compounds (Demeyer *et al.*, 2004). The most commonly isolated species in Greek, Spanish, and Italian traditional products is *S. xylosus*, representing from 17 to 100% of the isolated species (Papamanoli *et al.*, 2003; Iacumin *et al.*, 2006a; Metaxopoulos, 2007). *S. saprophyticus* and *S. equorum* are the second and third most dominant species, depending on the geographical location of production (García Fontán *et al.*, 2007a, 2007b; Talon *et al.*, 2007). In some Greek and Italian products, *S. saprophyticus* (Drosinos *et al.*, 2005) and *S. equorum* (Corbière Morot-Bizot *et al.*, 2006; Fonseca *et al.*, 2013) make important contributions to the microbial ecology. Other than in French and Spanish products, they are almost always present in Italian sausages as well, along with *S. succinus*. However, this species was not detected in the traditional “Androlla” and “Botillo” Spanish products, or its counts were consistently lower than those obtained for *S. xylosus* (García Fontán *et al.*, 2007a, 2007b). Some authors suggest that *S. equorum* has been underestimated in the past because of its phenotypic similarities

to *S. xylosus* (Corbière Morot-Bizot *et al.*, 2006). Other minor species also participate in the fermentation of different traditional products, including *S. carnosus*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lentus*, *S. pasteurii*, *S. warneri*, *S. intermedius*, *S. vitulus*, *S. auricularis*, *S. sciuri*, *Macrococcus caseolyticus*, *Micrococcus luteus*, *M. lylae*, *Kocuria varians*, and *K. kristinae* (Fontana *et al.*, 2005; Iacumin *et al.*, 2006a, 2012b). Studies of the genetic characterization of the isolated LAB and CNCPC strains have affirmed that considerable strain variability exists and have recognized that, in some cases, these strains are plant- or product-specific, confirming the theory that in a production plant there is always a specific selection of microflora via process conditions and ingredients that may influence the final characteristics of the products (Iacumin *et al.*, 2006b; Ammor *et al.*, 2005).

The surface flora, which consists of molds and yeasts, is also important for the sensory properties of these pork products (Santos *et al.*, 2001; Andrade *et al.*, 2010). Yeasts are usually detected in the batter at levels varying from 2.0 to 4.5 log cfu/g (Lebert *et al.*, 2007). Samelis *et al.* (1993) and Metaxopoulos *et al.* (2001) determined a stable number of 10^5 cfu/g in all stages of production. Yeasts prefer to grow internally, while molds develop on the surface because of oxygen availability. *D. hansenii* is the dominant yeast species associated with fermented sausages, but *Candida krisii*, *Willopsis saturnus*, and *C. sake* or *austromarina* are dominant in other Italian fermented sausages; *D. hansenii* was never detected (Flores *et al.*, 2004; Rantsiou *et al.*, 2005; Cocolin *et al.*, 2006; Silvestri *et al.*, 2007). *C. physchrophila* and *Saccharomyces barnettii* were also occasionally found (Silvestri *et al.*, 2007).

Molds such as *Penicillium nalgiovense*, *P. chrysogenum*, *P. farinosum*, *P. viridicatum*, and *Mucor racemosus* participate in the transformation process because of their capacities for producing lipases and proteases, creating micropores on the casing that facilitate the

dehydration process, and protecting lipids from oxidation in the presence of light (Lücke, 1974; Leistner, 1986; Mutti *et al.*, 1992; Incze, 2004). Nevertheless, other prejudicial molds can grow on sausages, making them unhealthy or producing spoilage (Mutti *et al.*, 1992; Larsen *et al.*, 2001). Among the mycotoxigenic species, strains of *P. verrucosum*, *P. nordicum*, and *Aspergillus ochraceus* are able to produce ochratoxin A (OTA), under specific environmental and substrate conditions (Toscani *et al.*, 2007; Iacumin *et al.*, 2009, 2011, 2012a).

The trend of enterococci is different and depends on the different types of sausages. The initial enterococci level was observed between 10^2 and 10^4 cfu/g, and they usually grew during early fermentation, particularly in high-pH traditional sausages, and then remained constant at a level of 10^4 – 10^6 cfu/g (Rebecchi *et al.*, 1998; Samelis *et al.*, 1998; Metaxopoulos *et al.*, 2001; Comi *et al.*, 2005). Conversely, in other cases, a decline was observed, leading to a final count of approximately 10^2 cfu/g. Total enterobacteria and *E. coli* were usually present only during the first days of fermentation, and their counts declined as a result of the LABs' acidification process (Aymerich *et al.*, 2003; Cocolin *et al.*, 2001a, 2001b; Papamanoli *et al.*, 2003). Sulphite-reducing clostridia were usually not present (Samelis *et al.*, 1998; Aymerich *et al.*, 2003; Papamanoli *et al.*, 2003; Comi *et al.*, 2005). However, considering the pathogenic bacteria, several studies investigated the presence of *L. monocytogenes* and *Salmonella* spp. during the different stages of the production. It has been determined that, generally, these food-borne pathogens are absent in 25 g of product at the end of the production process (>20% weight loss), although they can be present in the raw meat batter (Samelis *et al.*, 1998; Metaxopoulos *et al.*, 2001; Rantsiou *et al.*, 2005).

Regarding dry-cured ham (and other whole-meat products), very few studies have described the bacterial ecology; the available studies confirm that homofermentative and facultative

heterofermentative lactobacilli are the main groups in both Spanish and Italian hams and that *L. plantarum* is the most commonly isolated species (Silla *et al.*, 1985; Molina *et al.*, 1989; Lorenzo *et al.*, 2010).

The total aerobic mesophilic microflora, mainly represented by CNCPC, *Enterobacteriaceae*, and *Pseudomonadaceae*, is mostly present on the surface of the product, ranging from 10^4 to 10^7 cfu/g and increases significantly during the post-salting stage and during the first days of the drying–ripening stage (Huerta *et al.*, 1987; Lorenzo *et al.*, 2010). *E. agglomerans* and *Serratia liquefaciens* (able to grow at 2°C and resistant at salt concentrations up to 7.5%) can be responsible for taint development.

LABs ranged from 10^3 to 10^5 cfu/g on the surface of the product and from 80 to 150 cfu/g in the interior part of the meat (Huerta *et al.*, 1987; Marín Riaño *et al.*, 1993), indicating that they do not play an important role in this product (Molina *et al.*, 1989). Among LAB, *Lactobacillus curvatus*, *L. sakei*, *Enterococcus faecium*, *Lactococcus garvieae*, *E. durans*, *E. faecalis*, *E. inter faecalis-faecium*, *L. alimentarius*, and *Lactobacillus plantarum* were isolated from the surface of dry-cured lacón, whereas *L. sakei*, *L. alimentarius*, *L. curvatus*, *Lactococcus garvieae*, and *L. plantarum* were from the interior of the pieces (Lorenzo *et al.*, 2010). Other species identified were *L. farciminis*, *E. faecalis*, *E. faecium*, *E. durans*, *E. interfaecalis-faecium*, and *Leuconostoc mesenteroides* subsp. *mesenteroides*.

The ecology of molds on the surface of dry-cured ham has been widely studied (Leistner and Ayres, 1968; Hadlock *et al.*, 1976; Dragoni and Cantoni, 1979; Dragoni *et al.*, 1980a, 1980b; Montel *et al.*, 1986; Rojas *et al.*, 1991; Spotti *et al.*, 1988, 1989, 2001a, 2001b; Nuñez *et al.*, 1996; Comi *et al.*, 2004; Wang *et al.*, 2006; Battilani *et al.*, 2007; Comi and Iacumin, 2013). *Penicillium*, *Aspergillus*, and *Eurotium* species seem to be present during all stages of ham production and represent more than 97% of the isolates in Istrian cured ham. *Eurotium repens*

was the only species isolated among that genera, whereas in San Daniele ham, *E. amstelodami* was isolated (Comi *et al.*, 2004; Comi and Iacumin, 2013). Some authors have also noted the succession of *Penicillium* spp. by *Aspergillus* spp. and *Eurotium* spp. during the ripening period in hams with low a_w and those that are ripened under low relative humidity (Leistner and Ayres, 1968; Spotti *et al.*, 1989; Hernandez and Huerta, 1993). *Penicillium frequentans*, *P. verrucosum*, *P. lanosocoeruleum*, *P. lanosogriseum*, *P. chrysogenum*, *P. commune*, *P. herquei*, *P. citreonigrum*, *P. diversum*, *P. radicum*, and *P. expansum* were the most frequently isolated *Penicillium* strains at both pre-ripening and end-ripening periods, but *P. citrinum* and *Penicillium marneffei* were also found occasionally. *P. verrucosum* can dominate the mold flora of dry hams only when they are ripened in high relative humidity. In particular, *Penicillium* spp. and *Penicillium commune* are recognized to be responsible for producing off-flavors and the phenic acid defect, respectively (Northolt and Bullerman, 1982; Spotti *et al.*, 1988). Among *Aspergillus* spp., *Aspergillus flavus*, *A. repens*, *A. parasiticus*, *A. candidus*, *A. ochraceus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. nidulans*, *A. mellesius*, and *A. flavipes* were the most commonly detected species (Comi *et al.*, 2004; Comi and Iacumin, 2013; Rojas *et al.*, 1991). Other species, such as *Beauveria bassiana*, *Cladosporium cladosporioides*, *C. herbarum*, *Fusarium acutatum*, *F. annulatum*, *F. solani*, *Nigrospora oryzae*, *Paecilomyces variotii*, *Phlebia radiata*, *Spaerothryium filicinum*, *Sporidesmiella fusiformis*, *Trametes versicolor*, and *Trichoderma viride*, were also retrieved either from the surface of the product or from the air of the ripening rooms (Comi and Iacumin, 2013). The prevalence of a specific genus on the surface of dry-cured hams depends on the ripening temperature, the moisture of the meat, and the relative humidity of the environment; the presence of moldy areas on a ham is a sign of improper management of aging conditions and can lead to negative consequences (Comi *et al.*, 2004).

23.8 Microbial ecology of high-pressure preserved pork meat

High-pressure processing (HPP) technology is a very promising mild preservation technique for preserving meat, particularly sliced, cooked, and cured meat products. The first experiments with this technology date back to the late 1970s, when the technique was first applied to increase the meat tenderness (Bouton *et al.*, 1977; Cheftel and Culioli, 1996); later, its potential for extending shelf life was understood. The process strongly depends on the type of food being preserved; in the case of meat, it has to be taken into consideration that high-pressure treatment can induce irreversible changes in the components and properties of meat (Cheftel and Culioli, 1996; Hugas *et al.*, 2002). At pressures higher than 150 MPa, the color of the meat becomes similar to that of cooked meat. Increasing the pressure to values above 400 MPa denatures the globin protein. The higher the pressure, the more changes occur in protein structure and texture, color and lipid oxidation (Angsupanich and Ledward, 1998; Angsupanich *et al.*, 1999; Bouton *et al.*, 1977; Jung *et al.*, 2000; Ma *et al.*, 2007). The effects on the microbial flora and the inactivation kinetics depend on several parameters, such as the type of microorganism, pressure level, treatment time, pH, a_w , and meat composition (Hugas *et al.*, 2002; Carlez *et al.*, 1993, 1994). In general, there is a strict positive correlation between increased pressure and microorganism inactivation; however, when using real pork meat products, moderate pressure is recommended to avoid deterioration of the product and for economic reasons. Clearly, this compromise is unable to achieve a substantial inactivation of microorganisms, but it will allow the extension of the product's shelf life (Malinowska-Panczyk and Kolodziejska, 2013). As a general rule, gram-positive microorganisms are more resistant to HPP than gram-negative ones (Hugas *et al.*, 2002). Psychotrophic bacteria are more sensitive than mesophilic bacteria because

they lose their capacity to grow at refrigeration temperatures (Yuste *et al.*, 1998). Cell morphology also determines the effect; bacilli are more sensitive than cocci. Cells exposed to various stresses become more resistant and cells in the stationary growth phase seems to be more resistant compared with those in the exponential growth phase (Archer, 1996; Hugas *et al.*, 2002). Pathogenic species, such as *L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella typhimurium*, have different pressure sensitivities; moreover, the sensitivity seems to be strain-dependent (Alpas *et al.*, 1999, 2000; Moerman *et al.*, 2001; Garriga *et al.*, 2004; Malinowska-Panczyk *et al.*, 2009). HPP was very effective on *Pseudomonas* spp. and was able to reduce those species to below detection level ($<10^2$ cfu/g) (Linton *et al.*, 2004; Diez *et al.*, 2008). The same was not true for LAB populations, which were only marginally affected by the treatment. Diez *et al.* (2008) observed that the main spoilage bacteria were *W. viridescens*, *W. confusa*, *L. lactis*, and *L. mesenteroides*, although they differed in prevalence, compared with the untreated samples. The results indicated that *L. mesenteroides* and *Brochotrix thermosphacta* are more sensitive to high pressures compared with *W. viridescens*; in fact, these species were not present in the treated samples, a result that was confirmed also by other authors (Basak *et al.*, 2002; Tahiri *et al.*, 2006; Diez *et al.*, 2008).

Additionally, the use of moderate pressure at sub-zero temperatures proved to be very effective, minimizing the undesired effects on the meat. Using 193 MPa and -20°C , Malinowska-Panczyk *et al.* (2009) observed considerable inactivation and inhibition of the growth of psychrophilic microflora (>4 log), along with significant inactivation of coliforms and a relatively high degree of inactivation of coagulase-positive *Staphylococcus*. As far as sporulating microorganisms are concerned, their pressure sensitivity corresponds with their temperature sensitivity, meaning that *Streptococcus faecalis* is more sensitive than *Bacillus subtilis*, followed by *Geobacillus stearothermophilus*. The high pressure seems to make an important

contribution to the total effect of the combination of temperature and high pressure, but the optimal treatment condition was too high to be applied to pork meat products without causing damage (400 MPa, 50°C , 30 min for commercial sterilization; 250–350 MPa, 65 – 75°C , for pasteurization) (Moerman *et al.*, 2001).

References

- Ahn, S.H., Lee, Y.J., Lee, J.-I., and Paik, H.-D. 2012. Microbiological investigation of ready-to-cook pork Bulgogi on Korean markets. *Korean Journal of Food Science*, **32**, 441–447.
- Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C. P., and Ray, B. 1999. Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Applied and Environmental Microbiology*, **65**, 4248–4251.
- Alpas, H., Kalchayanand, N., Bozoglu, F., and Ray, B. 2000. Interactions of high hydrostatic pressure, pressurization temperature and pH on death an injury of pressure-resistant and pressure-sensitive strains of food borne pathogens. *International Journal Food Microbiology*, **60**, 33–42.
- Ammor, S., Rachman, C., Chaillou, S., Prévost, H., *et al.* 2005. Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. *Food Microbiology*, **22**, 373–382.
- Andrade, M.J., Córdoba, J.J., Casado, E.M., Córdoba, M.G., and Rodríguez, M. 2010. Effect of selected strains of *Debaryomyces hansenii* on the volatile compounds production of dry fermented sausage “salchichón”. *Meat Science*, **85**, 256–264.
- Andres, C. 1977. Starter culture for sausage has two microorganisms for better performance. *Journal of Food Protection*, **38**, 132–133.
- Angsupanich, K. and Ledward, D.A. 1998. High pressure treatment effects on cod (*Gadus morhua*) muscle. *Food Chemistry*, **63**, 39–50.
- Angsupanich, K., Edde, M., and Ledward, D.A. 1999. Effects of high pressure on the myofibrillar

- proteins of cod and turkey muscle. *Journal of Agricultural and Food Chemistry*, **47**, 92–99.
- Antic, D., Blagojevic, B., Ducic, M., Nastasijevic, I., Mitrovic, R., and Buncic, S. 2010. Distribution of microflora on cattle hides and its transmission to meat via direct contact. *Food Control*, **21**, 1025–1029.
- Archer, D.L. 1996. Preservation microbiology and safety: evidence that stress enhances virulence and triggers adaptive mutations. *Trends in Food Science and Technology*, **7**, 91–95.
- Augustin, J.-C. and Minvielle, B. 2008. Design of control charts to monitor the microbiological contamination of pork meat cuts. *Food Control*, **19**, 82–97.
- Aymerich, T., Martin, B., Garriga, M., and Hugas, M. 2003. Microbial quality and direct PCR identification of lactic acid bacteria and non-pathogenic staphylococci from artisanal low-acid sausages. *Applied and Environmental Microbiology*, **69**, 4583–4594.
- Aymerich, T., Martin, B., Garriga, M., Vidal-Carou, M.C., Bover-Cid, S., and Hugas, M. 2006. Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *Journal of Applied Microbiology*, **100**, 40–49.
- Bacus, J.N. 1986. Fermented meat and poultry products. In: *Advances in Meat and Poultry Microbiology* (ed. Pearson, A.M.D.), pp. 123–164. Macmillan, London, UK.
- Basak, S., Ramaswamy, H.S., and Piette, J.P.G. 2002. High pressure destruction kinetics of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and concentrated orange juice. *Innovative Food Science and Emerging Technologies*, **3**, 223–231.
- Battilani, P., Pietri, V.A., Giorni, P., Formenti, S., et al. 2007. *Penicillium* populations in dry-cured ham manufacturing plants. *Journal of Food Protection*, **70**, 975–980.
- Bjorkroth, J. 2005. Microbiological ecology of marinated meat products. *Meat Science*, **70**, 477–480.
- Blickstad, E. and Molin, G. 1983. Carbon dioxide as a controller of the spoilage flora of pork, with special reference to temperature and sodium chloride. *Journal of Food Protection*, **46**, 756–763.
- Blixt, Y. and Borch, E. 2002. Comparison of shelf life of vacuum-packed pork and beef. *Meat Science*, **60**, 371–378.
- Bonardi, S., Bassi, L., Brindani, F., D'Incau, M., Barco, L., Carra, E., and Pongolini, S. 2013. Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and *Yersinia enterocolitica* in pigs at slaughter in Italy. *Meat Science*, **163**, 248–257.
- Bonomo, M.G., Ricciardi, A., Zotta, T., Parente, E., and Salzano G. 2008. Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy). *Meat Science*, **80**, 1238–1248.
- Borch, E., Kant-Muermans, M.L., and Blixt, Y. 1996. Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, **33**, 103–120.
- Bouton, P.E., Ford, A.L., Harris, P.V., MacFarlane, J.J., and O'Shea, J.M. 1977. Pressure-heat treatment of postrigor muscle: effects on tenderness. *Journal of Food Science*, **42**, 132–135.
- Bouvet, J., Bavai, C., Rossel, R., Le Roux, A., et al. 2002. Effects of cutting process on pork meat contamination by verotoxin-producing *Escherichia coli* (VTEC) and *E. coli* O157.H7. *International Journal of Food Microbiology*, **77**, 91–97.
- Branen, A.L. 1983. Introduction to use of antimicrobials. In: *Antimicrobials in Food* (eds Branen, A.L. and Davidson, P.M.), pp. 1–9. Marcel Dekker, Inc., New York.
- Byrne, B., Lyng, J., Dunne, G., and Bolton, D.J. 2008. An assessment of the microbial quality of the air within a pork processing plant. *Food Control*, **19**, 915–920.
- Carlez, A., Rosec, J.P., Richard, N., and Cheftel, J.C. 1993. High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle. *LWT - Food Science and Technology*, **26**, 357–363.
- Carlez, A., Rosec, J.P., Richard, N., and Cheftel, J.C. 1994. Bacterial growth during chilled storage of pressure-treated minced meat. *LWT - Food Science and Technology*, **27**, 48–54.
- Cheftel, J.C. and Culioli, J. 1996. Effect of high pressure on meat: a review. *Meat Science*, **46**, 211–236.

- Chevallier, I., Ammor, S., Laguet, A., Labayle, S., *et al.* 2006. Microbial ecology of a small-scale facility producing traditional dry sausage. *Food Control*, **17**, 446–453.
- Choi, Y.M., Bae, Y.Y., Kim, K.H., Kim, B.C., and Rhee, M.S. 2009. Effects of supercritical carbon dioxide treatment against generic *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *E. coli* O157:H7 in marinades and marinated pork. *Meat Science*, **82**, 419–424.
- Choi, Y.M., Park, H.J., Jang, H.I., Kim, S.A., Imm, J.Y., Hwang, I.G., and Rhee, M.S. 2013. Changes in microbial contamination levels of porcine carcasses and fresh pork in slaughterhouses, processing lines, retail outlets, and local markets by commercial distribution. *Research in Veterinary Science*, **94**, 413–418.
- Cocolin, L., Manzano, M., Aggio, D., Cantoni, C., and Comi, G. 2001a. A novel polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) for the identification of Micrococcaceae strains involved in meat fermentations. Its application to naturally fermented Italian sausages. *Meat Science*, **58**, 59–64.
- Cocolin, L., Manzano, M., Cantoni, C., and Comi, G. 2001b. Denaturing gradient gel electrophoresis analysis of the 16S rRNA gene V1 region to monitor dynamic changes in the bacterial population during fermentation of Italian sausages. *Applied and Environmental Microbiology*, **67**, 5113–5121.
- Cocolin, L., Urso, R., Rantsiou, K., Cantoni, C., and Comi, G. (2006) Dynamics and characterization of yeasts during natural fermentation of Italian sausages. *FEMS Yeast Research*, **6**, 692–701.
- Comi, G. and Iacumin, L. 2013. Ecology of moulds during the pre-ripening and ripening of San Daniele dry cured ham. *Food Research International*, **54**, 1113–1119.
- Comi, G., Orlic, S., Redzepovic, S., Urso, R., and Iacumin, L. 2004. Moulds isolated from Istrian dried ham at the pre-ripening and ripening level. *International Journal of Food Microbiology*, **96**, 29–34.
- Comi, G., Urso, R., Iacumin, L., Rantsiou, K., Cattaneo, P., Cantoni, C., and Cocolin, L. 2005. Characterization of naturally fermented sausages produced in North East of Italy. *Meat Science*, **69**, 381–392.
- Coppola, S., Mauriello, G., Aponte, M., Moschetti, G., and Villani, F. 2000. Microbial succession during ripening of Naples-type salami, a southern Italian fermented sausage. *Meat Science*, **56**, 321–329.
- Corbière Morot-Bizot, S., Leroy, S., and Talon, R. 2006. Staphylococcal community of a small unit manufacturing traditional dry fermented sausages. *International Journal of Food Microbiology*, **108**, 210–217.
- Dainty, R.H., Shaw, B.G., and Roberts, T.A. 1983. Microbial and chemical changes in chill-stored red meats. In: *Food Microbiology: Advances and Prospects* (eds Roberts, T.A. and Skinner, F.A.), pp. 151–178. Academic Press, London, UK.
- Dainty, R.H. and MacKey, B.M. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and storage processes. *Journal of Applied Bacteriology Symposium Supplement*, **73**, 103S–114S.
- Demeyer, D.I. (1982) Stoichiometry of dry sausage fermentation. *Antonie van Leeuwenhoek*, **48**, 414–416.
- Demeyer, D. (2004) Meat fermentation: principles and application, Chapter 20. In: *Handbook of Food and Beverage Fermentation Technology* (eds Hui, Y.H., Meunier-Goddik, L., Hansen, A.S., Josephsen, J., *et al.*), pp. 353–368. Marcel Dekker Inc., New York, USA.
- Diez, A.M., Urso, R., Rantsiou, K., Jaime, I., Rovira, J., and Cocolin, L. 2008. Spoilage of blood sausages morcilla de Burgos treated with high hydrostatic pressure. *International Journal of Food Microbiology*, **123**, 246–253.
- Dragoni, I. and Cantoni, C. 1979. Le muffe negli insaccati crudi stagionati. *Industrie Alimentari*, **18**, 281–285.
- Dragoni, I., Marino, C., and Cantoni, C. 1980a. Muffe in prodotti carnei salati e stagionati (bresaole e prosciutti crudi). *Industrie Alimentari*, **19**, 405–407.
- Dragoni, I., Ravenna, R., and Marino, C. 1980b. Descrizione delle specie di *Aspergillus* isolate dalla superficie di prosciutti stagionati di Parma

- e San Daniele. *Archivio Veterinario Italiano*, **31**, 1–56.
- Drosinos, E.H., Mataragas, M., Xiraphi, N., Moschonas, G., Gaitis, F., and Metaxopoulos, J. 2005. Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science*, **69**, 307–317.
- Fadda, S., Vignolo, G., Holgado, A.P.R., and Oliver, G. 1998. Proteolytic activity of *Lactobacillus* strains isolated from dry-fermented sausages on muscle sarcoplasmic proteins. *Meat Science*, **49**, 11–18.
- Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M.C., Oliver, G., and Toldrá, F. 1999a. Characterization of muscle sarcoplasmic and myofibrillar protein hydrolysis caused by *Lactobacillus plantarum*. *Applied and Environmental Microbiology*, **65**, 3540–3546.
- Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M.C., Oliver, G., and Toldrá, F. 1999b. Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *Applied and Environmental Microbiology*, **65**, 578–584.
- Fadda, S., Oliver, G., and Vignolo, G. 2002. Protein degradation by *Lactobacillus plantarum* and *Lactobacillus casei* in a sausage model system. *Journal of Food Science*, **67**, 1179–1183.
- Flores, M., Durá, M.A., Marco, A., and Toldrá, F. 2004. Effect of *Debaryomyces* spp. on aroma formation and sensory quality of dry-fermented sausages. *Meat Science*, **68**, 439–446.
- Fonseca, S., Cachaldora, A., Gómez, M., Franco, I., and Carballo, J. 2013. Monitoring the bacterial population dynamics during the ripening of Galician chorizo, a traditional dry fermented Spanish sausage. *Food Microbiology*, **33**, 77–84.
- Fontana, C., Cocconcelli, P.S., and Vignolo, G. 2005. Monitoring the bacterial population dynamics during fermentation of artisanal Argentinian sausages. *International Journal of Food Microbiology*, **103**, 131–142.
- Friedrich, A., Dorn, C., Schroeter, A., Szabo, I., et al. 2010. Report on *Salmonella* isolates in livestock, food and feed, received at the German National Reference Laboratory for *Salmonella* during 2004–2008. *Berliner und Münchener Tierärztliche Wochenschrift*, **123**, 265–277.
- García Fontán, M.C., Lorenzo, J.M., Martínez, S., Franco, I., and Carballo, J. 2007a. Microbiological characteristics of Botillo, a Spanish traditional pork sausage. *LWT – Food Science and Technology*, **40**, 1610–1622.
- García Fontán, M.C., Lorenzo, J.M., Parada, A., Franco, I., and Carballo, J. 2007b. Microbiological characteristics of “androlla”, a Spanish traditional pork sausage. *Food Microbiology*, **24**, 52–58.
- Garriga, M., Grebol, N., Aymerich, M.T., Monfort, J.M., and Hugas, M. 2004. Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science and Emerging Technologies*, **5**, 451–457.
- Gill, C.O. 1979. A review: intrinsic bacteria in meat. *Journal of Applied Bacteriology*, **47**, 367–378.
- Gill, C.O. and Tan, K.H. 1980. Effect of carbon dioxide on growth of meat spoilage bacteria. *Applied and Environmental Microbiology*, **39**, 317–319.
- Greco, M., Mazette, R., De Santis, E.P.L., Corona, A., and Cosseddu, A.M. 2005. Evolution and identification of lactic acid bacteria isolated during the ripening of Sardinian sausages. *Meat Science*, **69**, 733–739.
- Hadlock, R., Samson, R., Stolk, A., and Schipper, M. 1976. Mould contamination of meat products. *Fleischwirtschaft*, **56**, 322–327.
- Hammes, W.P. and Knauf, H.J. 1994. Starters in processing of meat products. *Meat Science*, **36**, 155–168.
- Hammes, W.P., Bantleon, A., and Min, S. 1990. Lactic acid bacteria in meat fermentation. *FEMS Microbiology Reviews*, **87**, 165–174.
- Hammes, W.P., Haller, D., and Gänzle, M.G. 2008. Fermented meat. In: *Handbook of Fermented Functional Foods* (ed. Farnworth, E.R). CRC Press, Boca Raton, FL, USA.
- Hauser, E., Tietze, E., Helmuth, R., Junker, E., et al. 2010. Pork contaminated with *Salmonella enterica* serovar 4,[5],12:i:-, an emerging health risk for humans. *Applied and Environmental Microbiology*, **76**, 4601–4610.
- Hawken, P., Weese, J.S., Friendship, R., and Warriner, K. 2013. Carriage and dissemination

- of *Clostridium difficile* and methicillin resistant *Staphylococcus aureus* in pork processing. *Food Control*, **31**, 433–437.
- Hellstrom, S., Laukkanen, R., Siekkinen, K.M., Ranta, J., Maijala, R., and Korkeala, H. 2010. *Listeria monocytogenes* contamination in pork can originate from farms. *Journal of Food Protection*, **73**, 641–648.
- Hernández, E. and Huerta, T. 1993. Evolución de los parámetros microbiológicos del jamón curado. *Microbiología SEM*, **9**, 10–19.
- Holley, R.A., Peirson, M.D., Lam, J., and Tan, K.B. 2004. Microbial profiles of commercial, vacuum-packaged, fresh pork of normal or short storage life. *International Journal of Food Microbiology*, **97**, 53–62.
- Hong, E., Doumith, M., Duperrier, S., Giovannacci, I., et al. 2007. Genetic diversity of *Listeria monocytogenes* recovered from infected persons and pork, seafood and dairy products on retail sale in France during 2000 and 2001. *International Journal of Food Microbiology*, **114**, 187–194.
- Huerta, T., Sanchis, V., Hernández, J., and Hernández, E. 1987. Mycoflora of dry-salted Spanish ham. *Microbiologie – Aliments – Nutrition*, **5**, 247–252.
- Hugas, M., Garriga, M., Aymerich, T., and Monfort, J.M. 1995. Inhibition of *Listeria* in dry fermented sausages by the bacteriocinogenic *Lactobacillus sakei* CTC494. *Journal of Applied Bacteriology*, **79**, 322–330.
- Hugas, M., Garriga, M., and Monfort, J.M. 2002. New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, **62**, 359–371.
- Iacumin, L., Comi, G., Cantoni, C., and Cocolin, L. 2006a. Ecology and dynamics of coagulase-negative cocci isolated from naturally fermented Italian sausages. *Systematic and Applied Microbiology*, **29**, 480–486.
- Iacumin, L., Comi, G., Cantoni, C., and Cocolin, L. 2006b. Molecular and technological characterization of *Staphylococcus xylosus* isolated from Italian naturally fermented sausages by RAPD, Rep-PCR and Sau-PCR analysis. *Meat Science*, **74**, 281–288.
- Iacumin, L., Chiesa, L., Boscolo, D., Manzano, M., Cantoni, C., Orlic, S., and Comi, G. 2009. Moulds and ochratoxin A on surfaces of artisanal and industrial dry sausages. *Food Microbiology*, **26**, 65–70.
- Iacumin, L., Milesi, S., Pirani, S., Comi, G., and Chiesa, L.M. 2011. Ochratoxigenic moulds and Ochratoxin A in sausages from different areas in Northern Italy: occurrence, elimination or prevention with ozonated air. *Journal of Food Safety*, **31**, 538–545.
- Iacumin, L., Manzano, M., and Comi, G. 2012a. Prevention of *Aspergillus ochraceus* growth on and Ochratoxin A contamination of sausages using ozonated air. *Food Microbiology*, **29**, 229–232.
- Iacumin, L., Manzano, M., and Comi, G. 2012b. Catalase-positive cocci in fermented sausage: variability due to different pork breeds, breeding systems and sausage production technology. *Food Microbiology*, **29**, 178–186.
- ICMSF (International Commission on Microbiological Specifications for Foods). 1980. *Microbial Ecology of Foods*, Vol. I, *Factors Affecting Life and Death of Microorganisms*. Academic Press, London, UK.
- Incze, K. 2004. Mould ripened sausages. In: *Handbook of Food and Beverage Fermentation Technology* (eds Hui, Y.H., Meunier-Goddik, L., Hansen, Å.S., Josephsen, J., Nip, W.-K., Stanfield, P.S., and Toldra, F.), pp. 480–491. CRC Press, Boca Raton, FL, USA.
- Jiang, Y., Gao, F., Xu, X.L., Su, Y., Ye, K.P., and Zhou, G.H. 2010. Changes in the bacterial communities of vacuum-packaged pork during chilled storage analyzed by PCR-DGGE. *Meat Science*, **86**, 889–895.
- Jung, S., Ghoul, M., and De Lamballerie-Anton, M. 2000. Changes in lysosomal enzyme activities and shear values of high pressure treated meat during ageing. *Meat Science*, **56**, 239–246.
- Koort, J., Murros, A., Coenye, T., Eerola, S., Vandamme, P., Sukura, A., and Bjorkroth, J. 2005. *Lactobacillus oligofermentans* sp nov., associated with spoilage of modified atmosphere-packaged poultry products. *Applied and Environmental Microbiology*, **71**, 4400–4406.
- Larsen, T.O., Svendsen, A., and Smedsgaard, J. 2001. Biochemical characterization of Ochratoxin

- A-producing strains of the Genus *Penicillium*. *Applied and Environmental Microbiology*, **67**, 3630–3635.
- Lawrie, R.A. and Ledward, D.A. 2006. The spoilage of meat by infecting organisms. In: *Lawrie's Meat Science*, 7th edn, pp. 157–188. CRC Press, Boca Raton, FL, USA.
- Lebert, I., Leroy, S. and Talon, R. 2007. Microorganisms in traditional fermented meats, Chapter 11. In: *Handbook of Fermented Meat and Poultry*, Vol. 8 (eds Toldra, F., Hui, Y.H., Astiasaran, I., Nip, W.K., Sebranek, J.G., Silveira, E.T.F., Sthanke, L.H., and Talon, R.), pp. 113–124. Blackwell Publishing Ltd, Oxford, UK.
- Lee, B.H., Simard, R.E., Laleye, L.C., and Holley, R.A. 1985. Effects of temperature and storage duration on the microflora, physicochemical and sensory changes of vacuum- or nitrogen-packed pork. *Meat Science*, **13**, 99–112.
- Leistner, L. 1986. Mold-ripened foods. *Fleischwirtschaft*, **66**, 1385–1388.
- Leistner, L. and Ayres, J.C. 1968. Molds and meats. *Fleischwirtschaft*, **1**, 62–65.
- Lerche, M. and Reuter, G. 1960. A contribution to the method of isolation and differentiation of aerobic "lactobacilli" (genus *Lactobacillus beijerinckii*). *Archiv für Hygiene und Bakteriologie*, **179**, 354–370.
- Leser, T.D., Lindecrone, R.H., Jensen, T.K., Jensen, B.B., and Møller, K. 2000. Changes in bacterial community structure in the colon of pigs fed different experimental diets and after infection with *Brachyspira hyodysenteriae*. *Applied and Environmental Microbiology*, **66**, 3290–3296.
- Li, M.Y., Zhou, G.H., Xu, X.L., Li, C.B., and Zhu, W.Y. 2006. Changes of bacterial diversity and main flora in chilled pork during storage using PCR-DGGE. *Food Microbiology*, **23**, 607–611.
- Linares, M.B., Garrido, M.D., Martins, C., and Patarata, L. 2013. Efficacies of garlic and *L. sakei* in wine-based marinades for controlling *Listeria monocytogenes* and *Salmonella* spp. in Chouriço de Vinho, a dry sausage made from wine-marinated pork. *Journal of Food Science*, **78**, 719–724.
- Linton, M., McClements, J.M.J., and Patterson, M.F. 2004. Changes in the microbiological quality of vacuum-packaged, minced chicken treated with high hydrostatic pressure. *Innovative Food Science and Emerging Technologies*, **5**, 151–159.
- Lorenzo, J.M., García Fontán, M.C., Cachaldora, A., Franco, I., and Carballo, J. 2010. Study of the lactic acid bacteria throughout the manufacture of dry-cured lacón (a Spanish traditional meat product). Effect of some additives. *Food Microbiology*, **27**, 229–235.
- Lücke, F.K. 1974. Fermented sausages. In: *Microbiology of Fermented Foods* (ed. Wood, B.J.B.), pp. 41–49. Applied Science Publishers, London, UK.
- Ma, H.J., Ledward, D.A., Zamri, A.I., Frazier, R.A., and Zhou, G.H. 2007. Effects of high pressure/thermal treatment on lipid oxidation in beef and chicken muscle. *Food Chemistry*, **104**, 1575–1579.
- Malinowska-Panczyk, E. and Kolodziejska, I. 2013. The influence of moderate pressure and subzero temperature on the shelf life of minced cod, salmon, pork and beef meat. *Food Technology and Biotechnology*, **51**, 570–576.
- Malinowska-Panczyk, E., Kolodziejska, I., Murawska, D., and Wolosewicz, G. 2009. The combined effect of moderate pressure and chitosan on *Escherichia coli* and *Staphylococcus aureus* cells suspended in a buffer and on natural microflora of apple juice and minced pork. *Food Technology and Biotechnology*, **47**, 202–209.
- Marín Riaño, M.E., Carrascosa, A.V., and Cornejo Contreras, I. 1993. Micropoblación saprofita y patógena en la elaboración de jamón serrano. *Alimentaria*, **240**, 31–36.
- McMullen, L.M. and Stiles, M.E. 1993. Microbial ecology of fresh pork stored under modified atmosphere at –1, 4.4 and 10°C. *International Journal of Food Microbiology*, **18**, 1–14.
- Metaxopoulos, I. 2007. Phenotypic and technological diversity of lactic acid bacteria and staphylococci isolated from traditionally fermented sausages in Southern Greece. *Food Microbiology*, **24**, 260–270.
- Metaxopoulos, J., Samelis, J., and Papadelli, M. 2001. Technological and microbiological evaluation of traditional processes as modified for the

- industrial manufacturing of dry fermented sausage in Greece. *Italian Journal of Food Science*, **13**, 3–18.
- Meyer, C., Thiel, S., Ullrich, U., and Stolle, A. 2010. *Salmonella* in raw meat and by-products from pork and beef. *Journal of Food Protection*, **73**, 1780–1784.
- Moerman, F., Mertens, B., Demey, L., and Huyghebaert, A. 2001. Reduction of *Bacillus subtilis*, *Bacillus stearothermophilus* and *Streptococcus faecalis* in meat batters by temperature-high hydrostatic pressure pasteurization. *Meat Science*, **59**, 115–125.
- Molin, G. 2000. Modified atmospheres. In: *The Microbiological Safety and Quality of Food* (eds Lund, B.M., Baird-Parker, T.C., and Gould, G.W.), Vol. 1, pp. 214–234. Aspen Publishers, Gaithersburg, MD, USA.
- Molin, G. and Terström, A. 1986. Phenotypically based taxonomy of psychrotrophic *Pseudomonas* isolated from spoiled meat, water, and soil. *International Journal of Systematic Bacteriology*, **36**, 257–274.
- Molina, I., Silla, H., and Flores, J. 1989. Study of the microbial flora in dry-cured ham. Lactic acid bacteria. *Fleischwirtschaft*, **69**, 1708–1710.
- Montel, E., Villanueva, J.R., and Dominguez, A. 1986. Fungal profiles of Spanish country-cured hams. *International Journal of Food Microbiology*, **3**, 355–359.
- Mutti, P., Previdi, M.P., Quintavalla, S., and Spotti, E. 1992. Toxigenicity of mould strains isolated from salami as a function of culture medium. *Industria Conserve*, **63**, 142–145.
- Netschajew, A., Fredriksson-Ahomaa, M., Sperner, B., and Stolle, A. 2009. Detection of *Listeria* monocytogenes in vacuum-packed meat products using real-time PCR, immunoassay and culturing. *Archiv für Lebensmittelhygiene*, **60**, 12–17.
- Nieminen, T.T., Vihavainen, E., Paloranta, A., Lehto, J., Paulin, L., Auvinen, P., Solsmaa, M., and Björkroth, K.J. (2011) Characterization of psychrotrophic bacterial communities in modified atmosphere-packaged meat with terminal restriction fragment length polymorphism. *International Journal of Food Microbiology*, **144**, 360–366.
- Northolt, M.D. and Bullerman, L.B. 1982. Prevention of mould growth and toxin production through control of environmental condition. *Journal of Food Protection*, **45**, 519–523.
- Núñez, F., Rodríguez, M.M., Bermúdez, M.E., Córdoba, J.J., and Asensio, M.A. 1996. Composition and toxigenic potential of the mould population on dry-cured Iberian ham. *International Journal of Food Microbiology*, **32**, 185–197.
- Nychas, G.J.E. and Skandamis, P. 2005. Fresh meat spoilage and modified atmosphere packaging (MAP). In: *Improving the Safety of Fresh Meat* (ed. Sofos, J.N.), pp. 461–502. CRC/Woodhead Publishing, Cambridge, UK.
- Olsson, C., Ahrné, S., Pettersson, B., and Molin, G. 2003. The bacterial flora of fresh and chill-stored pork: analysis by cloning and sequencing of 16S rRNA genes. *International Journal of Food Microbiology*, **83**, 245–252.
- Papadopoulou, O.S., Doulgeraki, A.I., Botta, C., Cocolin L., and Nychas, G.J.E. 2012. Genotypic characterization of *Brochothrix thermosphacta* isolated during storage of minced pork under aerobic or modified atmosphere packaging conditions. *Meat Science*, **92**, 735–738.
- Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., and Kotzekidou, P. 2003. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science*, **65**, 859–867.
- Pearce, R.A., Bolton, D.J., Sheridan, J.J., McDowell, D.A., Blair, I.S., and Harrington, D. 2004. Studies to determine the critical control points in pork slaughter hazard analysis and critical control point systems. *International Journal of Food Microbiology*, **90**, 331–339.
- Pichner, R., Schönheit, C., Kabisch, J., and Böhnlein, C. 2014. Assessment of microbiological quality and safety of marinated pork products from German retail during shelf life. *Food Control*, **46**, 18–25.
- Prändl, O., Fischer, A., Schmidhofer, T., and Sinell, H.-J. 1988. *Fleisch. Technologie und Hygiene der Gewinnung und Verarbeitung*. Eugen Ulmer GmbH & Co., Stuttgart, Germany.

- Prendergast, D.M., Duggan, S.J., Fanning, S., Cormican, M., *et al.* 2008. Prevalence and numbers of *Salmonella* spp. and *Enterobacteriaceae* on pork cuts in abattoirs in the Republic of Ireland. *Journal of Applied Microbiology*, **105**, 1209–1219.
- Rabsch, W., Simon, S., and Humphrey, T. 2013. Public health aspects of *Salmonella* infections, Chapter 18. In: *Salmonella in Domestic Animals* (eds Barrow, P.A. and Methner, U.), pp. 351–376. Cabi Publishing, Oxfordshire, UK.
- Rantsiou, K., Urso, R., Iacumin, L., Cantoni, C., *et al.* (2005) Culture dependent and independent methods to investigate the microbial ecology of Italian fermented sausages. *Applied and Environmental Microbiology*, **71**, 1977–1986.
- Rebecchi, A., Crivori, S., Sarra, P.G., and Cocconcilli, P.S. 1998. Physiological and molecular techniques for study of bacterial community development in sausage fermentation. *Journal of Applied Microbiology*, **84**, 1043–1049.
- Reuter, G. 1972. Experimental ripening of dry sausages using lactobacilli and micrococci starter cultures. *Fleischwirtschaft*, **52**, 465–468, 471–473.
- Rojas, F.I., Jodral, M., Gosalvez, F., and Pozo, R. 1991. Mycoflora and toxigenic *Aspergillus flavus* in Spanish dry-cured ham. *International Journal of Food Microbiology*, **13**, 249–256.
- Säde, E., Murros, A., and Björkroth, J. 2013. Predominant enterobacteria on modified-atmosphere packaged meat and poultry. *Food Microbiology*, **34**, 252–258.
- Samelis, J. 2006. Managing microbial spoilage in the meat industry. In: *Food Spoilage Microorganisms* (ed. Blackburn, C.), pp. 213–286. Woodhead Publishing, Cambridge, UK.
- Samelis, J., Aggelis, G., and Metaxopoulos, J. 1993. Lipolytic and microbial changes during the natural fermentation and ripening of Greek dry sausages. *Meat Science*, **35**, 371–385.
- Samelis, J., Metaxopoulos, J., Vlassi, M., and Pappa, A. 1998. Stability and safety of traditional Greek salami – a microbiological ecology study. *International Journal of Food Microbiology*, **44**, 69–82.
- Santos, N.N., Santos-Mendonça, R.C., Sanz, Y., Bolumar, T., Aristoy, M.C., and Toldrá, F. 2001. Hydrolysis of pork muscle sarcoplasmatic proteins by *Debaryomyces hansenii*. *International Journal of Food Microbiology*, **68**, 199–206.
- Schillinger, U., Holzapfel, W.H., and Björkroth, J. 2006. Lactic acid bacteria. In: *Food Spoilage Microorganisms* (ed. Blackburn, C.), pp. 541–578. Woodhead Publishing, Cambridge, UK.
- Schirmer, B.C., Heir, E., and Langsrud, S. 2009. Characterization of the bacterial spoilage flora in marinated pork products. *Journal of Applied Microbiology*, **106**, 2106–2116.
- Shaw, B.G. and Latty, J.B. 1982. A numerical taxonomy study of the *Pseudomonas* strains from spoiled meat. *Journal of Applied Bacteriology*, **52**, 219–228.
- Silla, M.H., Innerarity, A., and Flores, J. 1985. Características de jamones con cristales de tirosina. *Revista de Agroquímica y Tecnología de Alimentos*, **25**, 95–103.
- Silvestri, G., Santarelli, S., Aquilanti, L., Beccaceci, A., *et al.* 2007. Investigation of the microbial ecology of Ciauscolo, a traditional Italian salami, by culture-dependent techniques and PCR–DGGE. *Meat Science*, **77**, 413–423.
- Spotti, E., Mutti, P., and Campanini, M. 1988. Indagine microbiologica sul difetto dell'acido fenico del prosciutto durante la stagionatura. *Industria Conserve*, **63**, 343–346.
- Spotti, E., Mutti, P., and Campanini, M. 1989. Presenza di muffe sui prosciutti durante la pre-stagionatura e la stagionatura: contaminazione degli ambienti e sviluppo sulla porzione muscolare. *Industria Conserve*, **64**, 110–113.
- Spotti, E., Chiavaro, E., Pari, E., and Busolli, C. 2001a. Sviluppo di *Penicillium verrucosum* in sistemi modello di prodotti carnei stagionati. Parte II. *Industria Conserve*, **76**, 167–183.
- Spotti, E., Chiavaro, E., Lepidani, A., and Colla, F. 2001b. Contaminazione da muffe e ocratossina A in prosciutti stagionati e in fase di stagionatura. *Industria Conserve*, **76**, 341–354.
- Stanbridge, L.H. and Davies, A.R. 1998. The microbiology of chill-stored meat. In: *The Microbiology of Meat and Poultry* (eds Davies, A. and

- Board, R.), pp. 174–219. Blackie Academic and Professional, London, UK.
- Susiluoto, T., Korkeala, H., and Bjorkroth, K.J. 2003. *Leuconostoc gasicomitatum* is the dominating lactic acid bacterium in retail modified-atmosphere-packaged marinated broiler meat strips on sell-by-day. *International Journal of Food Microbiology*, **80**, 89–97.
- Tahiri, I., Makhoulf, J., Paquin, P., and Fliss, I. 2006. Inactivation of food spoilage bacteria and *Escherichia coli* O157:H7 in phosphate buffer and orange juice using dynamic high pressure. *Food Research International*, **39**, 98–105.
- Talon, R., Leroy, S., and Lebert, I. 2007. Microbial ecosystems of traditional fermented meat products: the importance of indigenous starters. *Meat Science*, **77**, 55–62.
- Toscani, T., Moseriti, A., Dossena, A., Dall'Asta, C., Simoncini, N., and Virgili, R. 2007. Determination of ochratoxin A in dry-cured meat products by HPLC-FLD quantitative method. *Journal of Chromatography B*, **855**, 242–248.
- Tsigarida, E., Skandamis, P., and Nychas, G.J.E. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5°C. *Journal of Applied Microbiology*, **89**, 901–909.
- USDA (United States Department of Agriculture). 2014. *USDA National Nutrient Database for Standard Reference* [Online]. Available at: <http://ndb.nal.usda.gov> (accessed 9 June 2014).
- Vogel, R.F., Phole, B.S., Tichaczek, P.S., and Hammes, W.P. 1993. The competitive advantage of *L. curvatus* LTH1174 in sausage fermentations is caused by formation of curvacin A. *Applied Microbiology*, **15**, 129–136.
- Wang, X., Ma, P., Jiang, D., Peng, Q., and Yang, H. 2006. The natural microflora of Xuanwei ham and the no-mouldy ham production. *Journal of Food Engineering*, **77**, 103–111.
- Yeh, K.S., Chen, S.P., and Lin, J.H. 2005. One-year (2003) nationwide pork carcass microbiological baseline data survey in Taiwan. *Journal of Food Protection*, **68**, 458–461.
- Young, L.L. and Lyon, C.E. 1997. Effect of calcium marination on biochemical and textural properties of peri-rigor chicken breast meat. *Poultry Science*, **76**, 197–201.
- Yuste, J., Mor-Mur, M., Capellas, M., Guamis, B., and Pla, R. 1998. Microbiological quality of mechanically recovered poultry meat treated with high hydrostatic pressure and nisin. *Food Microbiology*, **15**, 407–414.
- Zhou, G.H., Xu, X.L., and Liu, Y. 2010. Preservation techniques for fresh meat – a review. *Meat Science*, **86**, 119–128.
- Zickrick, K., Wegner, K., Schreiter, M., Schiefer, G., Saupe, C., and Münch, H.D. 1986. *Mikrobiologie tierischer Lebensmittel*. 2. Auflage. VEB Fachbuchverlag, Leipzig, Germany.
- Zweifel, C., Fischer, R., and Stephan, R. 2008. Microbiological contamination of pig and cattle carcasses in different small-scale Swiss abattoirs. *Meat Science*, **78**, 225–231.

CHAPTER 24

Microbial ecology of poultry and poultry products

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24.1 Introduction

Poultry meat consumption per capita has increased markedly over recent decades and reached marked levels, e.g., 24 kg in the European Union (EU) in 2008 (FAO, 2010). The reasons include not only comparably more efficient and economical poultry production, but also changes in consumers' preferences toward poultry meat due to its quicker/easier cooking, lower saturated fat content, better tenderness and relatively neutral flavor compared to beef, lamb, and pork.

In respect to the role of poultry meat in overall microbial food safety and food-borne diseases, available data vary widely between world regions and countries. As an illustration, recently reported data from monitoring zoonoses and zoonotic agents in 2012 in the EU (EFSA/ECDC, 2014) based on strong-evidence food-borne disease outbreaks indicate that broiler meat/meat products were food vehicles in 4.2% of all outbreaks. More specifically, broiler meat/meat products were implicated in 3.7% of *Salmonella* outbreaks, 44.0% of *Campylobacter* outbreaks, 2.6% of *Bacillus cereus* outbreaks, 3.7% of *Clostridium* (including *C. botulinum*) outbreaks, and 8.6% of *Staphylococcus aureus* outbreaks. However, it should be kept in mind that these numbers for broilers do not include outbreaks caused by other poultry meats and that an unknown proportion of outbreaks

reported for "mixed foods" or "buffet meals" probably included poultry meats as well.

When considering global trends in the microbial safety of poultry meats in the EU in recent years, two aspects particularly draw attention. Firstly, the two top hazards together causing the large majority of food-borne disease cases have been *Campylobacter* (e.g., 214 268 in total or 55.5/100 000 population in 2012) followed by *Salmonella* (e.g., 91 034 in total or 22.2/100 000 population in 2012), and poultry meats as vehicles were implicated predominantly in the former and significantly in the latter. Secondly, over recent years, reported *Campylobacter* cases have been increasing steadily, but *Salmonella* cases have been decreasing due to systematic control measures applied at poultry farm and abattoir levels. The risk- and food chain-based approach to microbial risk reduction strategies for poultry meats summarized in this chapter primarily reflects the European situation in which the authors professionally operate.

24.2 Microbial hazard identification and prioritization

Zoonotic hazards can be transmitted to humans via: (a) non-food-borne routes, e.g., inhalation, direct contact, environment, or (b) food(meat)-borne routes.

The main examples of the first group include actual or suspected zoonotic hazards: (i) bacteria, e.g., *Chlamydomphila* (*Chlamydia*) *psittaci*, *Erysipelothrix rhusiopathiae*, *Brucella*, *Arcobacter* spp., Haemolytic streptococci, *Mycobacterium avium*, *Yersinia pseudotuberculosis*, *Burkholderia pseudomallei*; (ii) viruses, e.g., Avian influenza, Hepatitis E, West Nile, Newcastle disease; and (iii) fungi, e.g., *Cryptococcus neoformans*, *Trychophyton gallinae*, *Histoplasma capsulatum* (Dale and Brown, 2013; EFSA, 2012a; PAHO, 2001; Hui *et al.*, 2001). The main control strategies for those hazards are occupational health- and environmental protection-based, and hence will not be dealt with in this meat safety-orientated chapter.

Microbial hazards that are transmitted from poultry to humans primarily via the meat-borne route are indicated in Table 24.1. Although the integrated poultry meat chain approach to control of meat-borne hazards is universally applicable, the main focus of control strategies differs between two groups of meat-borne hazards. Those hazards that are primarily fecally shed by birds and do not require multiplication to cause human disease are most effectively controlled during farm-to-chilled carcass stages (e.g., *Salmonella*, *Campylobacter*). Those hazards that require growth/toxin production in foods to cause human disease (and/or originate primarily from post-abattoir environments) are most effectively controlled during meat processing–retail–food preparation stages (e.g., *L. monocytogenes*, *Clostridium*, *B. cereus*, *S. aureus*).

Some poultry meat-borne hazards are more important for meat safety and public health than others. They represent a priority and require more intensive efforts and larger resources directed at their control, as their high risk for humans requires a high level of risk-reduction. To prioritize poultry meat-borne hazards, several aspects need to be carefully considered for each hazard. One aspect is the incidence of corresponding human diseases in a given population. In addition, the public health impact of the hazard also depends on disease

severity, which can be measured via, for example, case fatality and/or disability adjusted life years (DALY). Further, source attribution of the disease, the proportion of cases caused via poultry meat (e.g., indicated via subtyping, epidemiological data), should also be used. Furthermore, the incidence of hazards on respective meats is an important parameter. By combining all the above-mentioned parameters for the individual meat-borne hazards, they can be qualitatively ranked into high/medium/low priorities (Table 24.1) in the context of microbial poultry meat safety assurance. However, any such prioritization is geographically and temporally limited, and should be revisited periodically as the presence/levels of hazards may change over time and between regions/countries.

24.3 Microbial aspects of poultry processing at abattoirs

Poultry abattoirs can operate on small to large volume scales, with line capacities from a few hundred to >10 000 birds per hour. In small abattoirs, processing of birds is largely conducted manually, using individual machines in some steps. However, the efficiency of processing is the first priority for most large volume abattoirs, which use a moving overhead conveyor with most slaughter and dressing operations fully automated. Regardless of the degree of automation, poultry processing includes the main steps briefly considered below. The main focus is on contamination with the two top hazards, *Salmonella* and *Campylobacter*.

24.3.1 Poultry abattoir process steps

The live birds arriving from farm to abattoir are fecal carriers of microbial pathogens (Bolder, 2007). Consequently, further spread of pathogens such as *Salmonella* and *Campylobacter* occurs via various direct or indirect routes at most process steps in the poultry abattoir. On

Table 24.1 Microbial hazards transmissible from poultry to humans via a meat-borne route (compiled from Buncic, 2014; Fredriksson-Ahomaa, 2014; EFSA, 2012a; Bhunia, 2008; FSANZ, 2005; Cox *et al.*, 2005).

Hazard (main poultry hosts)*	Type of disease in humans	Hazard's growth in foods required for respective disease?	Stages of the poultry meat chain where control measures have biggest risk-reduction potential	Priority (arbitrary prioritization in the context of poultry meat safety)
<i>Bacillus cereus</i> (1, 5)	Intoxication, toxicoinfection	Yes (toxin production)	Processing-retail-food preparation	Low to medium
<i>Campylobacter</i> spp. (thermophilic) (1, 4, 5)	Infection	No	Farm-abattoir	High
<i>Clostridium botulinum</i> (1, 4, 5)	Intoxication	Yes (toxin production)	Processing-retail-food preparation	Low to medium
<i>Clostridium difficile</i> (1, 4)	Infection/toxicoinfection	No	Insufficient data for clear conclusion	Low
<i>Clostridium perfringens</i> (1, 4, 5)	Toxicoinfection	Yes	Processing-retail-food preparation	Medium
Verocytotoxigenic <i>Escherichia coli</i> (1, 4, 5)	Toxicoinfection	No	Farm-abattoir	Low
Extended spectrum and/ or AmpC β -lactamases (ESBL/AmpC) <i>Salmonella enterica</i> (1)	Ineffective medication treatments for pathogens	No	Farm-abattoir	Medium
<i>Listeria monocytogenes</i> (1, 4, 5)	Infection	Yes	Processing-retail-food preparation	Low
<i>Salmonella</i> spp. non-typhoidal (1, 4, 5)	Infection	No	Farm-abattoir	Low-to-medium
<i>Staphylococcus aureus</i> (1, 4, 5)	Intoxication	Yes (toxin production)	Farm-abattoir	High
<i>Yersinia enterocolitica</i> (1)	Infection	No	Processing-retail-food preparation	Low-to-medium
			Insufficient data for clear conclusion	Low

* 1, chickens; 2, ducks; 3, geese; 4, turkeys; 5, Anseriformes.

the other hand, some process steps are designed to control microbial contamination, e.g., sequential carcass washing along the slaughter-line and carcass chilling (FAO/WHO, 2009). The main steps, in the same order as they are performed during poultry abattoir processing, are briefly considered in the text below, accompanied by available data on corresponding microbial contamination indicated in Table 24.2.

24.3.1.1 Transport of birds

The birds are delivered to abattoirs in crates (up to 30 birds each) placed in metal frames on multiple levels on trucks. The prevalence of *Salmonella* and *Campylobacter* on birds may increase during transportation and handling due to increased stress and/or prolonged feed withdrawal and/or cross-contamination via crates (Bolder, 2007; FAO/WHO, 2002, 2009). After unloading, crates should be washed and disinfected to reduce the contamination (EFSA, 2011).

24.3.1.2 Inspection of live birds

In the EU, as part of official meat inspection, food chain information (FCI) including reported data on farm management, epidemiological intelligence, and veterinary treatments are analyzed first (EC, 2004; EFSA 2012a). However, the content of FCI is not fully detailed in current EU legislation and varies between the member states; the only exception is the on-farm *Salmonella* status of broiler and turkey flocks. During *ante-mortem* inspection, birds are visually observed on a flock basis (conducted either on the farm or within crates at the abattoir) to prevent sick or abnormal birds entering the slaughter-line, and to ensure detection of animal health and welfare problems. As birds carrying the main meat-borne hazards (Table 24.1) show no symptoms, or exhibit these only very rarely, the main contribution of antemortem inspection to meat safety is detection of any birds heavily contaminated with feces (which would subsequently excessively contaminate the slaughter-line).

Table 24.2 Microbial contamination of poultry carcasses at the main processing steps in the abattoir (compiled from Abu-Ruwaida *et al.*, 1994; Altekruze *et al.*, 2009; Berrang *et al.*, 2000; FAO/WHO, 2002, 2009; Whyte *et al.*, 2004).

Process step after	Indicator organisms*			Microbial hazards*	
	Aerobic bacteria	<i>Enterobacteriaceae</i>	<i>Escherichia coli</i>	<i>Salmonella</i> spp. [§]	<i>Campylobacter</i> spp.
Unloading	–	–	–	–	4.9–9.6
Bleeding	6.8 (5.2 [†])	4.8 [†]	4.3	5.8–6.3 [†] (19–100%)	2.2–5.8
Scalding	5.0	–	2.1	3.1–3.3 [†] (12–80%)	1.0–2.4
Plucking	5.0 (7.2 [†])	3.3–5.6 [†]	2.8 (4.9 [†])	(12–100%)	2.0–4.5
Evisceration	4.5 (7.2 [†])	5.4 [†]	2.2 (5.0 [†])	(14–66%)	1.6–4.0
Final washing	3.6 (4.9–6.3 [†])	3.2–5.2 [†]	1.5 (3.3–4.4 [†])	(14–89%)	1.0–3.3
Water chilling	2.9	–	1.1	0.1–2.0 (11–77%)	1.2–2.3
Air chilling	4.6–5.2 [†]	3.3–3.5 [†]	3.2–3.3 [†]	(17–29%)	0.1–0.3

* Where not indicated differently, results expressed as mean log cfu/ml of rinse from whole carcass.

[†] Mean log cfu/g of sampled carcass skin.

[§] Only prevalence values available for *Salmonella* in some process steps (%).

24.3.1.3 Unloading and shackling

Birds are not lairaged; after unloading they are immediately hung upside down on shackles on a moving line, if an electrical stunning system is used; if a gas stunning system is used, then birds within crates are transported to a gas chamber. Shackling the live birds is associated with fecal leakage and wing-flapping leading to aerial dust dispersion and heavy air-borne microbial contamination (Bolder, 2007).

24.3.1.4 Stunning and bleeding

Stunning renders a bird unconscious, enabling humane slaughter. Most often, birds' heads are automatically dragged through an electrically charged water bath. Alternatively, gas stunning is used, as it causes comparably less physical damage to the birds, enables easier shackling of unconscious birds, and prevents live birds entering the scalding tank. Stunned birds are usually slaughtered by automatic neck-cutting whilst the head is guided across a circular blade, and then are bled out.

24.3.1.5 Scalding

Scalding involves submerging birds in hot water, usually 50–51 °C for 2.5–3 min, but both parameters can vary depending on the birds' size and whether they are intended for fresh or frozen carcasses. The process facilitates removal of feathers and cuticles (during a subsequent defeathering step) but also reduces microorganisms, dirt, litter, and feces on the birds. However, at the same time, scalding can contribute to a higher microbial contamination of carcasses (Buncic and Sofos, 2012), as the birds deposit large loads of microorganisms from the feathers, skin, and from faeces that are sometimes passively released into the scalding water. After an initial buildup, a proportion of the microbial load in scalding water dies off, and levels of bacteria on the carcass skin in the scalding tank generally do not exceed 4.7 log cfu/ml (NACMCF, 1997). Overall, depending on conditions applied, scalding can decrease microbial levels by roughly 2–3 logs compared to the bleeding step (Table 24.2).

24.3.1.6 Defeathering (plucking)

Feather removal is carried out in a series of plucking machines by rotation of multiple metal disks with rubber "fingers". Defeathering mediates cross-contamination between carcasses through soil scattering and spreading in the pluckers via the rubber "fingers" and the air. In addition, there is often a buildup of microorganisms on the "fingers", which are difficult to clean and sanitize (Buncic and Sofos, 2012), and hence can be a source of pathogens (NACMCF, 1997). Using water in pluckers may cause the formation of highly contaminated aerosols, enhancing the cross-contamination. Subsequently, carcasses are spray-washed to remove dirt, but this also helps to remove a proportion of bacteria and to reduce bacterial attachment to the carcasses (Bolder, 2007). Then the heads and feet are automatically cut off before the carcasses are hung on a second moving line leading to evisceration, which is located in a separate area.

24.3.1.7 Evisceration

Viscera are removed from the carcass during a highly automated operation involving a series of interconnected machines, which have to be properly adjusted to the birds' sizes (which are ideally uniform) so that no rupture of tissue or guts occurs. In the process, the body is cut from the tip of the breastbone to the cloaca, the cavity is opened, all edible and inedible offal are removed, and the edible giblets (heart, liver, and gizzard) are trimmed and washed (Buncic and Sofos, 2012). If carried out improperly, evisceration poses a high risk of microbial carcass contamination due to gut rupture and spillage of gut contents. Finally, the neck is cut off.

24.3.1.8 Meat inspection

Official postmortem inspection of internal organs and external and internal surfaces of the carcasses is carried out only visually. Any carcass with gross abnormalities affecting the safety or wholesomeness of meat is removed from the food chain. Because of high line speeds, supplementary inspection technologies are

sometimes used (a mirror is placed opposite the inspector, cameras are linked to image analyzing software) to increase the sensitivity of detection of lesions. The detected conditions are primarily of animal health and meat quality relevance, as the presence of the main meat-borne hazards (*Salmonella*, *Campylobacter*) is not associated with macroscopic lesions. Hence, the value of current postmortem inspection is in detection of fecal contamination that indicates an increased public health risk of enteric hazards, but has no value in actual detection of the main hazards themselves (EFSA, 2012a).

24.3.1.9 Final washing

Eviscerated carcasses are thoroughly washed inside and outside to remove blood spots and other organic debris and to reduce microbial

contamination. Although the carcasses are washed at frequent intervals along the line, this final pressurized washing is important, as it may contribute to significant reduction of *Salmonella* prevalence on carcasses, e.g., by 50–90% (Buncic and Sofos, 2012) or around 0.5–1.0 log of bacteria (Tables 24.2 and 24.3).

24.3.1.10 Carcass decontamination

In addition to fundamental process hygiene-based microbial risk reduction measures, decontamination treatments (physical, chemical, or biological) can be applied during slaughter and dressing to reduce prevalence/levels of pathogens and other microorganisms on carcasses (Loretz *et al.*, 2010; Buncic and Sofos, 2012). In the EU (EC, 2004), meat decontamination treatment can be used if pre-approved by the EU

Table 24.3 Antimicrobial efficiency of decontamination treatments of poultry carcasses (adapted from Loretz *et al.*, 2010).

Decontamination treatment†	Microbial reductions achieved (log cfu)*				
	Aerobic bacteria	Enterobacteriaceae	<i>Escherichia coli</i>	<i>Salmonella</i> spp.	<i>Campylobacter</i> spp.
Cold and warm water (<55 °C)	0.1–1.5	0.3–0.4	0.1–0.9	0.7–1.2	0.5–1.4
Hot water (55–80 °C)	0.3–1.3	0.5–1.0	0.1–1.3	–	0.1–1.7
Hot water + spray chilling	0.7–1.3	0.2–1.6	–	0.6–1.3	1.1–1.6
Steam (90 °C)	0.4–0.8	0.6–0.7	1.7–2.8	–	0.5–3.3
Electrolyzed water	1.0–2.4	–	1.5–1.7	2.1–2.7	0.6–3.0
Irradiation (1–5 kGy)	3.0	–	–	3.0	–
Acetic acid	0.2–2.0	0.6–2.3	2.8	0.8–1.4	–
Lactic acid	0.8–1.4	–	–	1.8	0.2–1.7
Chlorinated water	0.1–2.3	–	0.2–2.1	0.9–1.1	0.5–2.6
Acidified sodium chlorite	0.8–1.1	–	2.3	–	2.6
Cetylpyridinium chloride	2.0	–	–	2.0	>4.2
Trisodium phosphate (TSP)	0.5–2.2	1.1–3.8	0.5–4.0	0.9–1.8	0.2–1.7
Sodium carbonate/TSP + hot water	–	–	–	1.8–1.9	–
Sodium hypochlorite + acidic electrolyzed water	0.3	–	0.5	0.8	1.0
Chlorine + acetic acid/TSP	1.4	–	1.6–1.8	2.0	–
Tripotassium phosphate + lauric acid/myristic acid	1.1–1.5	–	0.6–1.1	–	1.4–2.7

* Antimicrobial reduction from different studies calculated and presented as log cfu/ml of rinsed liquid from carcass, log cfu/g of carcass skin or log cfu/cm² of carcass surface.

† Within one group of treatment, there were significant variations of conditions applied, such as the mode of application (spraying/immersion), the concentration of chemicals used, the application temperature, the exposure time, the point of application during processing, or the nature of microbial contamination (natural or artificial).

Commission and if not used to conceal poor hygiene practices. Before approval, a risk assessment and a positive scientific opinion on safety and efficacy of the treatment has to be provided by the European Food Safety Authority (EFSA). On the other hand, chemical decontamination treatments of poultry carcasses are commonly applied in the United States, but only after their efficacy has been validated; thereafter, they are regularly verified for continuous effectiveness during implementation (Buncic and Sofos, 2012). The antimicrobial efficiencies of various decontamination treatments reported in different studies are briefly summarized in Table 24.3.

24.3.1.11 Chilling

Finally, carcasses are chilled using either water-chilling or air-chilling methods. The method used depends on the market; if carcasses are to be sold as frozen, they are mostly continuously submerged in a counterflow current of cold water until the internal meat temperature reaches $\leq 4^{\circ}\text{C}$, thus inhibiting growth of most microbial food-borne pathogens. Due to the rinsing-off effect of being submerged in water, bacterial levels can be reduced by around 1 log (Table 24.2). However, chilling water can also mediate cross-contamination of carcasses (Buncic and Sofos, 2012). If intended for the fresh carcass market, carcasses are mostly chilled in air-chillers, using a continuous air blast system with or without water sprays at the beginning to improve the chilling process. A disadvantage of air chilling is that no removal of bacteria due to a rinsing-off effect occurs (Buncic and Sofos, 2012), but some reducing effects on bacteria, including *Campylobacter*, do take place (Table 24.2) due to desiccation of carcass surfaces. Spoilage of chilled poultry meat under extended aerobic storage conditions is due to aerobic psychrotrophic bacteria, predominantly *Pseudomonas* spp. and, to a lesser extent, *Acinetobacter*, *Moraxella*, and *Psychrobacter* spp. Sometimes cold-tolerant strains of *Enterobacteriaceae* (e.g., *Enterobacter* and *Serratia* spp.) are also isolated from spoiled

poultry meat. Generally, signs of spoilage become noticeable at bacterial levels of >7 log/g (off-odour) and >8 log/g (slime), but the levels in spoiled meat markedly vary with the predominant species and their metabolic properties. Unlike red meats, poultry meat is not normally stored anaerobically (e.g., in vacuum or carbon dioxide atmosphere) as it affects the meat color negatively.

24.3.2 Meat safety management in poultry abattoirs

The control of the main poultry meat-borne hazards in the abattoir is based on controlling the process hygiene. In practice, this is achieved through: (a) development and implementation of a meat safety system by the company, comprising prerequisite programs (good manufacturing practice (GMP) and good hygiene practice (GHP)) and a hazard analysis and critical control points (HACCP) plan; and (b) auditing of the system by a regulatory authority. The HACCP system should identify, evaluate, and control significant meat safety hazards, and is science-based and company-, product-, and process-specific. It is founded on seven principles: conduct a hazard analysis, identify the critical control points (CCPs) in the process, establish critical limits for each CCP, establish a monitoring system for each CCP, establish corrective actions if monitoring procedures indicate that a CCP is out of control, establish verification procedures to ensure that the HACCP system is operating correctly, and establish documentation and records.

Poultry abattoir HACCP plans treat the main enteric pathogens (*Salmonella*, *Campylobacter*) as high-risk hazards. CCPs are specific for each individual process, but very common CCPs in poultry abattoirs include multistage washing of carcasses (after de-feathering, after evisceration, and an inside/outside wash before chilling) and offal, with the chlorine level being monitored as a critical limit. Also, chilling of carcasses/offal is inevitably a CCP in poultry abattoirs, with temperature being monitored as a critical limit. If carcasses are subject to freezing, this step may

be a CCP, as freezing reduces *Campylobacter* levels on carcasses. Other possible CCPs include scalding, evisceration, and obviously – if allowed and applied – a decontamination step (Anonymous, 2000; Mead, 2000).

24.3.3 Assessment for process hygiene in poultry abattoirs

In the EU, assessment of the hygienic performance of abattoirs (aimed at reducing fecal and other contamination) is conducted through monitoring whether the legislated process hygiene criteria (PHC), which indicate whether the production process is functioning in an acceptable manner, are consistently met (EC, 2005). Currently, *Salmonella* occurrence is used to demonstrate that contamination of final poultry carcasses does not exceed acceptable levels. Neck skins of fifteen chilled carcasses must be sampled on one day on a weekly basis and pooled in groups of three to result in five pooled samples in which *Salmonella* presence or absence are then determined. The results are recorded for a rolling period of ten weeks and if no more than 5 pools out of the 50 are *Salmonella* positive, the process of hygiene of that abattoir is considered satisfactory (EC, 2011). In the case of unsatisfactory results, the cause has to be investigated to prevent the recurrence of the unacceptable microbiological contamination, including reviewing the HACCP-based procedures in the abattoir and the practices on farms from which the poultry originated.

Nevertheless, these current EU PHCs are being scientifically criticized because they provide only process outcomes and not information on initial versus final contamination and thereby neither characterize nor differentiate between the abattoir processes (EFSA, 2007). Furthermore, in poultry abattoirs, only the detection of *Salmonella* is required, as opposed to red meat abattoirs where indicator microorganisms are also measured. Indicators of fecal contamination (*Enterobacteriaceae* and/or generic *E. coli*) are widely considered more suitable for abattoir process hygiene assessment due to the multifactorial and highly variable

presence of pathogens and numerous difficulties with methods for their detection and/or enumeration (EFSA, 2012a). Nevertheless, the potential use of *Campylobacter* counts as a microbiological criterion for poultry abattoir process hygiene has been recently proposed by EFSA (2011), due to the frequent high contamination levels of this pathogen on poultry carcasses and the availability of suitable *Campylobacter* enumeration methods.

24.3.4 Generic framework for comprehensive risk-based poultry carcass meat safety assurance for priority hazards

In Europe, EFSA has recently developed a generic framework for a comprehensive poultry carcass meat safety assurance system, which is considered as the most effective approach to control the top priority microbial hazards (EFSA, 2012a). The key points of that framework in respect to *Salmonella* and *Campylobacter* controls are adapted and briefly summarized in Figure 24.1.

The starting point of the system is the use of measurable *Salmonella*- and *Campylobacter*-related targets at the carcass level, which appropriately correspond to the Food Safety Objective (FSO), which in turn is related to the Appropriate Level of Protection (ALOP); all these need to be pre-set by the regulatory authority. Targets at the carcass level (i.e., incidence or levels of hazards) would inform what has to be achieved at earlier steps in the food chain (i.e., targets to be met at the flock level). The target for each priority hazard should be derived from baseline surveys using mathematical modeling, be risk-based, and be accompanied by a sampling plan. Finally, a monitoring program needs to be designed so as to verify whether the targets are met.

A further important element of the system is risk categorization of: (a) poultry flocks based on the use of flock-related Harmonized Epidemiological Indicators (HEIs) and (b) abattoirs based on abattoir-related HEIs and data on their contamination reduction capacity, i.e.,

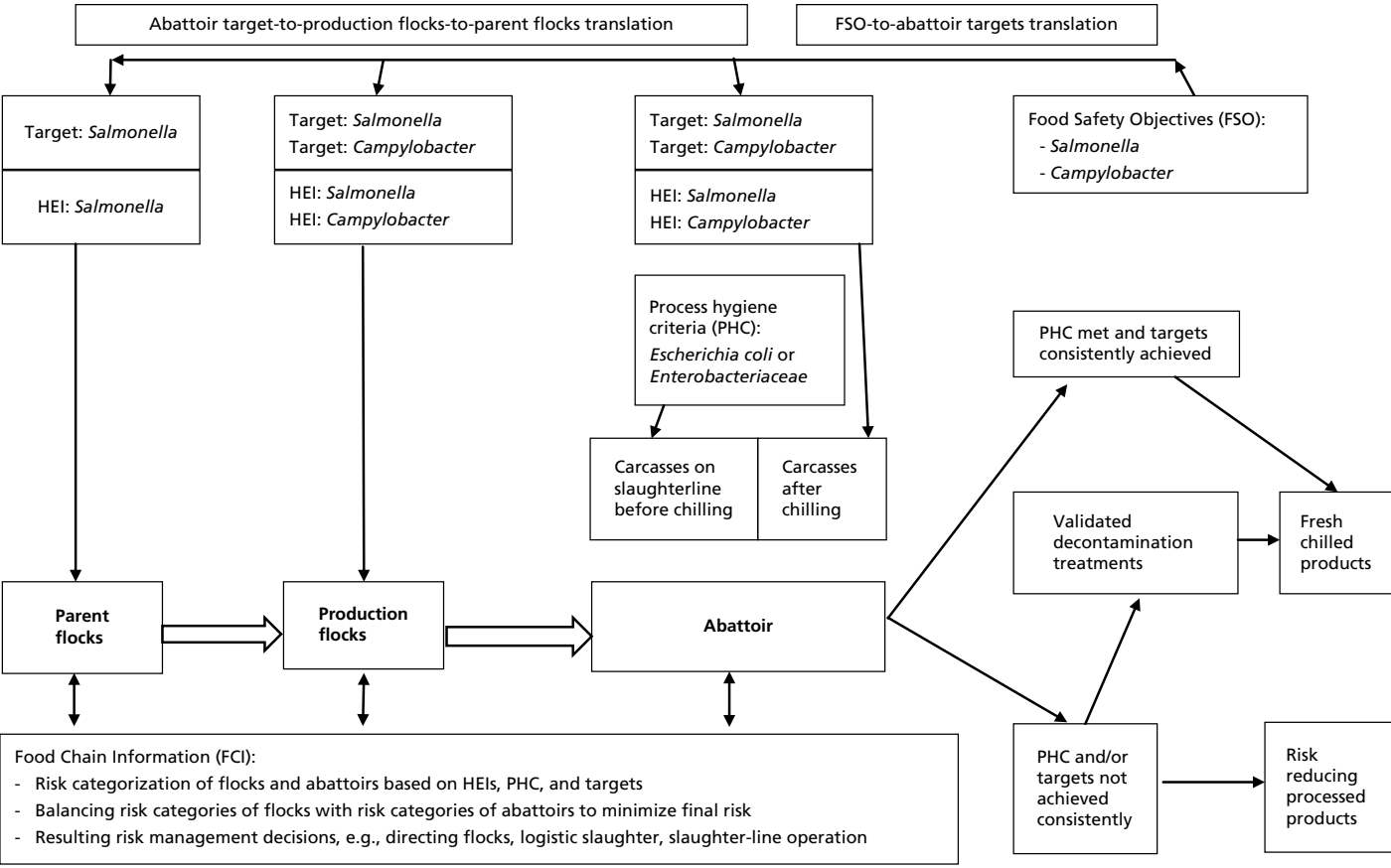


Figure 24.1 Generic framework for chilled poultry carcass meat safety assurance in respect to *Salmonella* and *Campylobacter*.

historical trends of meeting the process hygiene criteria. The HEIs (EFSA 2012b) can include:

- *Parent flock level: Salmonella* HEI (occurrence in pooled fecal samples) and parameters based on farm practices/biosecurity auditing.
- *Production flock level: Salmonella* and *Campylobacter* HEIs (occurrence in pooled fecal and counts in fecal dropping samples, respectively) and parameters based on farm practices/biosecurity auditing.
- *Abattoir level:* (i) *Salmonella* and *Campylobacter* HEIs (occurrence on neck-breast skin and counts in caecal content/neck-breast skin, respectively) and (ii) *E. coli* or *Enterobacteriaceae* levels on neck-breast skin as indicators of process hygiene.

All the information on risk categorization of flocks/abattoirs and other gathered information as indicated above should be managed through a centralized, systematic, electronically managed FCI system ensuring prompt and effective flow of information both “forward” and “backward” along the poultry meat chain.

Based on such FCI, the risk manager could make informed decisions (e.g., to direct high-risk flocks to appropriate risk category abattoirs having suitable equipment to reduce the contamination of carcasses) and/or to apply additional risk-reduction measures (e.g., decontamination

treatments of meat or subjecting meat to bactericidal processing steps) so as to ultimately meet the targets set for the final product (chilled carcass). Each additional risk-reduction treatment has to be validated and implemented within a verified/audited HACCP-based meat safety program.

24.4 Microbial aspects of derived poultry meat products

After the abattoir, poultry meat can reach consumers as a whole-bird product or as a wide range of further processed poultry products. The types and forms of these products vary greatly between countries and companies depending on consumers’ and markets’ demands. Nevertheless, common scenarios of further processing of chilled poultry carcasses are provided in Figure 24.2.

24.4.1 Microbial hazards in poultry meat products

Literature data on the occurrence of the pathogens associated with poultry meats/products at retail level are indicated in Table 24.4, whilst

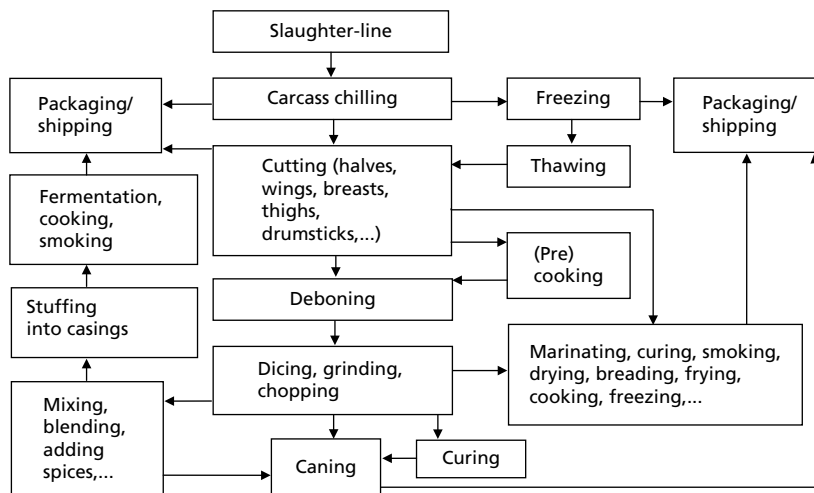


Figure 24.2 Common scenarios of further poultry meat processing.

Table 24.4 Microbial hazards in raw and ready-to-eat (RTE) poultry meat/products at retail.

Hazard	Raw poultry meat and products (N)	RTE poultry products (N)	Reference
<i>Salmonella</i>	9.6% (82 720)	0.4% (36 621)	Mataragas <i>et al.</i> , 2008
<i>Campylobacter</i>	35.1% (59 840)	0.07% (2813)	Mataragas <i>et al.</i> , 2008
<i>L. monocytogenes</i>	14.5% (1921)	1.5% (15 870)	Mataragas <i>et al.</i> , 2008
Pathogenic <i>E. coli</i>	0.5% (3134)	0% (801)	Mataragas <i>et al.</i> , 2008
<i>Y. enterocolitica</i>	7.6% (924)	1.6% (185)	Mataragas <i>et al.</i> , 2008
<i>B. cereus</i>	45% (60) – 56.7% (30)		Smith <i>et al.</i> , 2004; Abostate <i>et al.</i> , 2006
<i>C. perfringens</i>	Chicken: 38.1% (147) – 50.6% (285); turkey: 27.9% (68) – 28.1% (296)		FSANZ, 2005; Wen and McClane, 2004
<i>S. aureus</i>	Chicken: 25.0% (76) – 92.7% (110); turkey: 24.6% (57) – 71.4 (35)		FSANZ, 2005; Bhargava <i>et al.</i> , 2011

N=number of samples tested.

Table 24.5 *Salmonella* and *Campylobacter* in broiler meat at retail in the EU (adapted from EFSA/ECDC, 2014).

Hazard	Fresh broiler meat at retail		RTE broiler meat products at retail	
	2011	2012	2011	2012
<i>Salmonella</i>	6.2% (3076)*	7.7% (2660)	<0.1% (1353)	0% (740)
<i>Campylobacter</i>	34.0% (5059)	24.9% (3495)	no data	no data

* Number of samples tested.

data on *Salmonella* and *Campylobacter* specifically in broiler meat/products in the EU are shown in Table 24.5.

The occurrences of the two main poultry meat pathogens, *Salmonella* and *Campylobacter*, in further processed products are primarily dependent on their presence in/on incoming raw material (birds and carcasses); hence, their most effective control lies there. Nevertheless, in poultry meat products, both of these pathogens can be eliminated by heat treatments, while *Campylobacter* is particularly sensitive to freezing and drying treatments. The food-borne risk of some other pathogens (e.g., *L. monocytogenes*, *B. cereus*, *Clostridium*, *S. aureus*) in/on poultry products is primarily associated with higher concentrations of these microorganisms following their growth in the products. Hence, their most effective control is primarily focused on prevention of growth of these hazards during

the processing–retail–food preparation phase of the poultry meat chain. *L. monocytogenes* can multiply during refrigeration, but can be killed/reduced by proper cooking. Reported D-values in poultry meat include 13–14 min at 55°C, 5.6–8.7 min at 60°C, and 0.11–0.13 min at 70°C (Buncic and Avery, 2004). However, during post-cooking handling (slicing, packaging), ready-to-eat (RTE) products are the main concern as they can be re-contaminated with *L. monocytogenes*. *B. cereus* and *Clostridia* spp. form very resistant spores that can survive extreme conditions in food and afterwards the bacteria can produce toxins associated with growth. In addition, *Clostridium botulinum* produces a very potent but thermolabile toxin; improperly canned/cured products pose the highest risk food. *S. aureus* is heat sensitive and a poor competitor, but is also salt (i.e., low a_w) tolerant and markedly resistant to drying

and freezing; the main food safety concern is the thermostable toxins produced by this microorganism.

24.4.2 Microbiological criteria applicable to processed poultry meats and products

In the EU, PHCs for processed poultry meats (Table 24.6) indicate the acceptable functioning of an HACCP system, and are applied at the end of the manufacturing process. In the case where PHCs are not met, production hygiene and selection of raw materials have to be improved.

The EU food safety criterion (FSC) for poultry meat/products (Table 24.7) defines the acceptability of products/batch (EC, 2005) placed on the market within their shelf life (except for the absence of *L. monocytogenes* in RTE meat at the stage of processing). In the case that FSC is not met, the product/batch must be withdrawn or, in some circumstances, may be submitted to further processing by a treatment eliminating the hazard in question.

24.4.3 Principles of microbial safety assurance of poultry meat products

Microbial safety assurance of poultry meat products is based on process control implemented through an HACCP program, which is primarily hazard-based, i.e., aimed at eliminating or reducing exposure to such hazards. The main microbial hazard control measures applied within process- and product-specific HACCP-based programs for poultry meat

products include those (Table 24.8) that are also universally applicable for other processed products/foods.

However, recently there has been a growing recognition that a hazard-based food safety management system needs to be amended with a more risk-based approach, in which decisions, standards, and actions are based on specific knowledge of risks and are aimed at achieving an established level of health protection (ICMSE, 2002; CAC, 2007; EFSA, 2007). The approach includes using several elements within a hierarchical structure (Figure 24.3).

Appropriate Level of Protection (ALOP) states the level of protection of human health (and animal/plant life) deemed appropriate by a country. Food Safety Objective (FSO) is the maximum frequency and/or concentration of a hazard in a food at the time of consumption that enables an ALOP to be achieved. Performance Objective (PO) is the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain that enables the FSO to be achieved. Performance Criterion (PC) defines the effect in frequency and/or concentration of a hazard in a food that must be achieved to provide PO/FSO. Product/Microbiological Criterion (MC) defines the acceptability of a product, based on the absence/presence or level of microorganisms (and/or toxins) per unit or lot.

The generic framework illustrated in Figure 24.3 links the HACCP-based poultry meat products safety elements and the FSO-based elements into a complete food safety

Table 24.6 PHCs applicable to processing of poultry meat in the EU.

Food category	Microorganisms	Sampling plan		Limits	
		<i>n</i>	<i>c</i>	<i>m</i>	<i>M</i>
Minced meat and mechanically separated meat	Aerobic colony count	5	2	5 × 10 ⁵ cfu/g	5 × 10 ⁶ cfu/g
	<i>E. coli</i>	5	2	50 cfu/g	500 cfu/g
Meat preparations	<i>E. coli</i>	5	2	500 cfu/g or cm ²	5000 cfu/g or cm ²

n=number of units comprising the sample; *c*=number of sample units giving values between *m* and *M*.

Table 24.7 EU FSCs for poultry meat and products and non-compliance with them in 2012 (compiled from EC, 2005, 2011; EFSA/ECDC, 2014).

Microorganisms	Food category	Sampling plan		Limits	% non-compliant in single samples (N)	% non-compliant in batches (N)
		n	c			
<i>Listeria monocytogenes</i>	Ready-to-eat products of meat origin**	5	0	100 cfu/g	0.4% (7224)	<0.1% (5724)
<i>Listeria monocytogenes</i>	Ready-to-eat products of meat origin** at the stage of processing	5	0	Absence in 25 g	2.5% (12 216)	1.5% (34 947)
<i>Salmonella</i>	Minced meat and meat preparations* intended to be eaten raw	5	0	Absence in 25 g	0.2% (619)	0.5% (390)
<i>Salmonella</i>	Minced meat and meat preparations made from poultry meat intended to be eaten cooked	5	0	Absence in 25 g	8.7% (2246)	5.7% (689)
<i>Salmonella</i>	Mechanically separated meat*	5	0	Absence in 25 g	0% (3)	8.3% (12)
<i>Salmonella</i>	Meat products* intended to be eaten raw#	5	0	Absence in 25 g	1.5% (324)	0% (32)
<i>Salmonella</i>	Meat products made from poultry meat intended to be eaten cooked	5	0	Absence in 25 g	2.9% (413)	0% (370)
<i>Salmonella</i> Typhimurium, <i>Salmonella</i> Enteritidis	Fresh poultry meat	5	0	Absence in 25 g	7.9% (2701)	4.1% (677)

* Includes all meat species.

† Excludes fermented sausages.

Excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk
n = number of units comprising the sample; c = number of sample units allowed to exceed limits; N = number of samples tested.

Table 24.8 Main microbial hazard control measures for poultry meat products.

Measures at processing level	Measures at retail–food preparation level
Application of a microbicidal step (e.g., heating) wherever possible “Hurdle” concept using combinations of multiple antimicrobial factors Prevention of cross-contamination during post-cooking manipulation and handling, e.g., slicing, packaging Inclusion of post-processing pasteurization of sealed (“in-bag”) product Adequate final product refrigeration where necessary	Adequate refrigeration Proper cooking Prevention of cross-contamination from raw to ready-to-eat foods via contaminated refrigerators, hands, cutting boards, knives, and kitchen towels Adequate post-cooking handling, especially rapid cooling to refrigeration temperature or keeping food at >60 °C Educational programs targeting both operators and consumers

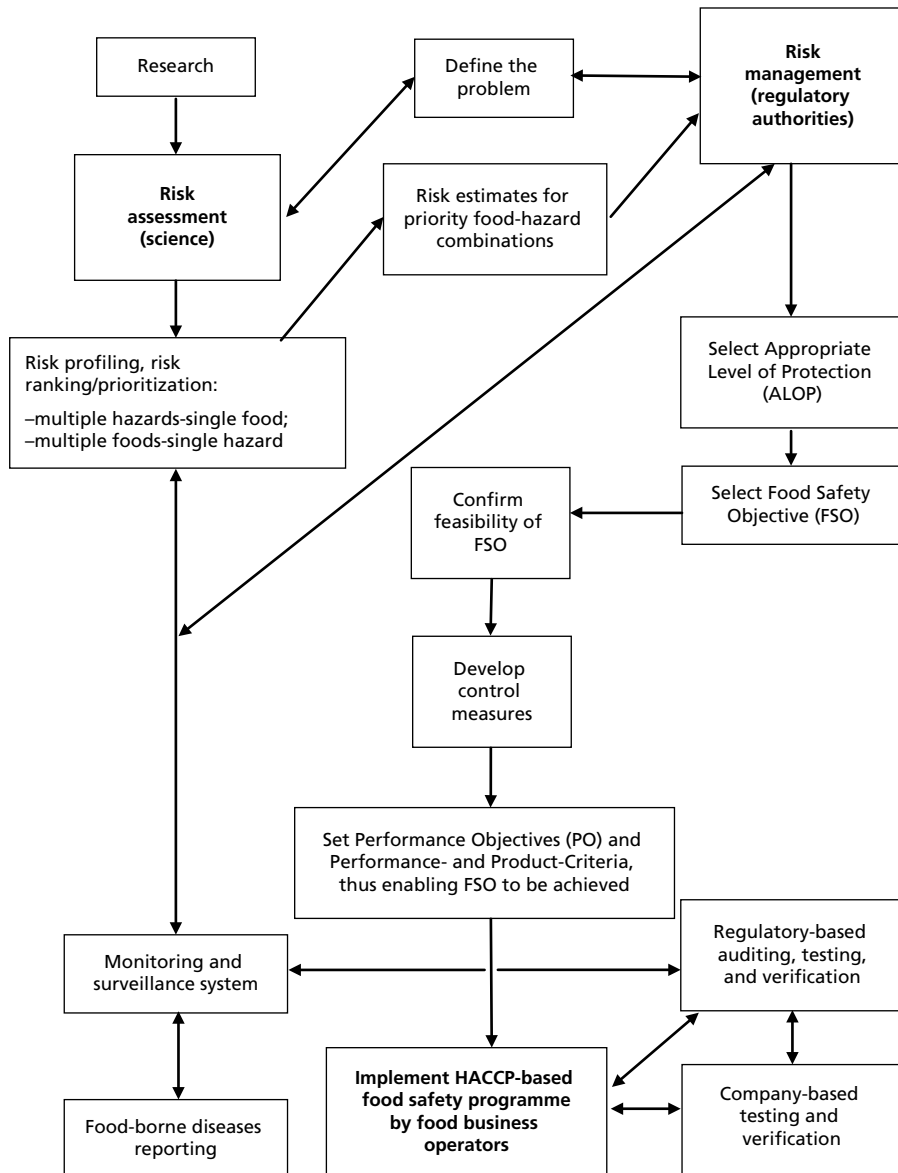


Figure 24.3 Generic framework linking poultry meat products safety assurance and risk-based food safety objective concept.

management system, and also indicates the general distribution of responsibilities for its appropriate functioning.

References

- Abostate, M.A.M., Zahran, D.A., and El-Hifnawi, H.N. 2006. Incidence of *Bacillus cereus* in some meat products and the effect of gamma radiation on its toxin(s). *International Journal of Agriculture and Biology*, **8**, 1–4.
- Abu-Ruwaida, A.S., Sawaya, W.N., Dashti, B.H., Murad, M., and Al-Othman, H.A. 1994. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. *Journal of Food Protection*, **57**, 887–892.
- Altekruse, S.F., Berrang, M.E., Marks, H., Patel Jr, B., Shaw, W. K., Saini, P., Bennett, P.A., and Bailey, J.S. 2009. Enumeration of *Escherichia coli* cells on chicken carcasses as a potential measure of microbial process control in a random selection of slaughter establishments in the United States. *Applied Environmental Microbiology*, **75**, 3522–3527.
- Anonymous. 2000. *A Guide to HACCP Systems in the Meat Industry*, Appendix IX.4: Slaughter, Dressing, Portioning, and Deboning of Chicken (Broilers). MAF Food Assurance Authority (Animal Products Group).
- Berrang, M.E., Dickens, J.A., and Musgrove, M.T. 2000. Effects of hot water application after defeathering on the levels of *Campylobacter*, coliform bacteria, and *Escherichia coli* on broiler carcasses. *Poultry Science*, **79**, 1689–1693.
- Bhargava, K., Wang, X., Donabedian, S., Zervos, M., da Rocha, L., and Zhang, Y. 2011. Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. *Emerging Infectious Diseases*, **17**, 1135–1137.
- Bhunja, A.K. 2008 *Foodborne Microbial Pathogens*. Springer, New York, USA.
- Bolder, N.M. 2007. Microbial challenges of poultry meat production. *World's Poultry Science Journal*, **63**, 401–411.
- Buncic, S. and Avery, S.M. 2004. Microbiological safety of meats: *Listeria monocytogenes*. In: *Encyclopaedia of Meat Sciences*, Vol. 2 (eds Jensen, W.K., Devine, C., and Dikeman, M.), pp. 804–814. Elsevier, Oxford, UK.
- Buncic, S. and Sofos, J. 2012. Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughtering. *Food Research International*, **45**, 639–653.
- Buncic, S. 2014. Control of biological meat-borne hazards. In: *Meat Inspection and Control in the Slaughterhouse* (eds Ninios, T., Fredriksson-Ahomaa, M., Lundén, J., and Korkeala, H.), pp. 334–353. Wiley Blackwell, Oxford, UK.
- CAC (Codex Alimentarius Commission). 2007. *Principles and Guidelines for the Conduct of Microbiological Risk Management (MRM)*, CAC/GL-63. FAO, Rome.
- Cox, N.A., Richardson, L.J., Bailey, J.S., Cosby, D.E., Cason, J.A., and Musgrove, M.T. 2005. Bacterial contamination of poultry as a risk to human health. In: *Food Safety Control in the Poultry Industry* (ed. Mead G.C.), pp. 21–43. Woodhead Publishing Limited, Cambridge, UK.
- Dale, E. and Brown, C. 2013. Zoonotic diseases from poultry. *Brazilian Journal of Veterinary Pathology*, **6**, 76–82.
- EC. 2004 European Commission Regulation 854/2004 of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption.
- EC. 2005. European Commission Regulation 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.
- EC. 2011 European Commission Regulation EC 1086/2011 of 27 October 2011 amending Annex II to Regulation (EC) No. 2160/2003 of the European Parliament and of the Council and Annex I to Commission Regulation (EC) No. 2073/2005 as regards *Salmonella* in fresh poultry meat.
- EFSA. 2007. Opinion of the Scientific Panel on Biological Hazards on Microbiological criteria and targets based on risk analysis. *The EFSA Journal*, **462**, 1–29.

- EFSA. 2011. Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *The EFSA Journal*, **9**, 2105.
- EFSA. 2012a. Scientific Opinion on the public health hazards to be covered by inspection of meat (poultry). *The EFSA Journal*, **10**, 2741.
- EFSA. 2012b. Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of poultry. *The EFSA Journal*, **10**, 2764.
- EFSA/ECDC. 2014. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. *The EFSA Journal*, **12**, 3547.
- FAO/WHO. 2002. Risk assessment of *Salmonella* in eggs and broiler chickens, Technical Report. *Microbiological Risk Assessment, Series 2*. Food and Agricultural Organisation, Rome, Italy. <http://www.who.int/foodsafety/publications/micro/en/salmonella.pdf> (accessed 13 July 2014).
- FAO/WHO. 2009. Risk assessment of *Campylobacter* spp. in broiler chickens, Technical Report. *Microbiological Risk Assessment, Series 12*. Food and Agricultural Organisation, Rome, Italy. http://www.who.int/foodsafety/publications/micro/MRA12_En.pdf (accessed 13 July 2014).
- FAO. 2010. *Agribusiness Handbook: Poultry Meat and Eggs*. Food and Agricultural Organisation, Rome, Italy.
- Fredriksson-Ahomaa, M. (ed.). 2014. Biological hazards. In: *Meat Inspection and Control in the Slaughterhouse* (eds Ninios, T., Fredriksson-Ahomaa, M., Lundén, J., and Korkeala, H.), pp. 277–333. Wiley Blackwell, Oxford, UK.
- FSANZ. 2005. *Scientific Assessment of the Public Health and Safety of Poultry Meat in Australia*. Food Safety Australia and New Zealand. http://www.foodstandards.govt.nz/code/primary-production/poultry/documents/P282_Poultry%20_%20DAR%20Attach3.pdf (accessed 13 July 2014).
- Hui, Y.H., Sattar, S.A., Murrell, K.D., Nip, W.-K., and Stanfield, P.S. (eds). 2001. *Foodborne Disease Handbook*, Vol. 2: *Viruses, Parasites, Pathogens and HACCP*. Marcel Dekker, New York, USA.
- ICMSF (International Commission on Microbiological Specifications for Foods). 2002. *Microorganisms in Food 7: Microbiological Testing in Food Safety Management*. Kluwer Academic/Plenum, New York, USA.
- Loretz, M., Stephan, R., and Zweifel, C. 2010. Antimicrobial activity of decontamination treatments for poultry carcasses: a literature survey. *Food Control*, **21**, 791–804.
- Mataragas, M., Skandamis, P.N., and Drosinos, E.H. 2008. Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. *International Journal Food Microbiology*, **126**, 1–12.
- Mead, G.C. 2000. Fresh and further-processed poultry. In: *The Microbiological Safety and Quality of Food* (eds Lund, B.M., Baird-Parker, T.C., and Gould, G.W.), pp. 445–471. Aspen Publication.
- NACMCF (National Advisory Committee on Microbiological Criteria for Foods). 1997. Generic HACCP application in broiler slaughter and processing. *Journal of Food Protection*, **60**, 579–604.
- PAHO. 2001. *Zoonoses and Communicable Diseases Common to Man and Animals*, Vol. 1. Pan American Health Organization, WHO, Washington, DC, USA.
- Smith, D.P., Berrang, M.E., Feldner, P.W., Phillips, R.W., and Meinersmann, R.J. 2004. Detection of *Bacillus cereus* on selected retail chicken products. *Journal of Food Protection*, **67**, 1770–1773.
- Wen, Q., and McClane, B.A. 2004. Detection of enterotoxigenic *Clostridium perfringens* type A isolates in American retail foods. *Applied and Environmental Microbiology*, **70**, 2685–2691.
- Whyte, P., McGill, K., Monahan, C., and Collins, J.D. 2004. The effect of sampling time on the levels of micro-organisms recovered from broiler carcasses in a commercial slaughter plant. *Food Microbiology*, **21**, 59–65.

CHAPTER 25

Microbial ecology of seafoods: a special emphasis on the spoilage microbiota of North Sea seafood

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25.1 Introduction

The microbiota of seafood is seafood specific and depends on many factors including the intrinsic properties of the fish species, the environment, water temperature, area of catch, and handling and processing procedures (Jay, 1986). Seafoods are mainly available under fresh condition, frozen, lightly preserved, or canned. Preservation and an extended shelf life is usually obtained, as it is for most food, based on the application of combined hurdles such as low temperature, heat treatments, lightly salted (less than 6% NaCl (w/w) in the water phase), marinated or lightly smoked, or the application of some preservatives like sorbate or benzoate. Many of the seafood products are also packed under modified atmosphere and stored and distributed under chilled conditions. Fresh and lightly preserved fish products are highly perishable and of limited shelf life. Therefore, the seafood microbial ecology is mainly studied based on the spoilage microbiota and shelf life, as microbial activities are the main factors limiting shelf life and can be manifested in visible growth, textural changes, or off-odors and off-flavors (Gram and Dalgaard, 2002).

Research has shown that only a fraction of the microbiota present, the “specific spoilage organisms”, or SSOs, are able to contribute to

changes associated with spoiled seafood (Dalgaard, 1995). Each fish/fishery product will have its own specific spoilage bacteria and the counts of these bacteria will, as opposed to the total count, be related to the shelf life. Microorganisms such as *Pseudomonas*, *Shewanella putrefaciens*, *Shewanella baltica*, *Photobacterium phosphoreum*, and *Brochothrix thermosphacta* are commonly known SSOs of seafood during cold storage (Dalgaard, 1995; Dalgaard *et al.*, 1997; Emborg *et al.*, 2002; Gram *et al.*, 1990; Gram and Huss, 1996; Lauzon *et al.*, 2009; Mejlholm *et al.*, 2005; Olafsdottir *et al.*, 2006a, 2006b; Paarup *et al.*, 2002; Vogel *et al.*, 2005).

25.2 Total viable counts (TVCs) and microorganisms identified depends on the method used

The total aerobic psychrotrophic count (APC) is most often used to study the microbial ecology, despite the knowledge that only the portion of the population that is able to form colonies on the specific media can be detected (Broekaert *et al.*, 2011; Hugenholtz *et al.*, 1998). In Table 25.1 is shown for some specific fish species which bacteria cannot grow on certain growth media.

Table 25.1 Identification of 121 isolates with different colony morphology from 11 North Sea fish species after 7 or 4 days (ray) of ice storage, which did not grow on a specific growth medium: plate count agar (PCA), marine agar (MA), Long and Hammer medium (LH), and iron agar with 0.5% NaCl (IA). Second column: the (range of) similarity values (*gyrB*) against type strains; when the *gyrB* sequence could not be used, the 16S rRNA similarity values are used. Third column: the abundance of isolation of these species by the number of isolates. Fourth column: the fish samples on which the microorganisms are found. (From Broekaert *et al.*, 2011. Reproduced by permission).

Identification	Similarity values (%)	Number of isolates	From fish species	Not growing on
<i>Photobacterium</i>				
<i>P. phosphoreum</i>	99.6	4	Salmon, cod, whiting	PCA
<i>P. iliopiscicarium</i>	99.1	3	Sole, plaice, whiting	PCA
<i>Shewanella</i>				
<i>S. frigidimarina</i>	94.6–99.2	47	All fish	PCA
<i>S. vesiculosa</i>	98.5–99.5	5	Angler fish, plaice, sole, salmon, pangasius	PCA
<i>S. baltica</i>	98.7–99.1	3	Angler fish, cod, mackerel	PCA
<i>S. glacialipiscicola</i>	98.7	1	Plaice	PCA
<i>Vibrio/Aliivibrio</i>				
<i>V. littoralis</i>	97.0 (16S)	2	Mackerel, sole	PCA
<i>A. lopei</i>	96.2	1	Salmon	PCA
<i>Pseudoalteromonas</i>				
<i>Psa. nigrifaciens</i>	99.8	33	All fish except cod	PCA
<i>Psychrobacter</i>				
<i>Psb. cibarius</i> -like	/*	1	Mackerel	PCA
<i>Psb. proteolyticus</i>	94.0–95.2	2	Angler fish	PCA
<i>Psb. fozii</i>	/†	1	Angler fish	LH
<i>Psb. maritimus</i>	/‡	1	Angler fish	LH
<i>Psb. cibarius</i>	/§	1	Mackerel	MA, LH & IA
<i>Pseudomonas</i>				
<i>Ps. fragi</i>	92.2–93.3	5	Angler fish, mackerel, sea bass	MA, LH & IA
<i>Ps. fluorescens</i>	89.2	1	Gilthead sea bream	PCA
<i>Acinetobacter</i>				
<i>Acinetobacter</i> sp.	80.8	1	Mackerel	MA, LH & IA
<i>Flavobacterium</i>				
<i>F. hydatis</i>	97.7–98.3 (16S)	4	Salmon, plaice, cod	MA
<i>F. hercynium</i>	96.6 (16S)	2	Angler fish, gilthead sea bream	MA
<i>Janthinobacterium</i>				
<i>J. lividum</i>	95.6	1	Cod	MA
<i>Brochothrix</i>				
<i>B. thermosphacta</i>	99.6 (16S)	2	Gilthead sea bream, sole	IA

*The isolate was identified based on *gyrB* gene sequence and phylogenetic tree analysis. FASTA results gave 96.2% similarity with the *Psb. immobilis* type strain, but phylogenetic tree analysis with inclusion of extra type strains confirmed a higher similarity with *Psb. cibarius*. Sequence comparison between both isolates and the type strain showed several mismatches with the sequence of the *Psb. cibarius*-like isolate.

†The isolate showed 93.6% similarity with *Psb. luti* type strain, but phylogenetic tree analysis gave a higher similarity with *Psb. fozii*.

‡FASTA results gave a similarity value of 93.6% with the *Psb. luti* type strain, while phylogenetic tree analysis gave a higher similarity with the *Psb. maritimus* type strain.

§FASTA results gave a similarity value of 97.4% with the *Psb. immobilis* type strain, while phylogenetic tree analysis gave a higher similarity with the *Psb. cibarius* type strain.

The choice of growth medium is therefore very important, especially since in seafood an APC underestimation of about 1 log₁₀ cfu/g is plausible when using growth media not containing sodium chloride (Figure 25.1), which was also shown in other studies (Broekaert *et al.*, 2011; Joffraud and Leroi, 2000; Van Spreekens, 1974). This can be explained by the fact that the microbiota of marine fish are often marine halophilic organisms e.g., *P. phosphoreum* and *S. putrefaciens*, which need a certain salt content to grow (Vallé *et al.*, 1998).

Because of the better results, most researchers use a combination of media to determine the total viable count of seafood. Very often, it is a combination of mesophilic and psychrophilic counts on several general media together with specific media for the detection of *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria (LAB) and H₂S producers (e.g., *S. putrefaciens*), microorganisms often found in seafood spoilage (Table 25.2).

Besides the choice of growth media, the use of microbiological techniques such as pour versus spread plating and incubation temperatures are also very variable between studies, both parameters that may create a slight dominance for certain microorganisms (Dalgaard *et al.*, 1997; Reynisson *et al.*, 2009). For instance, the most important SSO of fresh fish, *P. phosphoreum*, is not only

vulnerable to the salt content but also to temperature fluctuations (Dalgaard *et al.*, 1997; Emborg *et al.*, 2002).

25.3 The initial microbiota of marine fish

In general, seafood is contaminated by a wide range of microorganisms that occur naturally in the surrounding environment. The initial microbial contamination varies between 10² to 10⁷ cfu/cm² on the skin in the outer slime layer and between 10³ and 10⁹ cfu/g on the gills and in the intestines, while the fish flesh is considered to be sterile (Shewan, 1962). The degree of microbial contamination depends on several environmental factors such as the water temperature, salt content, the season of catch, fishing method, and the microbiological quality of the water, and also on fish-specific factors including the type of fish and their diet (Dalgaard *et al.*, 1997; Feldhusen, 2000; Grigorakis *et al.*, 2003; Huss, 1995; Jay, 1986; Kaspar and Tamplin, 1993; Tzikas *et al.*, 2007; Zaballos *et al.*, 2006).

According to standard literature, fish from cold and temperate waters contain mainly psychrotrophic Gram-negative, rod-shaped, strict aerobic or facultative anaerobic microorganisms of the genera *Aeromonas*, *Pseudomonas*,

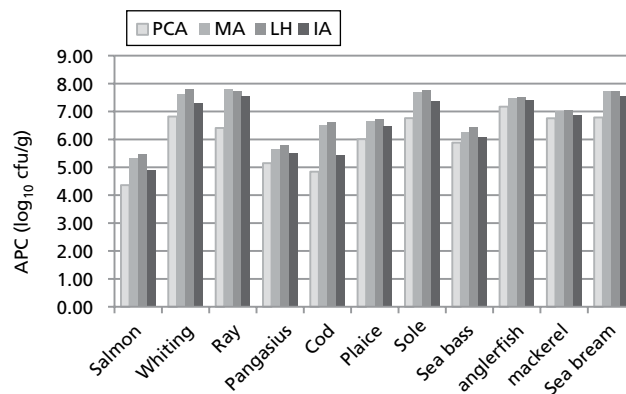


Figure 25.1 The total aerobic psychrotrophic count (APC) (log₁₀ cfu/g) on plate count agar (PCA), marine agar (MA), Long and Hammer medium (LH) and iron agar with 0.5% NaCl (IA) (mean of duplicate platings) for 11 fish samples after 7 days or 4 days (ray) of ice storage (right). (After Broekaert *et al.*, 2011. Reproduced by permission.)

Table 25.2 Different general and specific media used in North Sea seafood analysis over the last years and the wide range of incubation parameters used.

Microorganisms	Medium	Incubation parameters	Seafood type	References
General media				
Total viable count (incl. mesophilic count)	Plate count agar (PCA) [†] Tryptone soya agar + 0.6% yeast extract (TSAYE)	3d 31 °C or 2d 37 °C or 3d 30 °C or 2d 30 °C 2d 30 °C	Sea bass, sole, sardine product, hake Shellfish, herring	Baixas-Nogueras <i>et al.</i> , 2007; Carrascosa <i>et al.</i> , 2014; Kilinc and Cakli, 2005; Poli <i>et al.</i> , 2006; Teixeira <i>et al.</i> , 2014; Zambuchini <i>et al.</i> , 2008 Karimet <i>et al.</i> , 2011; Linton <i>et al.</i> , 2003
Total aerobic psychrophilic count	Long and Hammer medium (LH) [†] TSAYE Iron agar (IA), IA + 1% NaCl and no overlay) [‡] PCA	7d 15 °C or 3d 21 °C 10d 7 °C 7d 15 °C or 3d 20 °C or 5d 17 °C (pour) 2d 25 °C, 10d 7 °C, 3d 20–21 °C	Cod, several raw fish species, shrimp Shellfish, herring Production area fish fillets and shrimps, cod fillets, halibut Shell fish, sardine product, hake, several raw fish species, shrimp Halibut Shell fish, several raw fish species, shrimp	Broekaert <i>et al.</i> , 2011; Broekaert <i>et al.</i> , 2013; Hovda <i>et al.</i> , 2007a; Hovda <i>et al.</i> , 2007b; Hovda <i>et al.</i> , 2007c; Reynisson <i>et al.</i> , 2009 Karim <i>et al.</i> , 2011; Linton <i>et al.</i> , 2003 Gudbjornsdottir <i>et al.</i> , 2005; Hovda <i>et al.</i> , 2007a; Hovda <i>et al.</i> , 2007b; Mai <i>et al.</i> , 2011 Baixas-Nogueras <i>et al.</i> , 2007; Kahn <i>et al.</i> , 2005; Kilinc and Cakli, 2005 Hovda <i>et al.</i> , 2007a; Hovda <i>et al.</i> , 2007b; Hovda <i>et al.</i> , 2007c Broekaert <i>et al.</i> , 2011; Broekaert <i>et al.</i> , 2013; Kahn <i>et al.</i> , 2005
Specific media				
<i>Pseudomonas</i> spp.	<i>Pseudomonas</i> CFC STAA	2d 31 °C, 1d 30 °C or 3d 25 °C or 2d 20 °C 3d 25 °C	Sea bass, cod, sole, seafood Seafood	Bozariis <i>et al.</i> , 2013; Carrascosa <i>et al.</i> , 2014; Mai <i>et al.</i> , 2011; Zambuchini <i>et al.</i> , 2008 Bozariis <i>et al.</i> , 2013
<i>Brochothrix thermosphacta</i> <i>Photobacterium phosphoreum</i>	IA	14d 5 °C (spread) [‡]	Sea bass	Carrascosa <i>et al.</i> , 2014
<i>Enterobacteriaceae</i> H ₂ S producers (black colonies)	VRB(G)A IA, IA + 1% NaCl and no overlay) [‡]	2d 37 °C or 2d 30 °C 2–3d 20–21 °C or 5d 17 °C (pour)	Sea bass, sole Sea bass, cod, halibut, several raw fish species, shrimp	Carrascosa <i>et al.</i> , 2014; Poli <i>et al.</i> , 2006; Zambuchini <i>et al.</i> , 2008 Broekaert <i>et al.</i> , 2011; Broekaert <i>et al.</i> , 2013; Carrascosa <i>et al.</i> , 2014; Hovda <i>et al.</i> , 2007a; Hovda <i>et al.</i> , 2007b; Hovda <i>et al.</i> , 2007c; Mai <i>et al.</i> , 2011

[†] The reference medium by the International Organization for Standardization (ISO) for the psychrotrophic enumeration of microorganisms on food (ISO 17410 2001).

[‡] The NMKL 184 method (NMKL 184 2006).

[‡] Modified by Gram *et al.* (1987).

^{*} According to Dalgaard (1995).

Moraxella/*Acinetobacter*, *Shewanella*, *Flavobacterium*, or the family *Vibrionaceae* (Liston, 1980). Gram-positive bacteria, such as *Bacillus*, *Micrococcus*, *Clostridium*, *Lactobacillus*, and coryneforms may occur, but the Gram-negative microorganisms will dominate the microbiota (Elotmani *et al.*, 2004; Gennari *et al.*, 1999). Although it is said that seasonal differences occur in the contamination degree, significant differences in species variation have not been observed (Elotmani *et al.*, 2004). Also the influence of the water temperature on the species variation is disputed and may only depend on the fish species. Shewan (1977) described that fish from tropical waters contain more mesophilic Gram-positive microorganisms such as *Bacillus* and *Micrococcus*, but several other authors found a microbial contamination similar to those of temperate waters, but often with a slightly higher contamination degree of Gram-positives and enteric bacteria (Gram *et al.*, 1990; Jay, 1986; Liston, 1980). Because of the specificity of the microbiota depending on the seafood characteristics and origin, it was chosen to describe only North Sea (temperate water) seafood bacterial ecology in this contribution.

In general, most seafood in Europe is purchased raw or only slightly preserved. Nevertheless, it can be stated that all processing procedures will have an effect on the microbiota. Examples of changes in North Sea seafood bacterial ecology during storage are listed in Table 25.3. These changes may comprise delay of the growth/lag phase, changes in microbial ecology, or dominance of certain bacterial groups or particular species.

25.4 Raw seafood

25.4.1 Gutting and filleting

Even early handling and processing procedures may have an influence on the microbial ecology. Gutting of the fish often has several benefits, including a decrease in (mesophilic) aerobic counts and a lower amount of H_2S -producing

bacteria and *Enterobacteriaceae* (often gastrointestinal microorganisms), although the total viable psychrophilic count and the amount of *Pseudomonas* spp. may remain nearly the same (Erkan and Ozden, 2008). Some studies did not find any significant decrease in microbial amounts, but found that the specific spoilage organisms were detected faster and in higher amounts in gutted fish (Erkan and Ozden, 2008; Karl and Meyer, 2007). This observation highlights the problem of possible cross-contamination (from intestines and processing environment) during gutting. Filleting of fish will initially decrease the bacterial load as the outer layer (skin and slime layer containing up to $2-7 \log_{10}$ cfu/g) will be removed. However, due to the removal of the protective skin, which may slow down the microbial penetration, the increase in the microbial counts during storage will be faster than for the whole fish (Poli *et al.*, 2006).

25.4.2 Cooling (icing, superchilling, freezing)

One of the most important procedures to deliver high-quality seafood is immediate cooling/icing after the catch (Akankwasa, 1998). Different ways of icing (e.g., flake ice or slurry ice) can be used. The use of slurry ice may decrease the APC (mesophilic and psychrophilic) up to 1 log for some seafood (e.g., for fast spoiling ray) at the end of storage (Múgica *et al.*, 2007). For many food products including seafood, superchilling is used to result in a better quality compared to conventional chilling (Duun and Rustad, 2008; Fagan *et al.*, 2004; Wang *et al.*, 2008). When superchilled, food products are stored between the freezing point of the products and 1 to 2°C below this. The surrounding temperature is set below the initial freezing point of the food, which is between -0.5 and -2.8°C (Fennema *et al.*, 1973), and depending on the method used some ice is formed in the outer few millimeters. This is in contrast to conventional chilling on ice where the surface of the fish, when in direct contact with the ice, is $0 \pm 0.5^\circ\text{C}$. When the ice does not

Table 25.3 Changes in total aerobic psychrophilic count (APC in log₁₀ cfu/g) between T_0 (starting point of the experiments, mostly immediately after the catch) and T_{end} (most often at the end of shelf life), and shifts in the microbial ecology or in dominance (in % if possible) in microbiota between these two time points due to different storage or processing conditions (column 2) of several North Sea seafood products (column 1). NP: not provided. LS: Lightly salted (2.5%).

Fish species	Storage or processing conditions	APC T_0	Dominating microbiota at T_0	APC T_{end}	Dominating microbiota at T_{end}	References
Sea bream	Air	3,88	<i>Acinetobacter</i>	7,75	<i>Pseudomonas</i> , <i>Aeromonas</i> , <i>Shewanella</i>	Parlapani et al., 2013
Sardine	Air	4	<i>Flavobacterium</i> , <i>Psychrobacter</i> , <i>Pseudomonas fluorescens</i> , <i>Enterobacteriaceae</i>	6	<i>Pseudomonas fragi</i> , <i>Psychrobacter</i> , <i>Shewanella</i> , <i>Pseudomonas fluorescens</i>	Gennari et al., 1999
Salmon (raw)	MAP	3,5	NP	8	<i>P. phosphoreum</i> (31%), <i>Brochothrix thermosphacta</i> (22%), <i>Serratia</i> spp. (18%), <i>Carnobacterium divergens</i> (9%), <i>Carnobacterium maltaromaticum</i> (5%)	Macé et al., 2012
Salmon (raw)	Vacuum	3,5	NP	8	<i>Serratia</i> spp. (41%), <i>P. phosphoreum</i> (24%), <i>C. maltaromaticum</i> (14%), <i>B. thermosphacta</i> (10%)	Macé et al., 2012
Atlantic horse mackerel	MAP (48% CO ₂ , 50% N ₂ , 2% O ₂)	4,5	<i>Photobacterium</i> spp. (16, 1%), <i>Arthrobacter</i> spp. (12, 9%), <i>Carnobacterium</i> spp. (11%), <i>Pseudoclavibacter</i> (8, 4%), <i>Chryseobacterium</i> spp. (7, 1%)	5,7	<i>Serratia</i> (28, 6%), <i>Carnobacterium</i> spp. (22, 1%), <i>Shewanella</i> spp. (16, 9%), <i>Yersinia</i> spp. (11, 7%), <i>Vibrio</i> spp. (6, 5%)	Alfaro and Hernandez, 2013
Halibut	Air	2	<i>Pseudomonas</i> spp., <i>P. phosphoreum</i>	9	<i>Burkholderia</i> spp., <i>Pseudomonas</i> spp., <i>B. thermosphacta</i> , <i>Serratia</i> spp., <i>Yersinia</i> spp.	Hovda et al., 2007b
Halibut	50% CO ₂ , 50% N ₂	2	<i>Pseudomonas</i> spp., <i>P. phosphoreum</i> , <i>Micrococcus luteus</i>	7	<i>Staphylococcus</i> spp., <i>B. thermosphacta</i>	Hovda et al., 2007b
Halibut	50% CO ₂ , 50% O ₂			5,5	<i>Staphylococcus</i> spp., <i>B. thermosphacta</i> , <i>P. phosphoreum</i>	Hovda et al., 2007b
Cod	Air	4	<i>Chryseobacterium</i> sp., <i>Carnobacterium</i> sp., <i>Pseudomonas</i> sp., <i>Shewanella putrefaciens</i> , <i>Pseudomonas fragi</i> , <i>Photobacterium</i> spp., <i>Tiedjea artica</i> , <i>Serratia</i> sp.	9	<i>Pseudomonas</i> sp., <i>S. putrefaciens</i> , <i>P. phosphoreum</i> , <i>Pseudomonas putida</i>	Hovda et al., 2007a
Cod	50% CO ₂ , 50% N ₂			4,5	<i>S. putrefaciens</i> , <i>P. spp.</i> , <i>P. putida</i>	Hovda et al., 2007a
Cod	50% CO ₂ , 50% O ₂			4,5	<i>Pseudomonas</i> spp.	Hovda et al., 2007a

Cod	MAP, 2 °C	6	NP	7,5	<i>P. phosphoreum</i> (75%), <i>S. putrefaciens</i> (20%)	Guldager et al., 1998
Cod	MAP, thawed			6	<i>Carnobacterium</i> (55%), <i>Pseudomonas</i> (20%), <i>Coryneforms</i> (15%), <i>Shewanella</i> spp. (5%), <i>Moraxella</i> spp. (5%)	Guldager et al., 1998
Cod	MAP, 1.5 °C	4,7		7		Wang et al., 2008
Cod	MAP, -1 °C	4,7		6		Wang et al., 2008
Cod	LS, 0 °C, air	3,5	<i>Pseudomonas</i> spp., <i>Sphingomonas</i> spp., <i>Variovorax</i> sp., <i>Bradyrhizobium</i> spp.	7	NP	Reynisson et al., 2009
Cod	LS, -1 °C, air	3,5	<i>Photobacterium phosphoreum</i> (61%), <i>Psychrobacter</i> spp., <i>Janthinobacter lividum</i> , <i>Pseudomonas tolaasii</i>	7	<i>P. phosphoreum</i> (83%), <i>Pseudomonas</i> spp., <i>Psychrobacter arcticus</i> , <i>Vibrio logei</i>	Reynisson et al., 2009
Cod	LS, -1 °C, MAP	3,5	<i>P. phosphoreum</i> (100%)	5,5	<i>P. phosphoreum</i> (100%)	Reynisson et al., 2009
Norway lobster	Air, 1 °C	3,5	<i>Vibrio</i> spp. (82%), <i>Pseudoalteromonas</i> spp. (6%), <i>Photobacterium</i> spp. (6%), <i>Shewanella</i> (6%), <i>Psychrobacter</i> spp. (6%)	8	<i>Vibrio</i> spp. (45%), <i>Photobacterium</i> spp. (55%)	Gornik et al., 2013
Norway lobster	MAP, 1 °C			4	<i>Vibrio</i> spp. (6%), <i>Photobacterium</i> spp. (94%)	Gornik et al., 2013
King scallop	Air	5,5	<i>S. putrefaciens</i> , <i>Pseudomonas</i> spp., <i>Morganella morganii</i> , <i>Pseudoalteromonas haloplanktis</i> , <i>Psychrobacter</i> sp.	8	<i>B. thermosphacta</i> , <i>Pseudomonas</i> spp., <i>S. putrefaciens</i> , <i>Morganella morganii</i> , <i>Moellerella wisconsensis</i>	Coton et al., 2013
King scallop	MAP	5,5	<i>S. putrefaciens</i> , <i>Pseudomonas</i> spp., <i>M. morganii</i> , <i>Arthrobacter bergerei</i> , <i>P. haloplanktis</i>	8	<i>B. thermosphacta</i> , <i>Pseudomonas</i> spp., <i>S. putrefaciens</i> , <i>M. morganii</i> , <i>M. wisconsensis</i> , <i>C. maltaromaticum</i> , <i>Actinomyces radidentis</i>	Coton et al., 2013

have contact with the fish, the temperature will be slightly higher. This superchilling may decrease the microbial growth of microorganisms during storage by 1–2 log (TVCs and pseudomonads). Also *P. phosphoreum* and LAB can be decreased by 3–4 log (Olafsdottir *et al.*, 2006b; Wang *et al.*, 2008) while others, such as H₂S-producing bacteria – most likely *Shewanella* spp., may not be affected (Olafsdottir *et al.*, 2006b).

Freezing is also often used to store seafood for a larger amount of time. North Sea fish such as salmon, cod, and hake are often frozen/thawed after the catch to extend shelf life. Freezing of the fish may delay the microbial counts for up to a few days. According to Makarios-Laham and Lee (1993), psychrophilic microorganisms may even contribute to deterioration of seafood during frozen storage. In that study, slow microbial growth was measurable at –5 and –8°C. Growth near subfreezing temperatures was mainly by Gram-negative, oxidase, and catalase-positive, coccobacillary-shaped cells, which were identified as *Psychrobacter phenylpyruvicus* by API tests (Tsironi *et al.*, 2009).

P. phosphoreum and pseudomonads, however, will often be completely absent after freezing/thawing (Boknaes *et al.*, 2000; Emborg *et al.*, 2002; Guldager *et al.*, 1998) and also *Shewanella* spp. will be strongly reduced (Baixas-Nogueras *et al.*, 2007). LAB, such as *Carnobacterium* spp. may dominate the spoilage microbiota e.g. in frozen/thawed MAP salmon (Emborg *et al.*, 2002).

25.5 Processing – lightly preserved seafood

The initial microbiota strongly depends on the hygienic conditions within the processing areas (Gancel *et al.*, 2010; Leroi *et al.*, 2001; Truelstrup-Hansen and Huss, 1998), but often remains dominated by Gram-negative bacteria typical of fresh fish (Gonzalez-Rodriguez *et al.*, 2002; Leroi *et al.*, 1998; Paludan-Muller *et al.*, 1998).

Lightly preserved seafood is usually produced from fresh seafood and further processing involves one or a few additional steps that increase the risk of cross-contamination. The treatments reduce the microbiota but are not sufficient to eliminate them totally. These highly perishable products are stored usually at chilled temperatures under vacuum or MAP to extend shelf life. While the high postmortem pH, the low percentage of sugars, the high content of low molecular weight nitrogenous molecules, and the low temperature of temperate waters favor the rapid growth of psychrotolerant marine Gram-negative bacteria such as *Pseudomonas*, *Shewanella*, and *Photobacterium*, several preservation techniques may select for a shift in dominance between the present microbiota or favor the growth of other bacteria. These comprise LAB (modified atmosphere packaging (MAP) inclusive vacuum packaging, fermentation, brining), halophilic bacteria (lightly salted), *B. thermosphacta* (MAP), and Gram-positive bacteria (high-pressure treatment) (Table 25.3).

25.5.1 Lightly salted, marinated, and cold-smoked fish

The lowering of a_w by the addition of NaCl, equivalent to an a_w of 0.96, results in the inhibition of many Gram-negative bacteria, and allows growth of other more resistant organisms such as lactic acid bacteria (LAB) and *Micrococcus* (*Micrococcaceae*). At the end of storage, Gram-positive bacteria, especially LAB, become predominant, sometimes associated with *Enterobacteriaceae* and *B. thermosphacta* (cold-smoked salmon: Cardinal *et al.*, 2004; Leroi *et al.*, 2001; Truelstrup-Hansen, 1995; Maatjes herring: Lyhs *et al.*, 2007; cooked brined shrimp: Dalgaard *et al.*, 2003; Dalgaard and Jorgensen, 2000). Gonzalez-Rodriguez *et al.* (2002) found within the LAB the genera *Carnobacterium* and *Lactobacillus* most frequently present. The use of vacuum and MAP are other factors promoting LAB development since they are anaero-aero-tolerant bacteria (Leroi, 2010).

Besides salting, smoking of fish and fishery products is one of the oldest methods in food preservation and widely used in fish processing. The shelf life of smoked fish products depends largely on the initial bacterial contamination of the raw material, the decrease in a_w due to salting and drying, the heat treatment (cold <30°C or hot smoking >60°C), and the further storage conditions. Depending on these parameters the shelf life of the processed fish products will be affected. The temperature reached at the cold smoking process is too low to destroy bacteria. In cold smoked products the salt concentration (2.5–4%) in conjunction with refrigeration and the smoke has to reduce the growth of probable spoiling bacteria. Bozaris *et al.* (2013) found that the selection of the spoilage microorganisms in processed seafood is mainly determined by the pH and the salt content and resulted mainly in LAB and yeasts as SSOs. *Lactobacillus* spp. were indeed the predominant microorganisms found in marinated herring, sardines, and anchovies. In the latter product *Micrococcus* sp. were also reported (Fuselli *et al.*, 1998). Leroi *et al.* (1998, 2000, 2001) studied intensively smoked salmon and found besides lactobacilli and yeasts also *Enterobacteriaceae* and *B. thermosphacta*. Lyhs *et al.* (1999) characterized the lactobacilli present in cold smoked rainbow trout.

25.5.2 Hot-smoked fish products

Hot-smoking combines in a single process the effects of salting, drying and heating with those of the smoke itself. The microbiological status of the final product depends largely on the initial microbiota and the processes carried out. In this kind of processing, the intention is to cook the fish as well as to smoke it. Processing temperatures used in the industry vary significantly, and the minimum internal temperature for adequate processing of hot-smoked fish has been a major issue of concern for years. Proper heating helps to eliminate food-poisoning

bacteria and extend shelf life. Current guidance from health regulatory agencies recommends a minimum internal temperature of 63°C for 30 minutes. Following smoking, the products should be stored at or below 3°C. Hot-smoked products should also contain a minimum water phase salt concentration of 2.5% if aerobically packaged and 3.5% salt if anaerobically packaged. The thermal treatment has to be sufficient to destroy most of the non-spore-forming bacteria and microbial counts can be reduced, depending on the whole process, to less than 50 cfu/g in mackerel (Münckner and Meyer, 1994) or to about 2×10^2 cfu/g on sardine fillets (Beltran *et al.*, 1989), as reviewed by Sikorski and Kalodziejska (2002).

25.5.3 Packaging and storage conditions (temperature)

During storage, the composition of the initial microbial population may shift (Reynisson *et al.*, 2009; Shewan and Georgala, 1957). In general, seafood is kept in chilled storage conditions, preferably on ice. This makes that the psychrotrophs, which grow optimal at chilled conditions, are able to dominate the flora during storage since they outgrow non-psychrotrophic species of other genera (Castell and Mapplebeck, 1952; Moore *et al.*, 2006; Tryfinopoulou *et al.*, 2002). Air-stored chilled or iced conditions often benefit the growth of *Pseudomonas* spp., *S. putrefaciens*, and *Psychrobacter immobilis* (Gennari *et al.*, 1999). Temperature fluctuations will influence the microbiota during storage. For instance, abusive temperature can create an optimal environment for strong microbial spoilers such as *Photobacterium phosphoreum*, *Vibrionaceae*, and *Enterobacteriaceae* (Lindberg *et al.*, 1998; Molin and Stenström, 1984), or other microorganisms that are able to produce biogenic amines, and this may have consequences for human health (Olafsdottir *et al.*, 2006b).

The microbiota and shelf life of seafood will also be influenced by storage conditions such as

Table 25.4 Influence of the gas composition on specific spoilage organisms dominating fresh fish stored at 0 to 4°C under different gas atmospheres (Gram and Huss, 1996, 2000; Huss *et al.*, 1997; Lalitha *et al.*, 2005; Poli *et al.*, 2006). All these factors, together with the intrinsic features of the seafoods, create a seafood-specific microbiota.

Gas composition	Microorganisms in fish
Air	<i>Shewanella putrefaciens</i> , <i>Pseudomonas</i> spp.
>50% CO ₂ with O ₂	<i>Brochothrix thermosphacta</i> , <i>S. putrefaciens</i>
50% CO ₂	<i>Photobacterium phosphoreum</i> , lactic acid bacteria (LAB)
50% CO ₂ with O ₂	<i>P. phosphoreum</i> , LAB, <i>B. thermosphacta</i>
100% CO ₂	LAB
Vacuum packed	<i>Pseudomonas</i> spp., <i>P. phosphoreum</i>

aerobic storage, vacuum or modified atmosphere packaging (MAP), and the used gas composition (Table 25.4) (Poli *et al.*, 2006). MAP will extend the shelf life of fresh fish significantly due to its ability to retard microbial growth. It will also reduce certain undesirable physiological, chemical/biochemical, and physical changes in foods (Floros and Matsos, 2005). Numerous studies have been conducted concerning the effect of vacuum and MAP preservation on the shelf life and quality of North Sea fish and fishery products in general (Alfaro *et al.*, 2013; Sivertsvik *et al.*, 2002) and on specific North Sea seafood products such as cod (Debevere and Boskou, 1996), sardines (Ozogul *et al.*, 2004), sea bass (Torrieri *et al.*, 2006), salmon (Emborg *et al.*, 2002; Macé *et al.*, 2012), shellfish (Gornik *et al.*, 2013), and horse mackerel (Alfaro and Hernandez, 2013). The best-known specific spoilage organisms of fresh MAP fish are *P. phosphoreum*, *B. thermosphacta*, and LABs (Dalgaard *et al.*, 1993, 2003; Dalgaard and Jorgensen, 2000; Gram and Huss, 1996; Jeppesen and Huss, 1993; Rudi *et al.*, 2004). Based on the

gas mixture (Table 25.4), MAP packaging of fresh fish may eliminate the growth of several microorganisms important for the production of off-odors and off-flavors such as *Pseudomonas* spp., *Aeromonas* spp., and *S. putrefaciens* (Gennari *et al.*, 1999; Gram and Huss, 1996; Hansen *et al.*, 2007; Lalitha *et al.*, 2005; Leroi, 2010; Poli *et al.*, 2006).

25.5.4 High-pressure treatment (HPP)

HPP is established as a non-thermal technology that is capable of decreasing microbial numbers (Teixeira *et al.*, 2014) or delaying growth by extending the lag phase (Karim *et al.*, 2011; Teixeira *et al.*, 2014) and therefore extending shelf life and improving the microbiological safety of many foods, including seafood (Patterson, 2005). In seafood, such as herring and haddock (Karim *et al.*, 2011), sea bass (Teixeira *et al.*, 2014), and shellfish (Cruz-Romero *et al.*, 2008; Linton *et al.*, 2003), HPP treatments have been described. HPP is most often combined with chilled storage and MA packaging. Depending on the initial bacterial ecology, the dominant microbiota of HPP treated seafood may change towards a microbiota relatively resistant to high-pressure, mainly Gram-positive microorganisms, such as *Staphylococcus*, *Bacillus*, *Acinetobacter/Moraxella*, and LAB (Linton *et al.*, 2003). In herring and haddock with a dominant initial microbiota of *Staphylococcus*, *Bacillus*, and other Gram-positive bacteria (e.g., *Lactobacillus*, *Streptococcus*, and coryneforms), the percentage of *Enterobacteriaceae* decreased while *Micrococcus* spp. and *Bacillus* spp. dominated after HPP treatment and during shelf life spore-formers such as clostridia were able to outgrow, while psychrophilic Gram-negative bacteria stayed below the detection limit (Karim *et al.*, 2011). Also, H₂S producers (mainly Gram-negatives) often disappear below the detection limit (Teixeira *et al.*, 2014).

25.6 A case study: brown shrimp (*Crangon crangon*) (adapted from Broekaert *et al.*, 2013)

Brown shrimp can be found in cold to average temperature waters and are especially intensively caught in the North Sea. The shrimp landed in Belgian harbors are processed on board the vessels according to traditional methods. On board, shrimp are sorted and heated (20 kilo shrimp in approximately 100l of boiling sea water) for 2 to 4 minutes. When the cold shrimp are poured into the boiling water, the temperature immediately drops to 86–92°C. After cooking, the shrimp are immediately cooled down for about 5 minutes in a rinsing machine where cold clean sea water is poured over the cooked shrimp, which cools it down to approximately 15°C. The cooled shrimp are stored in a cold room covered with ice until landing the following morning. Between the cooking of the shrimp and consumption, diverse processing steps occur, which all may contribute to post-contamination of the product. To increase shelf life of this ready-to-eat (RTE) product, mainly preservatives such as 0.6% benzoic acid are added to the shrimp. In Belgium, a particular brand of shrimps is marketed without preservatives. Those shrimp are offered on the market within 24 hours after catch, unpeeled or mechanically (locally) peeled, in modified atmosphere packaging. In this case study, we will focus on the microbial load and ecological shifts in microbiota for brown shrimp processed either under optimal conditions (in the laboratory) or under market conditions, both without preservatives and air stored on ice.

Due to the cooking step, the microbial contamination can be a result of post-cooking recontamination or due to microorganisms resistant to the short heat treatment. Further processing, both on board and on land, will alter the microbiota. Especially cooling the shrimp with sea water will contaminate the shrimp with

Table 25.5 Microbiological counts on all growth media in log₁₀ cfu/g of raw shrimp and of cooked unpeeled shrimp sampled on board and processed under optimal lab conditions at T_0 (start of analyses). /: not performed. PCA = plate count agar, LH = modified Long and Hammer medium (Van Spreekens, 1974), MA = marine agar, CFC = *Pseudomonas* Cetrimide Fucidine Cephaloridine, MRS = Man Rogosa Sharp medium, VRBGA = violet red bile glucose agar, and IA = iron agar (Atlas, 2006). (From Broekaert *et al.*, 2013. Reproduced by permission.)

Microbiological counts of shrimp (log ₁₀ cfu/g) at T_0		
Medium	Uncooked	Cooked
Psychrophilic TVC		
PCA	4.9	2.9
LH	6.5	3.5
MA	6.5	4.1
<i>Pseudomonas</i> CFC	4.6	2.7
LAB (MRS)	2.6	2.2
Enterobacteriaceae (VRBGA)	1.0	<10
H ₂ S producers (IA)	2.2	<10

psychrophilic microbiota, but this also seems to be happening when the shrimp are cooled to the air or by ice (Van Spreekens and De Man, 1970). Broekaert *et al.* (2013) saw that cooking of the brown shrimp reduced the microbial load by 2 to 3 log depending on the growth medium used (Table 25.5); also *Pseudomonas* spp. and H₂S producers were strongly reduced, but LAB counts remained equal.

Peeling of the shrimp generally decreases the total count with about 1–2 log, indicating that the carapax of the shrimp is mainly the most contaminated area. However, cross-contamination between the carapax and the inner flesh of the shrimp during peeling seems unavoidable. In Broekaert *et al.* (2013), half of the cooked shrimp were peeled manually under sterile conditions (= optimal conditions) and half were not peeled, after arrival at the laboratory (T_0). In general, less microbiological counts (TVC, *Pseudomonas*, LAB, H₂S producers) were

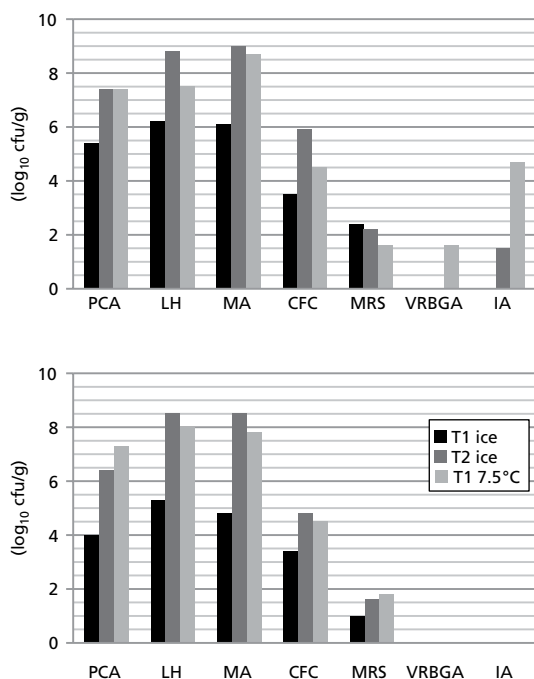


Figure 25.2 Microbiological counts on all growth media in $\log_{10}$ cfu/g of cooked unpeeled (top) and manually peeled (bottom) shrimp under the best possible conditions at T_1 (7 days of storage) and T_2 (13 days of storage) on ice and at 7.5°C . PCA = plate count agar, LH = modified Long and Hammer medium (Van Spreckens, 1974), MA = marine agar, CFC = *Pseudomonas* Cetrimide Fucidine Cephaloridine, MRS = Man Rogosa sharp medium, VRBGA = violet red bile glucose agar and IA = iron agar (Atlas, 2006). (After results from Broekaert *et al.*, 2013. Reproduced by permission.)

observed on peeled than on unpeeled shrimp during ice storage (Figure 25.2).

During storage on ice of unpeeled shrimp, the microbiota increased rapidly (Figure 25.2). After 7 days (T_1) a 2.5 (PCA) to 2.6 (LH) log increase was observed. After 13 days (T_2), an APC of 7.4 (PCA) or 8.8 log (LH) was reached. On CFC, the numbers of *Pseudomonas* spp. increased rather slowly during storage on ice; at T_1 the number increased by 0.8 log and at T_2 by 3.2 log. The number of LABs (MRS) was rather low and stayed equal during the whole study. Sulfide producers were absent during the

storage on ice until the end of storage (T_2). For the manually peeled shrimp, counts constantly remained lower than for unpeeled shrimp.

Today the use of machines for peeling shrimp is increasing. Machine peeled shrimp showed a less than 1 log lower count as compared with unpeeled shrimp. This lower difference compared to manually peeled shrimp might be due to environmental cross-contamination from the processing area (Gudbjornsdottir *et al.*, 2005). After 7 days of ice storage (T_1), the purchased shrimp were considered to be microbiologically spoiled with an APC of $8.3 \log_{10}$ cfu/g (unpeeled) and $7.6 \log_{10}$ cfu/g (peeled).

Chilled storage is very important for seafood conservation. For brown shrimp, results show that storage at 7.5°C revealed a much higher microbiological contamination on both the unpeeled (Figure 25.2 top) and the peeled (Figure 25.2 bottom) shrimp as compared to shrimp stored on ice. At T_1 , the shrimp stored at 7.5°C were considered spoiled due to a very high APC ($>7 \log_{10}$) and a strongly disagreeable odor.

Pseudoalteromonas and *Psychrobacter* dominate the microbiota of brown shrimp without preservatives regardless of differences in area and season of catch, early handling, processing procedures, and storage conditions (Table 25.6). Immediately after cooking (T_0) the microbiota consists of the genera *Psychrobacter*, *Pseudoalteromonas*, *Loktanella*, and *Planococcus* (all typical marine microorganisms). During air storage on ice, the microbiota on shrimp consists only of *Psychrobacter* and *Pseudoalteromonas* species. When stored at higher temperatures (7.5°C), a shift occurs towards *Psychrobacter* species and *Exiguobacterium* sp. Shrimps without preservatives purchased in the shop, both mechanically peeled and unpeeled, stored aerobically on ice, consisted of microorganisms from the genera *Psychrobacter* (40%) and *Pseudoalteromonas* (34%). Other genera present during storage were *Planococcus*, *Loktanella*, *Pseudomonas*, *Exiguobacterium*, and *Chryseobacterium*.

Table 25.6 Summarizing table after Broekaert *et al.* (2013) with all identified species or genera found on brown shrimp during storage, with indications of the sample from which they were found, the time points during storage, the processing procedures (optimal = best hygienic handlings applied in the laboratory), the storage temperature, detection/identification techniques, and growth medium for isolation/detection. T_0 = at day 1, T_1 = after 7 days storage, T_2 = after 13 days storage. Rep = rep-PCR fingerprinting; DGGE = denaturing gradient gel electrophoresis; 16S = 16S rDNA sequencing, S = microbiota recovered from plate swabs, D = microbiota recovered from direct DNA extraction from the shrimp matrix. LH = modified Long and Hammer medium, PCA = plate count agar, IA = Lyngby iron agar, MA = marine agar, CFC = *Pseudomonas* Cetrimide Fucidine Cephaloridine agar. (Reproduced by permission.)

Genus/species	Shrimp sample	Time point	Processing	Storage conditions	Detected/identified via	Growth media
<i>Bacillus</i> <i>Bacillus</i> sp.	Optimal processing	T_1	Both unpeeled and peeled	On ice and at 7.5 °C	DGGE (S) 16S-V3	LH
<i>Carnobacterium</i> <i>C. funditum</i> / <i>C. divergens</i>	Optimal processing	All timepoints	Both unpeeled and peeled	On ice and at 7.5 °C	DGGE (S, D) 16S-V3	PCA
<i>Chryseobacterium</i> <i>Chryseobacterium</i> sp.	Optimal processing	T_1	Peeled	At 7.5 °C	Rep and 16S	PCA
<i>Exiguobacterium</i> <i>Exiguobacterium</i> sp.	Optimal processing Optimal processing Purchased	T_1 All time points T_0	Unpeeled Both unpeeled and peeled Both unpeeled and peeled	At 7.5 °C On ice and at 7.5 °C On ice	Rep and 16S DGGE (D) 16S-V3 DGGE(D) 16S-V3	IA
<i>Loktanella</i> <i>Loktanella</i> sp.	Optimal processing	T_0	Unpeeled	On ice	Rep and 16S	MA
<i>Planococcus</i> <i>Planococcus</i> sp.	Optimal processing Optimal processing Optimal processing Purchased	T_0 T_1 All timepoints T_0 and T_1	Unpeeled Peeled Both unpeeled and peeled Both unpeeled and peeled	On ice At 7.5 °C On ice and at 7.5 °C On ice	Rep and 16S Rep and 16S DGGE (S,D) 16S-V3 DGGE (S,D) 16S-V3	MA MA PCA, LH LH

(Continued)

Table 25.6 (Continued)

Genus/species	Shrimp sample	Time point	Processing	Storage conditions	Detected/identified via	Growth media
<i>Pseudoalteromonas</i>						All media except PCA
<i>Psa. nigrifaciens</i>	Optimal processing	All timepoints	Both unpeeled and peeled	On ice and at 7.5 °C	rep and <i>gyrB</i>	
<i>Psa. elyakovii</i>	Optimal processing	T_1	Both unpeeled and peeled	On ice and at 7.5 °C	rep and <i>gyrB</i>	
<i>Psa. paragorgicola</i>	Optimal processing	T_1, T_2	Both unpeeled and peeled	On ice	rep and <i>gyrB</i>	
<i>Psa. translucida</i>	Optimal processing	T_0, T_1	Unpeeled	On ice	DGGE (S,D) 16S-V3	
	Purchased	T_1, T_2	Both unpeeled and peeled	On ice	DGGE (S) 16S-V3	
<i>Psa. arctica/Psa. fuliginea</i>	Optimal processing	T_1	Unpeeled	On ice	DGGE (S) 16S-V3	
	Purchased	T_1	Peeled	On ice	DGGE (S) 16S-V3	
<i>Pseudomonas</i>						
<i>Pseudomonas</i> sp.	Optimal processing	T_1	Peeled	On ice	Rep and 16S	CFC
<i>Psychrobacter</i>						
<i>Psychrobacter</i> sp.	Optimal processing	T_1, T_2	Both unpeeled and peeled	On ice	DGGE (S) 16S-V3	PCA, LH
	Optimal processing	T_0	Unpeeled	On ice	rep and <i>gyrB</i>	PCA, LH
<i>Psb. cibarius</i>	Optimal processing	All timepoints	Both unpeeled and peeled	On ice and at 7.5 °C	rep and <i>gyrB</i>	All media
<i>Psb. immobilis</i>	Optimal processing	All timepoints	Both unpeeled and peeled	On ice and at 7.5 °C	rep and <i>gyrB</i>	PCA
<i>Psb. okhotskensis</i>	Optimal processing	T_1	Peeled	On ice and at 7.5 °C	rep and <i>gyrB</i>	PCA
<i>Psb. marincola</i>	Optimal processing	T_0	Unpeeled	On ice	rep and <i>gyrB</i>	PCA
<i>Staphylococcus</i>						
<i>Staphylococcus</i> sp.	Purchased	T_0	Unpeeled	On ice	DGGE (D) 16S-V3	

References

- Akankwasa, A. 1998 The effect of delayed icing and gutting on the quality of freshwater arctic charr (*Salvelinus alpinus* L.). Report, Fisheries Training Programme, The United Nations University of Iceland, pp.1–17.
- Alfaro, B. and Hernandez, I. 2013. Evolution of the indigenous microbiota in modified atmosphere packaged Atlantic horse mackerel (*Trachurus trachurus*) identified by conventional and molecular methods. *International Journal of Food Microbiology*, **167**, 117–123.
- Alfaro, B., Hernandez, I., Le Marc, Y., and Pin, C. 2013. Modelling the effect of the temperature and carbon dioxide on the growth of spoilage bacteria in packed fish products. *Food Control*, **29**, 429–437.
- Arashisar, S., Hisar, O., Kaya, M., and Yanik, T. 2004. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets. *International Journal of Food Microbiology*, **97**, 209–214.
- Atlas, R.M. 2006. *Handbook of Microbiological Media for the Examination of Food*, 2th edn. CPC Press – Taylor and Francis Group, Boca Raton, FL, USA.
- Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogues, M.T., and Vidal-Carou, M.C. 2007. Effects of previous frozen storage on chemical, microbiological and sensory changes during chilled storage of Mediterranean hake (*Merluccius merluccius*) after thawing. *European Food Research and Technology*, **226**, 287–293.
- Beltran, A., Pelaez, C., and Moral, A. 1989. Keeping quality of vacuum-packed smoked sardine fillets: microbiological aspects. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, **188**, 232–236.
- Boknaes, N., Osterberg, C., Nielsen, J., and Dalgaard, P. 2000. Influence of freshness and frozen storage temperature on quality of thawed cod fillets stored in modified atmosphere packaging. *Lebensm.-Wiss.u.-Technol.*, **33**, 244–248.
- Bozariis, I.S., Stamatiou, A.P., and Nychas, G.J. 2013. Microbiological aspects and shelf life of processed seafood products. *Journal of Science, Food and Agriculture*, **93**, 1184–1190.
- Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., and Vlaemynck, G. 2011. Seafood quality analysis: molecular identification of dominant microbiota after ice storage on several general growth media. *Food Microbiology*, **28**, 1162–1169.
- Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., and Vlaemynck, G. 2013. Molecular identification of the microbiota of peeled and unpeeled brown shrimp (*Crangon crangon*) during storage on ice and at 7.5 degrees C. *Food Microbiology*, **36**, 123–134.
- Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J.L., and Leroi, F. 2004. Sensory characteristics of cold-smoked Atlantic salmon (*Salmo salar*) from European market and relationships with chemical, physical and microbiological measurements. *Food Research International*, **37**, 181–193.
- Carrascosa, C., Millan, R., Saavedra, P., Jaber, J.R., Montenegro, T., Raposo, A., Pérez, E., and Sanjuan, E. 2014. Predictive models for bacterial growth in sea bass (*Dicentrarchus labrax*) stored in ice. *International Journal of Food Science and Technology*, **49**, 354–363.
- Castell, C.H. and Mapplebeck, E.G. 1952. The importance of *Flavobacterium* in fish spoilage. *Journal of Fisheries Board Canada*, **9**, 148–156.
- Coton, M., Joffraud, J.J., Mekhtiche, L., Leroi, F., and Coton, E. 2013. Biodiversity and dynamics of the bacterial community of packaged king scallop (*Pecten maximus*) meat during cold storage. *Food Microbiology*, **35**, 99–107.
- Cruz-Romero, M., Kerry, J.P., and Kelly, A.L. 2008. Changes in the microbiological and physico-chemical quality of high-pressure-treated oysters (*Crassostrea gigas*) during chilled storage. *Food Control*, **19**, 1139–1147.
- Dalgaard, P. 1995. Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology*, **26**, 319–333.
- Dalgaard, P. and Jorgensen, L.V. 2000. Cooked and brined shrimps packed in a modified atmosphere have a shelf-life of > 7 months at 0°C, but spoil in

- 4–6 days at 25°C. *International Journal of Food Science and Technology*, **35**, 431–442.
- Dalgaard, P., Gram, L., and Huss, H.H. 1993. Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology*, **19**, 283–294.
- Dalgaard, P., Christiansen, T.J., and Huss, H.H. 1997. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. *Letters in Applied Microbiology*, **24**, 373–378.
- Dalgaard, P., Vancanneyt, M., Euras, V.N., Swings, J., Fruekilde, P., and Leisner, J.J. 2003. Identification of lactic acid bacteria from spoilage associations of cooked and brined shrimps stored under modified atmosphere between 0 degrees C and 25 degrees C. *Journal of Applied Microbiology*, **94**, 80–89.
- Debevere, J. and Boskou, G. 1996. Effect of modified atmosphere packaging on the TVB/TMA-producing microflora of cod fillets. *International Journal of Food Microbiology*, **31**, 221–229.
- Duun, A.S. and Rustad, T. 2008. Quality of super-chilled vacuum packed Atlantic salmon (*Salmo salar*) fillets stored at -1.4 and -3.6 degrees C. *Food Chemistry*, **106**, 122–131.
- Elotmani, F., Assobhei, O., Revol-Junelles, A.M., and Milliere, J.B. 2004. Microflora of fresh and ice-stored sardines (*Sardina pilchardus*) from the Moroccan Atlantic coast. *Ciencias Marinas*, **30**, 627–635.
- Emborg, J., Laursen, B.G., Rathjen, T., and Dalgaard, P. 2002. Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2 degrees C. *Journal of Applied Microbiology*, **92**, 790–799.
- Erkan, N. and Ozden, O. 2008. Quality assessment of whole and gutted sardines (*Sardina pilchardus*) stored in ice. *International Journal of Food Science and Technology*, **43**, 1549–1559.
- Fagan, J.D., Gormley, T.R., and Uimhuircheartaigh, M.M. 2004. Effect of modified atmosphere packaging with freeze-chilling on some quality parameters of raw whiting, mackerel and salmon portions. *Innovative Food Science and Emerging Technologies*, **5**, 214.
- Feldhusen, F. 2000. The role of seafood in bacterial foodborne diseases. *Microbes and Infection*, **2**, 1651–1660.
- Fennema, O.R., Powrie, W.D., and Marth, E.H. 1973. *Low-Temperature Preservation of Foods and Living Matter*. Dekker, New York, USA.
- Floros, J.D. and Matsos, K.I. 2005. Introduction to modified atmosphere packaging. In: *Innovations in Food Packaging*, pp. 159–172. Elsevier Ltd.
- Fuselli, S.R., Casales, M.R., Fritz, R., and Yeannes, M.D. 1998. Isolation and characterization of microorganisms associated with marinated anchovy (*Engraulis anchoita*). *Journal of Aquatic Food Product Technology*, **7**, 29–38.
- Gancel, F., Dzierszynski, F., and Tailliez, R. 2010. Identification and characterization of *Lactobacillus* species isolated from fillets of vacuum-packed smoked and salted herring (*Clupea harengus*). *Journal of Applied Microbiology*, **82**, 722–728.
- Gennari, M., Tomaselli, S., and Cotrona, V. 1999. The microflora of fresh and spoiled sardines (*Sardina pilchardus*) caught in Adriatic (Mediterranean) Sea and stored in ice. *Food Microbiology*, **16**, 15–28.
- Gonzalez-Rodriguez, M.N., Sanz, J.J., Santos, J.A., Otero, A., and Garcia-Lopez, M.L. 2002. Foodborne pathogenic bacteria in prepackaged fresh retail portions of farmed rainbow trout and salmon stored at 3 degrees C. *International Journal Food Microbiology*, **76**, 135–141.
- Gornik, S.G., Albalat, A., Theethakaew, C., and Neil, D.M. 2013. Shelf life extension of whole Norway lobster *Nephrops norvegicus* using modified atmosphere packaging. *International Journal of Food Microbiology*, **167**, 369–377.
- Gram, L. and Dalgaard, P. 2002. Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology*, **13**, 262–266.
- Gram, L. and Huss, H.H. 1996. Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology*, **33**, 121–137.
- Gram, L. and Huss, H. H. 2000. Fresh and processed fish and shellfish. In: *The Microbiological Safety and Quality of Foods* (eds Lund, B.M., Baird-Parker, A.C., and Gould, G.W.), pp. 472–506. Aspen Publishers, Inc., Gaithersburg, MD, USA.

- Gram, L., Trolle, G., and Huss, H.H. 1987. Detection of specific spoilage bacteria from fish stored at low (0-degrees-C) and high (20-degrees-C) temperatures. *International Journal of Food Microbiology*, **4**, 65–72.
- Gram, L., Wedellneergaard, C., and Huss, H.H. 1990. The bacteriology of fresh and spoiling Lake Victorian Nile perch (*Lates-Niloticus*). *International Journal of Food Microbiology*, **10**, 303–316.
- Grigorakis, K., Taylor, K.D.A., and Alexis, M.N. 2003. Seasonal patterns of spoilage of ice-stored cultured gilthead sea bream (*Sparus aurata*). *Food Chemistry*, **81**, 263–268.
- Gudbjornsdottir, B., Einarsson, H., and Thorkelsson, G. 2005. Microbial adhesion to processing lines for fish fillets and cooked shrimp: influence of stainless steel surface finish and presence of Gram-negative bacteria on the attachment of *Listeria monocytogenes*. *Food Technology and Biotechnology*, **43**, 55–61.
- Guldager, H.S., Boknaes, N., Osterberg, C., Nielsen, J., and Dalgaard, P. 1998. Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2 degrees C. *Journal of Food Protection*, **61**, 1129–1136.
- Hansen, A.A., Morkore, T., Rudi, K., Olsen, E., and Eie, T. 2007. Quality changes during refrigerated storage of MA-packaged pre-rigor fillets of farmed Atlantic cod (*Gadus morhua* L.) using traditional MAP, CO₂ emitter, and vacuum. *Journal of Food Science*, **72**, M423–M430.
- Hovda, M.B., Lunestad, B.T., Sivertsvik, M., and Rosnes, J.T. 2007a. Characterisation of the bacterial flora of modified atmosphere packaged farmed Atlantic cod (*Gadus morhua*) by PCR-DGGE of conserved 16S rRNA gene regions. *International Journal of Food Microbiology*, **117**, 68–75.
- Hovda, M.B., Sivertsvik, M., Lunestad, B.T., Lorentzen, G., and Rosnes, J.T. 2007b. Characterisation of the dominant bacterial population in modified atmosphere packaged farmed halibut (*Hippoglossus hippoglossus*) based on 16S rDNA-DGGE. *Food Microbiology*, **24**, 362–371.
- Hovda, M.B., Sivertsvik, M., Lunestad, B.T., and Rosnes, J.T. 2007c. Microflora assessments using PCR-denaturing gradient gel electrophoresis of ozone-treated and modified-atmosphere-packaged farmed cod fillets. *Journal of Food Protection*, **70**, 2460–2465.
- Hugenholtz, P., Goebel, B.M., and Pace, N.R. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology*, **180**, 4765–4774.
- Huss, H.H. 1995. Quality and changes in fresh fish. *Food and Agriculture Organization of the United Nations, FAO Fisheries technical paper* **348**, pp. 1–195.
- Huss, H.H., Dalgaard, P., and Gram, L. 1997. Microbiology of fish and fish products. *Development in Food Science*, **38**, 413–430.
- ISO 17410. 2001. Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of psychrotropic microorganisms.
- Jay, J.M. 1986. *Modern Food Microbiology*, 3th edn. Van Nostrand Reinhold Company, New York, USA.
- Jeppesen, V.F. and Huss, H.H. 1993. Characteristics and antagonistic activity of lactic acid bacteria isolated from chilled fish products. *International Journal of Food Microbiology*, **18**, 305–320.
- Joffraud, J.J. and Leroi, F. 2000. Spoilage and safety of cold-smoked fish. Final report of European Contract FAIR-PL-95-1207.
- Kahn, M.A., Parrish, C.C., and Shahidi, F. 2005. Enumeration of total heterotrophic and psychrotrophic bacteria using different types of agar to evaluate the microbial quality of blue mussels (*Mytilus edulis*) and sea scallops (*Placopecten magellanicus*). *Food Research International*, **38**, 751–758.
- Karim, N.U., Kennedy, T., Linton, M., Watson, S., Gault, N., and Patterson, M.F. 2011. Effect of high pressure processing on the quality of herring (*Clupea harengus*) and haddock (*Melanogrammus aeglefinus*) stored on ice. *Food Control*, **22**, 476–484.
- Karl, H. and Meyer, C. 2007. Effect of early gutting on shelf life of saithe (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*) and plaice (*Pleuronectes platessa*) stored in ice. *Journal für Verbraucherschutz und Lebensmittelsicherheit – Journal of Consumer Protection and Food Safety*, **2**, 130–137.

- Kaspar, C.W. and Tamplin, M.L. 1993. Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Applied Environmental Microbiology*, **59**, 2425–2429.
- Kilinc, B. and Cakli, S. 2005. Determination of the shelf life of sardine (*Sardina pilchardus*) marinades in tomato sauce stored at 4°C. *Food Control*, **16**, 639–644.
- Lalitha, K.V., Sonaji, E.R., Manju, S., Jose, L., Gopal, T.K.S., and Ravisankar, C.N. 2005. Microbiological and biochemical changes in pearl spot (*Etroplus suratensis* Bloch) stored under modified atmospheres. *Journal of Applied Microbiology*, **99**, 1222–1228.
- Lauzon, H.L., Magnusson, H., Sveinsdottir, K., Gudjonsdottir, M., and Martinsdottir, E. 2009. Effect of brining, modified atmosphere packaging, and superchilling on the shelf life of cod (*Gadus morhua*) loins. *Journal of Food Science*, **74**, M258–M267.
- Leroi, F. 2010. Occurrence and role of lactic acid bacteria in seafood products. *Food Microbiology*, **27**, 698–709.
- Leroi, F., Joffraud, J.J., Chevalier, F., and Cardinal, M. 1998. Study of the microbial ecology of cold-smoked salmon during storage at 8 degrees C. *International Journal of Food Microbiology*, **39**, 111–121.
- Leroi, F., Joffraud, J.J., and Chevalier, F. 2000. Effect of salt and smoke on the microbiological quality of cold-smoked salmon during storage at 5 degrees C as estimated by the factorial design method. *Journal of Food Protection*, **63**, 502–508.
- Leroi, F., Joffraud, J.J., Chevalier, F., and Cardinal, M. 2001. Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. *Journal of Applied Microbiology*, **90**, 578–587.
- Lindberg, A.M., Ljungh, A., Ahrne, S., Lofdahl, S., and Molin, G. 1998. *Enterobacteriaceae* found in high numbers in fish, minced meat and pasteurised milk or cream and the presence of toxin encoding genes. *International Journal of Food Microbiology*, **39**, 11–17.
- Linton, M., McClements, J.M.J., and Patterson, M.F. 2003. Changes in the microbiological quality of shellfish, brought about by treatment with high hydrostatic pressure. *International Journal of Food Science and Technology*, **38**, 713–727.
- Liston, J. 1980. Microbiology in fishery science. In: *Advances in Fish Science and Technology* (ed. Connel, J.J.), pp. 138–157. Fishing News Book Ltd, Farnham, Surrey, UK.
- Lyhs, U., Bjorkroth, J., and Korkeala, H. 1999. Characterization of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping. *International Journal of Food Microbiology*, **52**, 77–84.
- Lyhs, U., Lahtinen, J., and Schelvis-Smit, R. 2007. Microbiological quality of maatjes herring stored in air and under modified atmosphere at 4 and 10 degrees C. *Food Microbiology*, **24**, 508–516.
- Macé, S., Cornet, J., Chevalier, F., Cardinal, M., Pilet, M.F., Dousset, X., and Joffraud, J.J. 2012. Characterisation of the spoilage microbiota in raw salmon (*Salmo salar*) steaks stored under vacuum or modified atmosphere packaging combining conventional methods and PCR-TTGE. *Food Microbiology*, **30**, 164–172.
- Mai, N.T., Gudjonsdottir, M., Lauzon, H.L., Sveinsdottir, K., et al. 2011. Continuous quality and shelf life monitoring of retail-packed fresh cod loins in comparison with conventional methods. *Food Control*, **22**, 1000–1007.
- Makarios-Laham, I.K. and Lee, T.C. 1993. Protein hydrolysis and quality deterioration of refrigerated and frozen seafood due to obligately psychophilic bacteria. *Journal of Food Science*, **158**, 310–313.
- Mejlholm, O., Boknaes, N., and Dalgaard, P. 2005. Shelf life and safety aspects of chilled cooked and peeled shrimps (*Pandalus borealis*) in modified atmosphere packaging. *Journal of Applied Microbiology*, **99**, 66–76.
- Molin, G. and Stenström, I.-M. 1984. Effect of temperature on the microbial flora of herring fillets stored in air or carbon dioxide. *Journal of Applied Bacteriology*, **56**, 275–282.
- Moore, E.R.B., Tindal, B.J., Dos Santos, V.A.P.M., Pieper, D.H., Ramos, J.-L., and Palleroni,

- N.J. 2006. Nonmedical: *Pseudomonas*. In: *The Prokaryotes – A Handbook on the Biology of Bacteria*, 3rd edn, Vol. 6 (eds Dworkin, M., et al.), pp. 646–703. Springer, New York, USA.
- Múgica, B., Barros-Velázquez, J., Miranda, J.M., and Aubourg, S.P. 2007. Evaluation of a slurry ice system for the commercialization of ray (*Raja clavata*): effects on spoilage mechanisms directly affecting quality loss and shelf-life. *LWT – Food Science and Technology*, **41**, 974–981.
- Münckner, W. and Meyer, C. 1994. Untersuchungen zum Einsatz von Flüssigrauch bei der Herstellung geräucherter Fischerzeugnisse. *Fleischwirtschaft*, **74**, 547–553.
- NMKL 184. 2006. Aerobic count and specific spoilage organisms in fish and fish products.
- Olafsdottir, G., Lauzon, H.L., Martinsdottir, E., and Kristbergsson, K. 2006a. Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. *International Journal of Food Microbiology*, **111**, 112–125.
- Olafsdottir, G., Lauzon, H.L., Martinsdottir, E., Oehlenschläger, J., and Kristbergsson, K. 2006b. Evaluation of shelf life of superchilled cod (*Gadus morhua*) fillets and the influence of temperature fluctuations during storage on microbial and chemical quality indicators. *Journal of Food Science*, **71**, S97–S109.
- Ozogul, F., Polat, A., and Ozogul, Y. 2004. The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). *Food Chemistry*, **85**, 49–57.
- Paarup, T., Sanchez, J.A., Moral, A., Christensen, H., Bisgaard, M., and Gram, L. 2002. Sensory, chemical and bacteriological changes during storage of iced squid (*Todaropsis eblanae*). *Journal of Applied Microbiology*, **92**, 941–950.
- Paludan-Müller, C., Dalgaard, P., Huss, H.H., and Gram, L. 1998. Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5 degrees C. *International Journal of Food Microbiology*, **39**, 155–166.
- Parlapani, F.F., Meziti, A., Kormas, K.Ar., and Bozaris, I.S. 2013. Indigenous and spoilage microbiota of farmed sea bream stored in ice identified by phenotypic and 16S rRNA gene analysis. *Food Microbiology*, **33**, 85–89.
- Patterson, M.F. 2005. Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, **98**, 1400–1409.
- Poli, B.M., Messini, A., Parisi, G., Scappini, F., Vigiani, V., Giorgi, G., and Vincenzini, M. 2006. Sensory, physical, chemical and microbiological changes in European sea bass (*Dicentrarchus labrax*) fillets packed under modified atmosphere/air or prepared from whole fish stored in ice. *International Journal of Food Science and Technology*, **41**, 444–454.
- Reynisson, E., Lauzon, H.L., Magnusson, H., Jonsdottir, R., Olafsdottir, G., Marteinsson, V., and Hreggviðsson, G.O. 2009. Bacterial composition and succession during storage of North-Atlantic cod (*Gadus morhua*) at superchilled temperatures. *BMC Microbiology*, **9**, 250.
- Rudi, K., Maugesten, T., Hannevik, S.E., and Nissen, H. 2004. Explorative multivariate analyses of 16S rRNA gene data from microbial communities in modified-atmosphere-packed salmon and coalfish. *Applied and Environmental Microbiology*, **70**, 5010–5018.
- Shewan, J.M. 1962. The bacteriology of fresh and spoiling fish and some related chemical changes. In: *Recent Advances in Food Science*, J. (eds Hawthorn, J. and Muil Leitch, J.), pp. 167–193. Butterworth, Washington, DC, USA.
- Shewan, J.M. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: *Proceedings of the Conference of Handling, Processing and Marketing of Tropical Fish*. pp. 51–66. Tropical Products Institute, London, UK.
- Shewan, J.M. and Georgala, D.L. 1957. The effect of spoilage and handling on the bacterial flora of fish. *Proceedings of the Nutrition Society*, **16**, 161–163.
- Sikorski, Z.E. and Kalodziejska, I. 2002. Microbial risks in mild hot smoking of fish. *Critical Reviews in Food Science and Nutrition*, **42**, 35–51.

- Sivertsvik, M., Jeksrud, W.K., and Rosnes, J.T. 2002. A review of modified atmosphere packaging of fish and fishery products – significance of microbial growth, activities and safety. *International Journal of Food Science and Technology*, **37**, 107–127.
- Teixeira, B., Marques, A., Mendes, R., Goncalves, A., Fidalgo, L., Oliveira, M., Savaira, J.A., and Nunes, M.L. 2014. Effect of high-pressure processing on the quality of sea bass (*Dicentrarchus labrax*) fillets during refrigerated storage. *Food and Bioprocess Technology*, **7**, 1333–1343.
- Torrieri, E., Cavella, S., Villani, F., and Masi, P. 2006. Influence of modified atmosphere packaging on the chilled shelf life of gutted farm bass (*Dicentrarchus labrax*). *Journal of Food Engineering*, **77**, 1078–1086.
- Truelstrup-Hansen, L. 1995. *The Quality of Cold Smoked Salmon*. Danish Institute for Fisheries Research and The Royal Veterinary and Agricultural University of Copenhagen, Denmark.
- Truelstrup-Hansen, L. and Huss, H.H. 1998. Comparison of the microflora isolated from spoiled cold-smoked salmon from three smokehouses. *Food Research International*, **31**, 703–711.
- Tryfinopoulou, P., Tsakalidou, E., and Nychas, G.J. 2002. Characterization of *Pseudomonas* spp. associated with spoilage of gilt-head sea bream stored under various conditions. *Applied and Environmental Microbiology*, **68**, 65–72.
- Tsironi, T., Dermesonlouoglou, E., Giannakourou, M., and Taoukis, P. 2009. Shelf life modelling of frozen shrimp at variable temperature conditions. *LWT – Food Science and Technology*, **42**, 664–671.
- Tzikas, Z., Amvrosiadis, I., Soutos, N., and Georgakis, Sp. 2007. Seasonal variation in the chemical composition and microbiological condition of Mediterranean horse mackerel (*Trachurus mediterraneus*) muscle from the North Aegean Sea (Greece). *Food Control*, **18**, 251–257.
- Vallé, M., Eb, P., Tailliez, R., and Malle, P. 1998. Optimization of the enumeration of total aerobic bacterial flora in the flesh of seafish. *Journal of Rapid Methods and Automation in Microbiology*, **6**, 29–42.
- Van Spreekens, K.J.A. 1974. The suitability of a Long and Hammer's medium for the enumeration of more fastidious bacteria from fresh fishery products. *Archiv für Lebensmittelhygiene*, **25**, 213–219.
- Van Spreekens, K.J.A. and De Man, T.A. 1970. Enkele microbiologische aspecten van gekookte garnalen. *Voedingsmiddelentechnologie*, **1**, 290–292.
- Vogel, B.F., Venkateswaran, K., Satomi, M., and Gram, L. 2005. Identification of *Shewanella baltica* as the most important H₂S-producing species during iced storage of Danish marine fish. *Applied and Environmental Microbiology*, **71**, 6689–6697.
- Wang, T., Sveinsdottir, K., Magnusson, H., and Martinsdottir, E. 2008. Combined application of modified atmosphere packaging and super-chilled storage to extend the shelf life of fresh cod (*Gadus morhua*) loins. *Journal of Food Science*, **73**, S11–S19.
- Zaballos, M., Lopez-Lopez, A., Ovreas, L., Bartual, S.G., et al. 2006. Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea. *FEMS Microbiology Ecology*, **56**, 389–405.
- Zambuchini, B., Fiorini, D., Verdenelli, M.C., Orpianesi, C., and Ballini, R. 2008. Inhibition of microbiological activity during sole (*Solea solea* L.) chilled storage by applying ellagic and ascorbic acids. *LWT – Food Science and Technology*, **41**, 1733–1738.

CHAPTER 26

Microbial ecology of mayonnaise, margarine, and sauces

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26.1 Introduction

Fats/oils constitute a main proportion of many food materials, representing a high energy intake for their consumers. Mayonnaise, margarine, salad dressings, and sauces are composed of mainly fat/oil as raw material. Deterioration of this type of food is generally attributed to chemical processes such as hydrolysis and oxidation since these changes have been known to be responsible for the formation of new compounds (Alford *et al.*, 1971). On the other hand, these chemical changes are sometimes desirable for certain foods. In addition to chemical deteriorations, fats/oils can be attacked by various lipolytic microorganisms under favorable growth conditions. The ability to produce fat-splitting enzymes, lipases, is a property common to most bacteria (Lawrence, 1967). Some strains of yeasts and molds are also able to produce lipid hydrolyzing enzymes.

Temperature, moisture, and availability of easily digestible nutrients are critical parameters for microbial ecology of fat/oil rich foods. In water-in-oil systems including margarine, water is dispersed as droplets throughout the fat phase. This prevents the movement of microorganisms between the droplets, playing as a limiting factor for microbial growth (Foods ICMS, 2006) as well as lowering availability of water. The presence of antimicrobial agents such as salt, acids, and spices in product formulation

may also exhibit a microbiostatic or microbicidal effect. Correspondingly, there is no conclusive evidence for industrially produced fat- or oil-based foods if they had been associated with outbreaks of illnesses caused by food-borne microorganisms (Delamarre and Batt, 1999). However, some cases have been encountered that resulted from spoilage microorganisms. Therefore, good manufacture practices including hygiene procedures should be strictly performed by the manufacturers in order to prevent economic losses. This chapter summarizes microbial ecology and pathogenic microorganisms associated with major fat/oil-based foods, i.e., mayonnaise, margarine, and sauces.

26.2 Mayonnaise

Mayonnaise, which is one of the most widely consumed condiments in the world, can be used as a raw material in production of salads, sauces, and other delicacies. Mayonnaise is produced by emulsifying the aqueous phase and vegetable oil consisting of acidifying agents such as vinegar and lemon juice, and egg yolk emulsifier. In addition to vinegar and water, the aqueous phase of the mayonnaise is composed of salts, sugar, polysaccharide, additive, and various types of condiments and spices (Fialová *et al.*, 2008). A wide range of vegetable oils can be used in mayonnaise production. The soybean,

rapeseed, and sunflower oil are generally used in mayonnaise production while cotton seed and olive oil are rarely used in the formulation. Minimal fat and egg yolk content of the mayonnaise is regulated to be 70 and 5%, respectively, according to the standard *The Comité des Industries des Mayonnaises et Sauces Condimentaires de la Communauté Économique Européenne's* (CIMSCEE) (Foods ICMS, 2006). However, fat content and egg yolk should not be less than 78 and 6%, respectively. Different acidifying agents such as acetic, citric, lactic, and malic acid are used in mayonnaise formulations. However, vinegar is generally preferred to produce mayonnaise production as acidifying agents. Mayonnaise must contain at least 65% vegetable oil and the pH value must varied from about 3.6 to 4.0 with acetic acid as the predominant acid showing 0.29–0.5% of the total product according to the American standard (USUS-DHEW, 1975). The pH value of the mayonnaise generally varies from 3.0 to 4.5 (Foods ICMS, 2006).

26.2.1 Microbial ecology of mayonnaise

Mayonnaise and related products can be considered as microbiologically stable products due to their high oil content (70–80%) and presence of acidifying agents. Acidifying agents such as vinegar or lemon juice decrease the pH value of the final product as well as contributing the desired aroma (Fialová *et al.*, 2008; Depree and Savage, 2001). Smittle (2000) reported that pathogenic microorganisms such as *Salmonella*, *Escherichia coli* O157:H7, *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Yersinia enterocolitica* cannot survive when they are inoculated in mayonnaise and dressings. In addition to low water activity and pH level, good hygienic practice (GHP) can minimize spoilage of the commercially produced mayonnaise. In most cases, microbial spoilage of the mayonnaise results from a small group of acid-tolerant yeasts, especially *Zygosaccharomyces bailii* and lactic acid bacteria (LAB). However, spoilage of these groups of microorganism is not a

threatening factor but may decrease quality of products and cause economic losses (Smittle and Flowers, 1982).

26.2.1.1 *Salmonella* serotypes

Most of the outbreaks of *Salmonella* spp. have been reported from homemade mayonnaise (Zhu *et al.*, 2012; Perales and Garcia, 1990). Unpasteurized egg or egg yolk, and insufficient acidifying agents concentration may increase the possibility of the growing of *Salmonella* spp. Zhu *et al.* (2012) reported that types of acidifying agents such as citric or acetic acid and the presence or absence of preservative with acidifying agents affected the survival of *Salmonella* spp. It was observed in their study that the numbers of the *Salmonella* spp. in acetic acid or citric acid solution with the preservatives were significantly lower than those in vinegar, lemon juice, and the solution without the preservatives. Heo *et al.* (2010) conducted a study to observe the survival of *Salmonella enterica* subsp. *enterica* serovar Typhimurium in commercial mayonnaise at different temperature and pH values. They observed that inactivation rates were faster at the low pH and higher temperature values. Influence of the temperature and pH values on the survival of *Salmonella enterica* subsp. *enterica* serovar Enteritidis was studied by Perales and Garcia (1990). They reported that inactivation of *S. Enteritidis* was more rapid in mayonnaise made with vinegar (acetic acid) than lemon juice (citric acid) and at higher temperature, regardless of the acidulant type.

Lock and Board (1994) studied 24 varieties of commercial mayonnaise with different pH values (2.6–4.8) and types of acids. They observed rapid inactivation of *S. Enteritidis* PT 4 in all cases. *Salmonella* spp. could not be recovered after 48 h from products stored at 20°C in eight samples. However, they found that *Salmonella* spp. were undetectable after 20 min in a fat-free mayonnaise with pH 2.6 at 4°C, with *Salmonella* spp. generally surviving longer.

Radford *et al.* (1991) studied the influence of various types of oils on the survival of

S. Enteritidis in homemade mayonnaise. They observed that the death rate of *S. Enteritidis* was faster in mayonnaise made with extra virgin olive oils than in mayonnaise made with sunflower oil or a blend of olive oils.

Leuschner and Boughtflower (2001) developed a standardized laboratory-scale procedure in order to prepare mayonnaise. The objective of this work was to investigate the effects of formulation, processing, and storage conditions on the survival and growth of *S. Enteritidis* in artificially contaminated mayonnaise. They reported that cells of *S. Enteritidis* increased their stability in the mayonnaise when they were subjected to low pH in two stages, first to pH 5.8 and then to pH 4.5 before addition to the mayonnaise. The pH of the mayonnaise remained between 4.2 and 4.0 over the storage period. Low-level *S. Enteritidis* was stable in artificially contaminated mayonnaise for 4 weeks at 4 °C.

26.2.1.2 *Escherichia coli* O157:H7

Mayonnaise is generally considered as a microbiologically safe product in the case of the use of pasteurized eggs and acetic acid level in the aqueous phase of $\geq 1.4\%$. However, an outbreak of *Escherichia coli* O157:H7 was reported associated with mayonnaise and dressing (Weagant *et al.*, 1994). It was studied the survival of three strains of *E. coli* O157:H7 obtained from the mayonnaise implicated in the Oregon outbreak, using an inoculum size of 10^8 cfu/g in a mayonnaise product with pH of 3.65. It was observed that the pathogens died off quite rapidly at 25 °C to an undetectable level within 3 days. The pathogen was detectable for up to 35 days when inoculated mayonnaise was held at 7 °C.

Hathcox *et al.* (1995) investigated the influence of temperature on the rate of death of *E. coli* O157:H7 in commercially manufactured full-fat (real) and reduced-calorie mayonnaise dressings. The initial bacterial population was 2.23 log cfu/g while no viable cells was detected after 4 days at 30 °C, 7 days at 20 °C, and 58 days at 20 °C. The authors suggested that the survival of the pathogen in commercial mayonnaise and low-fat mayonnaise could be eliminated by good

manufacturing practice and appropriate storage conditions. Reduction of *E. coli* O157:H7 is most rapid at temperatures where mayonnaise is stored, distributed, and offered for sale at retail. Leuschner and Zamparini (2002) investigated the effects of spices on growth and survival of *E. coli* O157:H7 and *S. Enteritidis* in broth model systems and mayonnaise. They concluded that clove was the most effective antimicrobial followed by garlic, while mustard and ginger had only little bacteriostatic activity. They also reported that *E. coli* O157:H7 was more sensitive than *S. Enteritidis* against all spices studied except mustard.

26.2.1.3 *Listeria monocytogenes*

Acetic acid used in commercially produced mayonnaise reduces pH level and rapidly inactivate *Salmonella* spp. and *L. monocytogenes*. Therefore, the risk of any low-level pathogen (re)contamination is reduced in the case of sufficient acetic acid concentration (Foods ICMS, 2006). Erickson and Jenkins (1991) investigated *Salmonella* spp. and *L. monocytogenes* inactivation in commercial mayonnaise products. They observed that *L. monocytogenes* death rates were directly affected by aqueous phase acetic acid concentration. Another important result of this study was that the *L. monocytogenes* load decreased by 3 and 5 log cfu/g in 72 h in reduced calorie and cholesterol-free reduced calorie mayonnaise dressing, respectively.

Acetic acid concentration on the survival characteristic of *Salmonella* spp. and *L. monocytogenes* in cholesterol-free (CFM) and fat-reduced mayonnaise (RCM) was studied by Glass and Doyle (1991). Two formulations of CFM, prepared with 0.3 or 0.7% acetic acid in the aqueous phase, and four formulations of RCM, prepared with 0.1, 0.3, 0.5, or 0.7% acetic acid in the aqueous phase, were used. Products were inoculated with 10^6 cfu/g *L. monocytogenes* and *Salmonella* spp. and held at 23.9 °C for up to 2 weeks. The results of the study showed that *L. monocytogenes* did not grow at 23.9 °C and was inactivated by a factor of 10^4 within the 72 h

storage time in mayonnaise formulated with 0.7% acetic acid in the aqueous phase. They summarized that the reduced calorie mayonnaise products with 0.7% acetic acid concentration could be considered as microbiologically safe products.

26.2.1.4 Other pathogenic bacteria

A low pH level (≤ 4.5) of mayonnaise prevents the growth of some food-borne pathogens like *Clostridium botulinum*, *C. perfringens*, *S. aureus*, and *Bacillus cereus* (Foods ICMS, 2006; Smittle, 1977). Resistance of *S. aureus* to acid pH for different commercial products including mayonnaise was studied by Erdogrul *et al.* (2006). They reported that the *S. aureus* population in mayonnaise declined rapidly when held at 4 or 25°C and no viable cells of acid adapted and non-adapted *S. aureus* ATCC 6538 were recovered from mayonnaise after 48 h of storage. The effect of temperature and pH value to the possible growth of *S. aureus* and enterotoxin production in homemade mayonnaise was studied (Gomezlucia *et al.*, 1987,1990). Toxin was detected only in mayonnaise acidified with vinegar at a pH ≥ 5.0 and no toxin was found at pH 4.5. They also observed that the *S. aureus* population decreased by approximately 2 log cfu/g at pH ≤ 4.9 , reached about 5 log cfu/g at pH 5 and increased to 7 log cfu/g at pH ≥ 5.15 . *Campylobacter jejuni* was observed in egg yolk but the organism is unable to grow below 30°C and can be inactivated easily in the presence of acetic acid (Foods ICMS, 2006).

26.2.1.5 Lactic acid bacteria

An acid-tolerant microorganisms like lactobacilli can survive and may lead to undesirable changes, which negatively affect sensorial properties, reduce shelf life, and cause economical losses (Fialová *et al.*, 2008). *Lb. fructivorans* was reported as predominantly the *Lactobacillus* species causing spoilage of mayonnaise and related products, by Smittle and Flowers (1982). *Lb. brevis*, *Lb. casei*, *Lb. plantarum*, and *Lb. buchneri* were other lactobacillus species isolated from mayonnaise and related species

(Foods ICMS, 2006; Fialová *et al.*, 2008; Manios *et al.*, 2014; Kurtzman and Rogers, 1971).

The effect of food preservatives on the growth of spoilage lactobacilli isolated from mayonnaise-based sauces was studied by Fialova *et al.* (2008). In that study, non-contaminated and contaminated mayonnaise were tested with 2.7 and 1.5 log cfu/g initial lactobacilli loads. They reported that the maximum number of the lactobacilli increased to 5.4 log cfu/g for non-contaminated mayonnaise while it reached to 7.5 log cfu/g and then decreased to 5.4 log cfu/g for contaminated mayonnaise. They also observed that pH of the mayonnaise decreased during the first 44 days of storage.

26.2.1.6 Yeasts

Acetic acid-resistant yeasts such as *Z. bailii* and *Pichia membranaefaciens* are generally responsible for spoilage of mayonnaise and dressings. These microorganisms are resistant to weak acids such as acetic acid and can grow in the presence of 3% acetic acid medium. *Z. bailii* can also show resistance to a broad range of preservatives such as sorbic and benzoic acids (Steels *et al.*, 2000). *Z. rouxii*, *Saccharomyces cerevisiae*, and *Candida magnolia* are potential spoilage yeast species for mayonnaise. Most spoilage yeasts cause gas formation and brownish colonies (Foods ICMS, 2006). *Issatchenkia orientalis*, *C. inconspicua*, *C. parapsilosis*, *S. exiguous*, *S. dairensis*, *Debaryomyces hansenii*, *Rhodotorula mucilaginosa*, and *Torulopsis cutaneum* have been reported as other yeasts related to spoilage of mayonnaise (Smittle, 1977; Tornai-Lehoczi *et al.*, 2003).

26.2.1.7 Molds

Mayonnaise formulated with acetic acid is rarely spoiled by molds (Hocking, 1994). Most molds cannot grow in the presence of $\geq 0.5\%$ acetic acid. Growth of the molds is limited because of limited oxygen in the packaging material, which is consumed because of oil oxidations (Foods ICMS, 2006). Some mold species, such as *Geotrichum* spp, *Monascus*

ruber, *M. acetoabutans*, and *Penicillium glaucum*, have been reported to be associated with spoilage of the mayonnaise and related products in spite of their unfavorable environment to mold growth (Foods ICMS, 2006; Muys, 1971).

26.3 Margarine

Margarine was firstly produced by Hippolyte Mege Mouries, a French chemist, in 1896 (Zaeromali *et al.*, 2013). Margarine is considered as a cheap substitute for butter and can be used in a number of different forms such as cooking oil, dough shortening, and a spread on breads. Margarine can be defined as a food product in a water-in-oil emulsion or plastic form that includes any plastic fat composition emulsified with at least 80% fat and contains vitamin A not less than 15000 international units per pound (US-FDA, 2013).

Fat/oil is the main constituent of margarine. Other ingredients such as milks, preservatives, emulsifiers, acidulants, and salts have also been identified in margarine formulation (Delamarre and Batt, 1999). Vegetable oils such as soya oil, palm oil, and sunflower oil are widely used in margarine production after chemical modification by partial hydrogenation and interesterification (Petrauskaite *et al.*, 1998). Preservatives such as benzoate, sorbate, citric acid, and lactic acid are incorporated in margarine for an extended shelf life. However, addition of salt is not permitted because of health-related concerns in some countries (Swanson, 2005).

26.3.1 Microbiological properties of margarine

Microbiological properties of margarine are influenced by the ingredients, physicochemical properties, processing conditions, and packaging materials. In margarine production, initial ingredients are monitored for their microbiological safety specifications, which should be mainly by the suppliers:

- Water, one of the main ingredients of margarine, should always be in potable conditions.
- If oils are used, they are generally considered as microbiologically safe since the refining process inhibits their native microbiota.
- Milk and/or milk ingredients should be pasteurized before use to eliminate microbial risks (Zijl and Klapwijk, 2000).
- Some herbs and spices, which may be used as flavoring agents in some countries, may be a risk of contamination of pathogenic bacteria such as *B. cereus*, *E. coli*, and *S. aureus*, and mycotoxin producing fungi like *Aspergillus* and *Penicillium* (Hashem and Alamri, 2010; McKee, 1995). Therefore, they should be decontaminated before use.
- Other potential ingredients such as emulsifiers, stabilizers, and antioxidants may also be sources of microbial contamination.

Spoilage of margarines mainly originate from chemical events and margarine is generally considered as an inert product microbiologically. Considering the effect of the overall composition of margarine, growth of most microorganisms is prevented since the presence of micron-sized oil droplets in the emulsion structure is an effective factor that limits the microbial growth. Smaller droplets mean a smaller interior area and a limited amount of nutrient molecules, thereby lengthening the microbial replication time (Zijl and Klapwijk, 2000). In addition, water-in-oil emulsions such as margarines are more resistant to microbiological growth than oil-in-water emulsions like mayonnaise (Lück, 1990). Use of antimicrobial preservatives such as lactic acid, benzoic acid, and sorbic acid or their salts/combinations, which are legally permitted in the European Union (EC Council Regulation 2991/94), may limit microbial growth. Salt can be incorporated into margarine with an amount of 0–2% and also plays a role as a growth limiting factor.

The margarine production process is mainly composed of incorporation and emulsification of water (brine, preservatives, organic acids, etc.) and oil-soluble ingredients (flavors, emulsifiers,

colors, vitamins, etc.). As the aqueous phase is well dispersed in the oil phase, a stable fat-continuous mixture, namely pre-emulsion, is formed. The pre-emulsion is fed to a closed system by a high-pressure pump and the resulting structure is a fine emulsion. Following heat treatment and mixing processes, the product is packed in different ways in conformity with its consumption style (Swanson, 2005).

By considering the effect of production steps of margarine, it can be clearly seen that pathogenic microorganisms are unable to multiply during the processing. Even though initial ingredients have desirable microbiological properties, spoilage microorganisms also stay at negligible levels. Heat treatment (pasteurization) is optional in margarine processing and depends on the types of ingredients. It is especially performed for the aqueous phase. If materials such as dairy products and egg yolk are used as ingredients, pasteurization is required since they are susceptible to microbial spoilage. It is known that high levels of fats/oils present in foods show a protective effect on target microorganisms against heat treatments, especially at lower temperatures (Ma *et al.*, 2007). However, this is not applicable for foods with a high water content including margarine, the water amount of which is ~15–20%, resulting in little protective effect. An appropriate pasteurization process inactivates most pathogenic (i.e., *S. aureus*, *E. coli* O157:H7, and *L. monocytogenes*) and spoilage microorganisms (yeasts, molds, and vegetative bacteria); however, bacterial spores are not affected.

Packaging is the final processing step of margarine production. Margarines are put into tubs or wrappers depending of their usage (as table margarine and cooking or baking purposes) or stiffness (softness or hardness). Margarine wrappers are generally manufactured under hygienic conditions. However, molds are the most probable microorganisms to contaminate and they may find an opportunity to grow on the surface if favorable nutritive and moisture conditions are present.

26.3.2 Microbial spoilage and pathogenic microorganisms

Chemical stability of margarine depends on the level of hydrogenation and oil used as margarine spoilage is largely formed by chemical events. Molds are more frequent causes of margarine spoilage rather than bacteria. Mold growth is characterized by formation of mycelia apparently on the surface of the margarine. However, sometimes mold growth may be invisible, causing off-flavor with small water droplets. Salt is an important limiting factor for fungal growth. Regular salt margarines containing 2% salt are less susceptible to fungal spoilage since their water activity level is approximately 0.865–0.890, which is lower than that of the low-salt margarines containing approximately 1% salt (Richardson and George, 1998). *Cladosporium*, *Aspergillus*, *Alternaria*, *Paecilomyces*, *Trichoderma harzianum*, *T. viride*, *Penicillium expansum*, *P. roqueforti*, and *Geotrichum candidum* are among the mold species that have been associated with the spoilage of margarines (Beerens, 1980; Hocking, 1994). Two heat-resistant molds, *Aspergillus fumigatus* and *Paecilomyces variotii*, were also isolated from margarines produced in a company in Turkey and were held responsible for margarine spoilage, while some decontamination methods were also tested in the studies (Gumus *et al.*, 2008, 2010). Occasionally, lipolytic yeasts such as *Candida lipolytica* may cause problems in margarines.

In spite of fewer reports related to spoilage of margarines by bacteria, lipolytic bacteria constitute the predominant microbiota of bacterial spoiled margarines. Lipolytic spoilage arises by breakdown of the emulsion structure of margarines caused by extracellular enzymes including lipases. This type of spoilage generally progresses in aerobic conditions while an anaerobic environment retards the growth of most spoilage bacteria. Species belonging to genera *Micrococcus*, *Flavobacterium*, *Bacillus*, *Pseudomonas*, and *Zymomonas* have been found to be responsible for lipolytic spoilage of margarines (Beerens, 1980).

Considering pathogenic microorganisms, in spite of intrinsic conditions such as a pH value above 4.6 and water activity greater than 0.85 in margarine, which may be favorable for survival of pathogenic microorganisms, their growth in margarines is not likely. Guentert and Linton (2003) tested survival/growth of selected food-borne pathogens (*S. aureus*, *L. monocytogenes*, *S. Typhi*, and *E. coli* O157:H7) in margarine-style table spreads stored at different temperatures (5, 7, and 21 °C). Growth was observed only for *S. Typhi* in table spreads stored at 21 °C with a slow growth rate while *S. aureus*, *L. monocytogenes*, and *E. coli* O157:H7 were inactivated over time. Correspondingly, outbreaks of food-borne illnesses caused specifically by margarine consumption have not been reported. However, there have been some reports for similar products. For example, an outbreak of food intoxication involving over 265 cases was reported in the US, caused by consumption of a vegetable margarine and a margarine–butter blend that were contaminated by an enterotoxin-A producing strain of *Staphylococcus intermedius* (Khambaty *et al.*, 1994). In 2004, 450 cases of margarine blend products sold in 13 states of the US were recalled by the food distribution company because of possible *L. monocytogenes* contamination (Anonymous, 2004). In spite of no reports of outbreaks, general hygienic rules should be followed and sufficient control measures need to be performed for pathogens such as *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes*.

26.4 Sauces and salad dressings

Sauces and salad dressings are commonly used materials in daily life all over the world to add flavor to foods. The term “sauce” comes from French; however, it originates from the Latin “salsa”, which means salty. Sauces are semi-liquid foods that are used in food preparation and are not consumed as food materials themselves.

A wide variety of materials are used in sauce preparation depending on its intended purpose, palatal taste, and cultural attributes, while use of thickeners, flavorings, and emulsifiers is common. Due to variability of formulations, raw materials, and preparation processes, different kinds of sauces such as fish sauce, soy sauce, salad dressings, and other kinds of sauces have different physicochemical, sensorial, and textural properties in the world.

Soy sauce is a Japanese origin oriental traditional seasoning that is common in many countries worldwide. It has a sharp flavor and sharp taste, making it more popular among the sauces. The soy sauce production process is mainly composed of the following steps: incorporation of soybeans and wheat (1:1), fermentation, addition of saline water, brine fermentation, filtration, pasteurization, and sedimentation (van der Sluis *et al.*, 2001). Firstly, the fermentation process is carried out by *Aspergillus* spp. including *A. oryzae* and *A. sojae* at 30 °C for 2 days, when the molds produce extracellular hydrolysis enzymes that break down proteins and polysaccharides (van der Sluis *et al.*, 2001; Tanaka *et al.*, 2012). Following the first fermentation, the fermented batch is incorporated with brine (2–22% salt), resulting in a salt concentration of ~17% in the mixture. During the brine fermentation, hydrolyzed sugars and amino acids are used by halotolerant LAB and yeasts. After the fermentation, soy sauce is obtained following sequential filtration, pasteurization, and clarification processes (van der Sluis *et al.*, 2001).

Fish sauce is another common sauce that is especially well known in Southern Asian countries, having different names such as *nouc-mam* in Vietnam, *nampla* in Thailand, *patis* in the Philippines, *shottsuru* in Japan, and *aek-jeot* in Korea (Lopetcharat and Park, 2002). Similar to soy sauce, fish sauce is also produced by fermentation of the raw material with salt. However, it has a stronger and more complicated umami taste than soy sauce. Traditional processing of fish sauce is composed of three steps: salting, enzyme hydrolysis, and fermentation (Saisithi,

1994). Fish and salt are the main raw materials of fish sauce production. In the first step, fish and salt are mixed at varying concentrations from 2:1 to 6:1, depending on the region (Lopetcharat and Park, 2002; Park *et al.*, 2001).

Salad dressings are oil-in-water emulsions, which contain weak organic acids, such as acetic and citric acids, salts, sugar, chelators, thickening agents, surfactants, and preservatives (Casro *et al.*, 2009). The final product contains more than 30% of edible vegetable oil and 4% liquid egg yolk. The pH is 3.2–3.9 and acetic acid concentration is nearly 0.9–1.2% of the total product. The aqueous phase of the salad dressing includes 3–4% of salt and 20–30% of sugar (Foods ICMS, 2006).

26.4.1 Factors affecting microbial ecology of sauces and dressings

Microbiological properties of sauces and salad dressings may differ depending on various factors such as hygiene of handling, preparation processes, and intrinsic properties of food. Good manufacturing practices (GMPs) are required to have a safe product to consume as well as applying other factors including water activity and pH as barriers for microbial growth. The pH value is the major limiting factor affecting microbiological properties of sauces and inhibition of pathogenic microorganisms (Sikora *et al.*, 2008). Growth of most food-borne bacteria and/or their toxin formation is inhibited at pH values lower than 4 (FDA, 1996). Therefore, preservation of sauces depends on the use of acidifying agents such as vinegar (acetic acid). However, excessive use of acetic acid in percentages higher than 1.5% may result in an undesirable taste in the product (Sikora *et al.*, 2008). Water activity levels of sauces may differ among different types of products. For example, soy sauce, water activity of which is around 0.7 (FDA, 1984), prevents growth of most pathogenic bacteria. Salt is the major factor lowering the water activity. The osmotic effect of salt provides the bacteriostatic or bactericidal effect due to plasmolysis of the cells. Yeasts and molds are capable of causing spoilage

at lower water activity values as compared to bacteria. Some types of sauces, such as those made from fish, are produced by microbial fermentation, which has a preservative effect and enables inhibition of undesirable microorganisms. Use of salt and spices also may exhibit lower bacterial counts in fermented types of sauces while addition of glucose as the fermentation raw material may promote bacterial growth (Kilinc *et al.*, 2006).

Similar to mayonnaise and other sauces, salad dressings also have low pH levels generally provided by vinegar or lemon juice. Therefore, salad dressings do not represent a certain health risk in terms of pathogenic bacteria. A combination of intrinsic factors is a very important factor in commercial processing and preservation of salad dressings. High-temperature heat treatment is not preferred in salad dressings because possibly mild heat treatment is enough to reduce, control, or eliminate spoilage microorganisms (Beuchat *et al.*, 2006). Formulation of the water phase of the salad dressing is a critical factor affecting microbial growth. A decrease in salt and organic acid content may lead to microbial growth. The pH is adjusted with acetic acid. It has been reported that acetic acid concentration in the water phase is the most important factor in destroying pathogenic bacteria (Smittle, 2000). It was reported that a few species of *Lactobacillus*, *Saccharomyces*, and *Zygosaccharomyces* are the main microbiota of salad dressing (Castro *et al.*, 2009).

26.4.2 Microbial ecology of sauces and salad dressings

Halophilic aerobic spore-forming bacteria were shown to be the predominant microorganisms of fish sauce (Zhu *et al.*, 2012). *Bacillus*-type bacteria, aerobes, were found to dominate in nampla, a type of Thai fish sauce, and were effective on aroma formation by producing volatile acids. A *Staphylococcus* spp. and *Streptococcus* spp. were also found among the predominant bacteria contributing to aroma formation (Saisithi *et al.*, 1966). Noda *et al.* (1980) investigated the inhibitory effect of osmophilic LAB on

the growth of osmophilic yeasts during shoyu (Japanese fermented soy sauce) fermentation. In the result, osmophilic shoyu yeasts such as *Z. rouxii* and *Torulopsis versatilis* were inhibited by a metabolite produced by osmophilic LAB, namely *Tetragenococcus halophilus*. Acetic acid was considered as the primary inhibitor while lactic acid was slightly inhibitory. In another study, microbiota of Pacific whiting fish sauce during atmospheric fermentation at 25% salt and 50 °C were investigated (Lopetcharat and Park, 2002) and *Staphylococcus*, *Bacillus*, and *Micrococcus* were found to be the predominant microorganisms during fermentation.

The rates of death of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* in various types of commercial salad dressings were investigated by Beuchat *et al.* (2006). In this study, salad dressings were inoculated with low (2.4–2.5 log cfu/g) and high (5.3–5.9 log cfu/g) populations of five separate strain mixtures of each pathogen and held at 25 °C for up to 15 days. All tested pathogens above rapidly died in all salad dressings, independent of the initial inoculum population. It was reported that total counts and populations of LAB and yeasts and molds remained low or undetectable (<1 log cfu/ml) during the 15-day storage period. They summarized that shelf-stable, dairy-based, pourable ranch, and blue cheese salad dressings manufactured by three companies and stored at 25 °C did not support the growth of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*.

Erickson and Jenkins (1991) carried out a study to investigate the survival characteristics of *Salmonella* spp. and *L. monocytogenes* in mayonnaise- and dressing-based products. They reported that inactivation rates of *Salmonella* spp. were unaffected by the ingredient composition while the *L. monocytogenes* inactivation rate was directly correlated with aqueous phase acetic acid concentrations. The authors concluded that commercial mayonnaise, including reduced calorie mayonnaise dressing types, show negligible consumer safety risks.

Yeast growth in salad dressings at 10 and 25 °C storage temperatures was investigated

by differentiation on chromomeric agar (CHROMagar) for *Candida crusei*. The color of colonies indicated the presence of different yeast species. Analysis of three different brands of salad dressing also showed that the fastest growth rate belonged to *C. crusei*, especially at low temperatures (Fujikawa *et al.*, 1998).

Table 26.1 gives some information about the microbial ecology of sauces produced in different regions of the world. As concluded in the table, the population of microorganisms increases in the earlier stages of fermentation and then starts to decrease gradually within the extending storage periods. This decrease can be the result of depletion of nutrients over time, transmission of salt throughout the sauce, and decline in the water activity. Notwithstanding the sauce type, fermentation is the main determinant for diversity and predominance of the microorganisms. In general, halophilic and proteolytic bacteria, yeasts, and molds dominate the media. LAB also play a role in the fermentation and flavor formation. Survival of pathogenic or spoilage microorganisms does not take place because of an unfavorable environment and/or a lack of competence ability.

26.5 Conclusion

This chapter provides current and detailed information about the production processes, microbial ecology of margarine, mayonnaise, sauces, and salad dressings, and factors affecting their microbiological properties. In most cases, these products are considered as microbiologically safe due to their unfavorable properties, such as low pH and water activity and the presence of antimicrobial agents for microbial growth. Considering margarine, yeasts and molds are the main causes of the limited number of outbreaks. Microbial spoilage of the mayonnaise results from a small group of acid-tolerant yeasts and LAB although spoilage of these groups of microorganisms is not a prominent health concern. Sauces and dressings do not provide favorable conditions for undesirable

Table 26.1 Microbial ecology of sauces.

Sauce type	Fermentation conditions	Predominant microorganisms	Other microorganisms	Reference
Bakasang (Indonesian fish sauce)	37 °C for 40 days	<i>Micrococcus</i> , <i>Streptococcus</i> , and <i>Pediococcus</i> spp.	–	Ijong and Ohta, 1996
Fish sauce	27–35 °C for 12 months	TVC (2.93–5.73 log) Halophilic bacteria (2.20–4.30 log) Proteolytic bacteria (2.10–4.14 log)	–	Dissaraphong et al., 2006
Fish sauce from sardine	37 °C for 57 days	Increase at first 8 days of fermentation and then decrease in TVC and LAB	<i>S. aureus</i> and yeast–mold were not detected during fermentation	Kilinc et al., 2006
Yu-lu (Chinese fish sauce)	30 ± 5 °C for 180 days and 50 °C for 7 days	Increase in TVC during the first 120 days and then decrease (from 6 logs to 8 logs)		Jiang et al., 2007
Fish sauce from silver carp	RT for 12 months with addition of <i>Tetragenococcus halophilus</i>	TVC (<10 ⁷ – 3.0 × 10 ⁷ cfu/g); LAB (1.0 × 10 ⁵ – 5.7 × 10 ⁷ cfu/g); YM (<10 ⁴ – 2.0 × 10 ⁶ cfu/g); CB (<1 cfu/g)	<i>T. halophilus</i> -like bacteria were found to be predominant for all tanks fermented under the different starting conditions	Uchida et al., 2005
Soy sauce		<i>Z. rouxii</i> (early fermentation stage), <i>Candida etchellsii</i> and <i>C. versatilis</i> (middle fermentation stage), <i>C. etchellsii</i> (mature fermentation stage)	<i>Lactobacillus fermentum</i> , <i>Lb. plantarum</i> , <i>Lb. iners</i> , <i>Streptococcus thermophilus</i> , <i>Staphylococcus gallinarum</i> , and <i>S. kloosii</i>	Tanaka et al., 2012
Soy sauce mash	Samples collected from Thai factories	–	<i>T. halophilus</i> , <i>Lb. acidipiscis</i> , <i>Lb. acidipiscis</i> , <i>Lb. pentosus</i> , and <i>Lb. plantarum</i>	Tanasupawat et al., 2002
Soy sauce mash		–	<i>Staphylococcus carnosus</i> , <i>S. piscifermentans</i> , <i>S. aureus</i> , and <i>S. epidermidis</i>	Probst et al., 1998

TVC: total viable count (log cfu/g); LAB: lactic acid bacteria; YM: yeasts and molds; CB: coliform bacteria.

microorganisms; however, if microbial fermentation is available in the manufacturing process, mainly halophilic/halotolerant microorganisms dominate the product microbiota during the fermentation. In conclusion, although margarine, mayonnaise, and sauces are generally considered as microbiologically safe food products and spoilage is rare, good hygienic practices should be taken into consideration to manufacture safe and high-quality products.

References

- Alford, J.A., Smith, J.L., and Lilly, H.D. 1971. Relationship of microbial activity to changes in lipids of foods. *Journal of Applied Bacteriology*, **34**, 133–146.
- Anonymous. 2004. Margarine recalled over *Listeria* fears [Online]. Available at: http://articles.chicagotribune.com/2004-04-25/news/0404250391_1_recall-food-distribution-expiration-dates (accessed March 2015).
- Beerens, H. 1980. Sanitary state of processing and bacteriological properties of margarines. *Revue Francaise des Corps Gras*, **27**, 221–223.
- Beuchat, L.R., Ryu, J.H., Adler, B.B., Harrison, M.D. 2006. Death of *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* in shelf-stable, dairy-based, pourable salad dressings. *Journal of Food Protection*, **69**, 801–814.
- Castro, M.P., Rojas, A.M., Campos, C.A., and Gerschenson, L.N. 2009. Effect of preservatives, tween 20, oil content and emulsion structure on the survival of *Lactobacillus fructivorans* in model salad dressings. *LWT – Food Science and Technology*, **42**, 1428–1434.
- Delamarre, S. and Batt, C.A. 1999. The microbiology and historical safety of margarine. *Food Microbiology*, **16**, 327–333.
- Depree, J.A. and Savage, G.P. 2001. Physical and flavour stability of mayonnaise. *Trends in Food Science and Technology*, **12**, 157–163.
- Dissaraphong, S., Benjakul, S., Visessanguan, W., and Kishimura, H. 2006. The influence of storage conditions of tuna viscera before fermentation on the chemical, physical and microbiological changes in fish sauce during fermentation. *Bioresource Technology*, **97**, 2032–2040.
- Erdogrul, O., Erbilir, F., and Toroglu, S. 2006. Survival of acid-adapted and non-adapted *Staphylococcus aureus* in various food samples. *Annals in Microbiology*, **56**, 25–27.
- Erickson, J.P. and Jenkins, P. 1991. Comparative *Salmonella* spp. and *Listeria monocytogenes* inactivation rates in 4 commercial mayonnaise products. *Journal of Food Protection*, **54**, 913–916.
- FDA. 1984. Water Activity (a_w) in Foods (updated 06/14/2010; cited 2014). Available at: <http://www.fda.gov/iceci/inspections/inspectionguides/inspectiontechnicalguides/ucm072916.htm>.
- FDA. 1996. Bacterial Pathogen Growth and Inactivation, Appendix-4, pp. 417–438. Available at: <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM252447.pdf>.
- Fialová, J., Chumchalová, J., Miková, K., and Hrušová, I. 2008. Effect of food preservatives on the growth of spoilage lactobacilli isolated from mayonnaise-based sauces. *Food Control*, **19**, 706–713.
- Foods ICMS. 2006. *Microorganisms in Foods*, Vol. 6: *Microbial Ecology of Food Commodities*. Kluwer Academic/Plenum Publishers.
- Fujikawa, H., Maruyama, B., Wauke, T., Morozumi, S., and Itoh, T. 1998. Analysis of yeast growth in salad dressing during storage with chromogenic agar CHROMagar Candida (R). *Journal of the Food Hygienic Society of Japan*, **39**, 120–126.
- Glass, K.A. and Doyle, M.P. 1991. Fate of *Salmonella* and *Listeria monocytogenes* in commercial, reduced-calorie mayonnaise. *Journal of Food Protection*, **54**, 691–695.
- Gomezluca, E., Goyache, J., Blanco, J.L., Garayzabal, J.F.F., Orden, J.A., and Suarez, G. 1987. Growth of *Staphylococcus aureus* and enterotoxin production in home-made mayonnaise prepared with different pH values. *Journal of Food Protection*, **50**, 872–875.
- Gomezluca, E., Goyache, J., Orden, J.A., Domenech, A., et al. 1990. Influence of temperature of incubation on *Staphylococcus aureus* growth and enterotoxin production in

- home-made mayonnaise. *Journal of Food Protection*, **53**, 386.
- Guentert, A.M. and Linton, R.H. 2003. Growth and survival of selected pathogens in margarine-style table spreads. *Journal of Environmental Health*, **65**, 9–14.
- Gumus, T., Gecgel, U., Demirci, A.S., and Arici, M. 2008. Effects of gamma irradiation on two heat resistant moulds: *Aspergillus fumigatus* and *Paecilomyces variotii* isolated from margarine. *Radiation Physics and Chemistry*, **77**, 680–683.
- Gumus, T., Demirci, A., Sagdic, O., Arici, M. 2010. Inhibition of heat resistant molds: *Aspergillus fumigatus* and *Paecilomyces variotii* by some plant essential oils. *Food Science and Biotechnology*, **19**, 1241–1244.
- Hashem, M. and Alamri, S. 2010. Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. *Saudi Journal of Biological Sciences*, **17**, 167–175.
- Hathcox, A.K., Beuchat, L.R., Doyle, M.P. 1995. Death of enterohemorrhagic *Escherichia coli* O157:H7 in real mayonnaise and reduced-calorie mayonnaise dressing as influenced by initial population and storage-temperature. *Applied and Environmental Microbiology*, **61**, 4172–4177.
- Heo, S.K., Lee, J.Y., Jo, S.H., Bae, D.H., Kim, Y.S., Chung, M.S., and Ha, S.D. 2010. Inactivation of *Salmonella enterica* serovar Typhimurium in commercial mayonnaise at different temperatures and pH. *Journal of the Korean Society for Applied Biological Chemistry*, **53**, 351–355.
- Hocking, A.D. 1994. Fungal spoilage of high-fat foods. *Food Australia*, **46**, 30–33.
- Ijong, F.G. and Ohta, Y. 1996. Physicochemical and microbiological changes associated with baka-sang processing – a traditional Indonesian fermented fish sauce. *Journal of the Science of Food and Agriculture*, **71**, 69–74.
- Jiang, J.-J., Zeng, Q.-X., Zhu, Z.-W., and Zhang, L.-Y. 2007. Chemical and sensory changes associated Yu-lu fermentation process – a traditional Chinese fish sauce. *Food Chemistry*, **104**, 1629–1634.
- Khambaty, F.M., Bennett, R.W., and Shah, D.B. 1994. Application of pulsed-field gel electrophoresis to the epidemiological characterization of *Staphylococcus intermedius* implicated in a food-related outbreak. *Epidemiology and Infection*, **113**, 75–81.
- Kilinc, B., Cakli, S., Tolasa, S., and Dincer, T. 2006. Chemical, microbiological and sensory changes associated with fish sauce processing. *European Food Research and Technology*, **222**, 604–613.
- Kurtzman, C.P., Rogers, R., and Cw, H. 1971. Microbiological spoilage of mayonnaise and salad dressings. *Applied Microbiology*, **21**, 870–874.
- Lawrence, R.C. 1967. *Microbial Lipases and Related Esterases: Detection, Distribution and Production of Microbial Lipases*. New Zealand Dairy Research Institute.
- Leuschner, R.G.K. and Boughtflower, M.P. 2001. Standardized laboratory-scale preparation of mayonnaise containing low levels of *Salmonella enterica* serovar enteritidis. *Journal of Food Protection*, **64**, 623–629.
- Leuschner, R.G.K. and Zamparini, J. 2002. Effects of spices on growth and survival of *Escherichia coli* 0157 and *Salmonella enterica* serovar Enteritidis in broth model systems and mayonnaise. *Food Control*, **13**, 399–404.
- Lock, J.L. and Board, R.G. 1994. The fate of *Salmonella enteridis* PT4 in deliberately infected commercial mayonnaise. *Food Microbiology*, **11**, 499–504.
- Lopetcharat, K. and Park, J.W. 2002. Characteristics of fish sauce made from pacific whiting and surimi by-products during fermentation stage. *Journal of Food Science*, **67**, 511–516.
- Lück, E. 1990. Food applications of sorbic acid and its salts. *Food Additives and Contaminants*, **7**, 711–715.
- Ma, L., Kornacki, J.L., Zhang, G., Lin, C.-M., and Doyle, M.P. 2007. Development of thermal surrogate microorganisms in ground beef for implant critical control point validation studies. *Journal of Food Protection*, **70**, 952–957.
- Manios, S.G., Lambert, R.J.W., and Skandamis, P.N. 2014. A generic model for spoilage of acidic

- emulsified foods: combining physicochemical data, diversity and levels of specific spoilage organisms. *International Journal of Food Microbiology*, **170**, 1–11.
- McKee, L.H. 1995. Microbial contamination of spices and herbs: a review. *LWT – Food Science and Technology*, **28**, 1–11.
- Muys, G. 1971. Microbial safety in emulsions. *Process Biochemistry*, **6**, 25–28.
- Noda, F., Hayashi, K., and Mizunuma, T. 1980. Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. *Applied and Environmental Microbiology*, **40**, 452–457.
- Park, J.-N., Fukumoto, Y., Fujita, E., Tanaka, T., *et al.* 2001. Chemical composition of fish sauces produced in Southeast and East Asian countries. *Journal of Food Composition and Analysis*, **14**, 113–125.
- Perales, I. and Garcia, M.I. 1990. The influence of pH and temperature on the behavior of *Salmonella* Enteridis phage type-4 in homemade-mayonnaise. *Letters in Applied Microbiology*, **10**, 19–22.
- Petruskaite, V., De Greyt, W., Kellens, M., and Huyghebaert, A. 1998. Physical and chemical properties of trans-free fats produced by chemical interesterification of vegetable oil blends. *Journal of American Oil Chemical Society*, **75**, 489–493.
- Probst, A.J., Hertel, C., Richter, L., Wassill, L., Ludwig, W., and Hammes, W.P. 1998. *Staphylococcus condimenti* sp. nov., from soy sauce mash, and *Staphylococcus carnosus* (Schleifer and Fischer, 1982) subsp. *utilis* subsp. nov. *International Journal of Systematic Bacteriology*, **48**, 651–658.
- Radford, S.A., Tassou, C.C., Nychas, G.J.E., and Board, R.G. 1991. The influence of different oils on the death rate of *Salmonella* Enteridis in home-made mayonnaise. *Letters in Applied Microbiology*, **12**, 125–128.
- Richardson, K. and George, B. 1998. A bulletin for the Australian Food Industry – spoilage of low salt margarine. [Online]. Available at: <http://foodscience.csiro.au/fshbull/fshbull14g.htm> (accessed August 2015).
- Saisithi, P. 1994. Traditional fermented fish: fish sauce production. In: *Fisheries Processing* (ed. Martin, A.M.), pp. 111–131. Springer, USA.
- Saisithi, P., Kasemsarn, R.-O., Liston, J., and Dollar, A.M. 1966. Microbiology and chemistry of fermented fish. *Journal of Food Science*, **31**, 105–110.
- Sikora, M., Badrie, N., Deisingh, A.K., and Kowalski, S. 2008. Sauces and dressings: a review of properties and applications. *Critical Reviews in Food Science and Nutrition*, **48**, 50–77.
- Smittle, R.B. 1977. Microbiology of mayonnaise and salad dressing: a review. *Journal of Food Protection*, **40**, 415–422.
- Smittle, R.B. 2000. Microbiological safety of mayonnaise, salad dressings, and sauces produced in the United States: a review. *Journal of Food Protection*, **63**, 1144–1153.
- Smittle, R.B. and Flowers, R.S. 1982. Acid tolerant microorganism involved in the spoilage of salad dressing. *Journal of Food Protection*, **45**, 977–983.
- Steels, H., James, S.A., Roberts, I.N., and Stratford, M. 2000. Sorbic acid resistance: the inoculum effect. *Yeast*, **16**, 1173–1183.
- Swanson, K.M. 2005. Oil- and fat-based foods. In: *Microorganisms in Foods* (ed. Foods ICoMSf), Vol. **6**, pp. 480–521. Kluwer Academic/Plenum Publishers. New York, USA.
- Tanaka, Y., Watanabe, J., and Mogi, Y. 2012. Monitoring of the microbial communities involved in the soy sauce manufacturing process by PCR-denaturing gradient gel electrophoresis. *Food Microbiology*, **31**, 100–106.
- Tanasupawat, S., Thongsanit, J., Okada, S., and Komagata, K. 2002. Lactic acid bacteria isolated from soy sauce mash in Thailand. *The Journal of General and Applied Microbiology*, **48**, 201–209.
- Tornai-Lehoczki, J., Péter, G., Dlačny, D. 2003. CHROMagar *Candida* medium as a practical tool for the differentiation and presumptive identification of yeast species isolated from salads. *International Journal of Food Microbiology*, **86**, 189–200.

- Uchida, M., Ou, J., Chen, B.-W., Yuan, C.-H., *et al.* 2005. Effects of soy sauce koji and lactic acid bacteria on the fermentation of fish sauce from freshwater silver carp *Hypophthalmichthys molitrix*. *Fisheries Science*, **71**, 422–430.
- US-FDA. 2013. *CFR – Code of Federal Regulations, Requirements for Specific Standardized Margarine*, Revised as of April 1, 2013. Washington, DC, USA.
- USUS-DHEW. 1975. *Dressings for Food. Mayonnaise*, 21 CFR 251. US Government Printers Office, Washington, DC, USA.
- van der Sluis, C., Tramper, J., and Wijffels, R.H. 2001. Enhancing and accelerating flavour formation by salt-tolerant yeasts in Japanese soy-sauce processes. *Trends in Food Science and Technology*, **12**, 322–327.
- Weagant, S.D., Bryant, J.L., and Bark, D.H. 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. *Journal of Food Protection*, **57**, 629–631.
- Zaeromali, M., Maghsoudlou, Y., Aryaey, P., and Nateghi, L. 2013. Investigation of physicochemical, microbial and fatty acids profile of table margarine made with palm and soybean oils. *European Journal of Experimental Biology*, **3**, 178–182.
- Zhu, J.L., Li, J.R., and Chen, J.R. 2012. Survival of *Salmonella* in home-style mayonnaise and acid solutions as affected by acidulant type and preservatives. *Journal of Food Protection*, **75**, 465–471.
- Zijl, M.M.V. and Klapwijk, P.M. 2000. Yellow fat products (butter, margarine, dairy and nondairy spreads). In: *The Microbiological Safety and Quality of Food*, Vol. I (eds Lund, B.M., Baird-Parker, T.C., and Gould, G.W.), pp. 784–806. Aspen Publishers, Inc., Gaithersburg, MD, USA.

CHAPTER 27

Microbial ecology of confectionary products, honey, sugar, and syrups

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27.1 Introduction

This chapter addresses some low-moisture foods: cocoa, chocolate, nuts, peanut butter, sugar, syrups, and honey. It mainly focuses on their microbial ecology, impact of unit operations, and the characteristics of the processed products that allow growth or survival of microorganisms. An understanding of microorganisms present at low-moisture foods and how they react towards different processing conditions is important for high quality and safe food production. There is diversity in manufacturing processes of different low-moisture foods, but most of the unit operations during processing can be considered as deterministic factors of product safety. Therefore, efficacy of safe food production is dependent on the process history. The ideal approach of safe food production is to address each unit operation individually and determine whether they influence microbial content in the food.

27.2 Cocoa and chocolate

Chocolate is a quite popular confectionary product consumed worldwide, especially by children. Starting from opening of the cocoa bean to industrial processing of chocolate, the overall process is divided into three main steps: cocoa pre-processing on farms (harvest, fermentation,

drying, and storage); primary cocoa processing that transforms cocoa beans into semi-finished products (cocoa liquor, cocoa butter, cocoa cake, and cocoa powder); and finally chocolate and confectionary processing.

The first step of cocoa pre-processing consists of harvesting and opening of cocoa pods and fermentation of the pulp and seeds. Before opening the healthy fruit, the internal part of cocoa (i.e., the pulp) is practically free of microbial contamination. However, after opening, wide ranges of microorganisms are introduced to cocoa beans and cause complex fermentation in the pulp. They originate from a variety of environment sources (Ostovar and Keeney, 1973; Schwan and Wheals, 2004). In the early stages of fermentation, yeasts dominate and produce ethanol, secreting enzymes that break down pectin. The most common yeast genuses isolated during fermentation are *Saccharomyces*, *Candida*, *Hansenula*, *Klockera*, and *Kluyveromyces* (Ardhana and Fleet, 2003; Schwan and Wheals, 2004; Nielsen *et al.*, 2007). In the next phase, lactic acid bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*) proliferate and produce lactic acid, followed by acetic acid bacteria (*Acetobacter*, *Gluconobacter*), which produce acetic acid and cause a rise in temperature up to 50 °C (Ardhana and Fleet, 2003; Papalexandratou *et al.*, 2011). The last stage of fermentation is characterized by increased pH of the pulp to around 5.0, the

predominance of aerobic spore-forming bacteria (*B. pumilus*, *B. megaterium*, *B. licheniformis*, *B. subtilis*, and *B. cereus*), and the appearance of filamentous fungi, such as *Aspergillus*, *Mucor*, *Fusarium*, and *Penicillium* (Ostovar and Keeney, 1973; Schwan and Wheals, 2004; Afoakwa *et al.*, 2008). These last two microbial groups are responsible for the off-flavor in chocolate by producing lipases, which hydrolyze lipids to fatty acids. After fermentation, a natural or artificial drying process is carried out in order to reduce the moisture of the beans to 6–8% (ICMSE, 2011; Thompson *et al.*, 2013). Depending on the weather conditions, natural drying may be prolonged for weeks, whereas in artificial drying the process takes around 48–72 h (Beckett, 2009). The spoilage microorganisms present in dried beans are predominantly mesophilic and thermophilic sporeformers, yeast, and molds (Barrile *et al.*, 1971).

Pathogenic bacteria may contaminate cocoa beans due to poor hygiene conditions during material handling at pre-processing stages. From a public health standpoint, *Salmonella* and toxigenic fungi are the main pathogens that have concerned government and the cocoa–chocolate industry. Salmonellosis outbreaks associated with the consumption of chocolate have been reported since 1970. In most cases, the outbreaks were epidemic, spread geographically and reached a large number of people (Werber *et al.*, 2005; CDR-HPA, 2006). Dried cocoa beans and chocolate have characteristics such as low water activity ($a_w \approx 0.3$ to 0.5) and high fat content (>20 %), which result in *Salmonella* viability for long periods of time (Tamminga *et al.*, 1976; Komitopoulou and Peñaloza, 2009). In addition, the synergistic effect of low a_w and high fat content protect the pathogen against gastric acidity and thus results in a reduction dose–response curve with a low infectious dose (≤ 1 cell/g) (D'Aoust, 1977; Hockin *et al.*, 1989).

One exploratory study conducted in Brazil on *Salmonella* contamination during cocoa primary production stages found that 3% of dried

cocoa beans are contaminated with pathogenic bacteria (Nascimento *et al.*, 2010). A later study revealed that *Salmonella* behavior during the pre-processing stages is dependent on the time of contamination (Nascimento *et al.*, 2013). Therefore, they are affected mainly by pH during the fermentation and by a_w during the drying and storage steps. The most critical period is between the end of the fermentation and the beginning of the drying. Studies have shown that if contamination occurs, *Salmonella* can survive by several weeks in stored cocoa beans at ambient conditions (Komitopoulou and Peñaloza, 2009; Nascimento *et al.*, 2013).

Roasting is considered as the main step to reduce microbial contamination in cocoa beans. This process is commonly carried out between 110 and 140 °C for 30 min and 12 min for cocoa beans and nibs, respectively (Beckett, 2009). Usually after the roasting step mostly spore-forming bacteria (*B. licheniformis*, *B. subtilis*, *B. coagulans*, *B. circulans*, *B. brevis*, *B. megaterium*) are detected in the product (Barrile *et al.*, 1971). However, the synergistic effect of low water activity (0.3 to 0.5) and high fat content (>20%) of cocoa can increase heat resistance of *Salmonella* (D'Aoust, 1977; Podolak *et al.*, 2010). Depending on the initial load and the process parameters, cocoa roasting cannot guarantee the safety of the product. According to Nascimento *et al.* (2012), during dry roasting a decimal reduction of *Salmonella* occurs after 2.5 min at 140 °C and after 9 minutes at 110 °C. In another study, *Salmonella* spp. survived approximately 5 min at 130 °C during roasting of cocoa beans (Silva and Gibbs, 2012).

The last stage of the cocoa and chocolate processing is the production of chocolate and other confectionary products. This process comprises milling, mixing, conching, tempering, and molding. The conching process uses temperatures between 50 and 80 °C for 2–72 h depending on the equipment and the type and quality of chocolate (Beckett, 2008). It might reduce the microbial count of the chocolate mass. However, this process is not a reliable control step for heat-resistant bacteria

such as *Salmonella*. At 70 °C, 600 min (Krapf and Gantenbein-Demarchi, 2010), 700 min (Nascimento *et al.*, 2012), and 1200 min were determined as the time required to reduce a logarithmic cycle of *Salmonella* (Barrile and Cone, 1970).

Due to the absence of an effective killing step during the chocolate processing, the quality of the ingredients plays an important role for the safety of the final product (ICMSE, 2005). Cordier (1994) points out that raw cocoa beans are the foremost source of *Salmonella* during chocolate processing. Smedt *et al.* (1991) detected *Salmonella* in 79% of the analyzed dust samples from the cocoa bean cleaning zone. The processing environment has also been incriminated as the contamination source in a salmonellosis outbreak associated with chocolate (CDR-HPA, 2006). Craven *et al.* (1975) recovered *Salmonella* from six out of 286 chocolate processing environmental samples. In Mexico, Torres-Vitela *et al.* (1995) isolated *Salmonella* from two samples of wrapped chocolate.

Regarding toxigenic fungi, as observed for *Salmonella*, cocoa beans can be contaminated during pre-processing steps, mainly during drying and/or storage (Mounjouenpou *et al.*, 2008; Copetti *et al.*, 2010, 2011). During these stages, there is still sufficient free water to allow fungal growth and mycotoxin production. Ochratoxin A was recovered from 27% of the samples during fermentation, 51% during drying, and 52% during storage (Copetti *et al.*, 2010). However, ochratoxin A has not been considered a significant hazard (Amezqueta *et al.*, 2005) due to its significantly low amount (50%) during the shelling process (Gilmour and Lindblom, 2008). According to Copetti *et al.* (2013), 83% of the ochratoxin A is in the shell fraction. Nevertheless, this mycotoxin has been detected in around 90% of cocoa processing products (Bonvehí, 2004; Copetti *et al.*, 2013). Another unit operation that reduces mycotoxin levels in cocoa is roasting. This step was able to reduce between 24 and 40% of ochratoxin A in cocoa after 30 min at 140 °C (Manda *et al.*, 2009). However, the effectiveness of this unit operation

is directly influenced by process parameters and the initial level of contamination. High occurrence of aflatoxins and ochratoxin A in chocolate bars are reported worldwide, with 23 to 100% positive samples (Kumagai *et al.*, 2008; Brera *et al.*, 2011; Copetti *et al.*, 2012). However, the mycotoxin contamination rate is higher in chocolate with a high fat-free cocoa solid content (Copetti *et al.*, 2012).

27.3 Nuts and peanut butter

The tree nuts category that will be covered here includes almond (*Prunus amygdalus*), Brazil nut (*Bertholletia excels*), cashew nut (*Anacardium occidentale*), hazelnut (*Corylus avellana*), macadamia nut (*Macadamia ternifolia*), pecan (*Carya illinoensis*), pistachio (*Pistachia vera*), and walnut (*Juglans regia*). According to the botanical definition, peanuts or groundnuts (*Arachis hypogaea*) is a legume, but in this section, as has also been done by peer-reviewed authors, it will be considered as part of the nut group.

Nuts and peanuts are very much appreciated due to their high nutritional value and health benefits (Freitas-Silva and Venâncio, 2011; Pinkas *et al.*, 2009). They are commercialized in the shell or in the shelled condition. Also they are consumed as snacks after drying without any kind of heat treatment or can be roasted or fried before consumption. Nuts and peanuts are also used as an ingredient in a range of confectionary products, including peanut butter and hazelnut cream. Their production chain can be divided into three major stages: primary production, which includes pre- and post-harvesting steps, secondary processing, and manufacturing of confectionary products. Most varieties of tree nuts are cultivated in farms, but Brazil nuts are produced in pristine forests, making it more difficult to control microbiological contamination (Freitas-Silva and Venâncio, 2011). *Aspergillus flavus* and *Salmonella* are the most found microorganisms in nuts and their confectionary products. During

primary production, environmental conditions (temperature, rainfall, and humidity), contaminated soil, contaminated irrigation water, inadequate equipment hygiene, the presence of animals, and mechanical damage caused by insects and birds influence directly the type and load of microbiological contamination of nuts (Schatzki and Ong, 2001; ICMSE, 2011). On the other hand, fungi contamination can also occur in previous stages, especially during the inflorescence for Brazil nuts (Freire *et al.*, 2000) and cashew nuts (Costa *et al.*, 2009). For pistachio, the critical point for fungi contamination is the early split that occurs a month before harvesting (Codex Alimentarius, 2010). The outer shell protects the nutmeat from external contamination during nut growth. According to a study carried out throughout the peanut production chain, peanut samples showed a high *Enterobacteriaceae* count (up to 9.5 log cfu/g) in all steps before shelling; after this stage the counts were reduced to <4.8 log cfu/g, corroborating the hypothesis that bacteria contamination is concentrated in the external part (data not published). However, insect damage and drought stress may also expose kernel to soil microflora such as mycotoxigenic fungi (Pitt and Hocking, 2009) and *Salmonella* Typhimurium. It can internalize within major tissues of peanut plant originated from *Salmonella* contaminated seed (Deering *et al.*, 2012) and may end up in peanuts. Nuts and peanuts can be harvested manually or mechanically; it depends on the variety of the nut and social-economic condition of the production region. In Brazil, the mechanized threshing during primary production of peanuts was identified as the most important contamination source of *Salmonella* (Bernardi *et al.*, 2014). The molds, which are present in nuts and peanuts during harvesting, include *Alternaria* spp., *Fusarium* spp., *Cladosporium* spp., *Penicillium* spp. (Hocking, 1988), and aflatoxin producer species such as *A. flavus* and *A. parasiticus* (Horn *et al.*, 2000). After harvesting, nuts are dried naturally under sun or artificially in dryers to achieve 8% of moisture ($a_w \approx 0.7$). In two individual studies,

Salmonella was isolated from 0.67% (Miksch *et al.*, 2013) and 2.33% (Calhoun *et al.*, 2013) of raw peanut samples. During primary storage and transportation, temperature and humidity play an important role for microbiological stability of nuts, influencing the risk of spoilage and the production of mycotoxins. Low soluble carbohydrate and high lipid levels may lead to sharp rises in a_w with a slight increase in moisture content (ICMSE, 2005). Aflatoxin production is limited when a_w is less than 0.85 (ICMSE, 1996). The increase in fungal infection and aflatoxin levels has been reported during storage of nuts (Saleemullah *et al.*, 2006). While storing at refrigerator, moisture must be carefully controlled to avoid condensation. Codex Alimentarius (1972) recommends storage of nuts at 1 °C with 60–70% relative humidity.

During secondary processing, the steps may vary with the nut species and can include washing, artificial drying, cooking/boiling, shelling, sorting, blanching, roasting, and slicing. In some of these processing steps (such as washing, shelling, slicing) cross-contamination may happen. Washing can contribute in fungal growth and mycotoxin production, which may be carried out for some kind of nuts before shelling and drying. Dry roasting and oil roasting are two critical microbial control methods in peanut processing. Temperature during dry roasting varies according to the equipment and final product characteristic. During roasting moisture content decreases to as low as 1.25%, which is supposed to prevent all microbial activities (Woodroof, 1983). However, contamination of nuts and peanuts by pathogens has drawn attention in the last decades due to the emergence of *Salmonella* outbreaks (Isaacs *et al.*, 2005; CDC, 2007, 2009, 2012). This pathogen was detected (from 0.009 to 2 MPN/g) in almond and peanut samples (Danyluk *et al.*, 2007; Bansal *et al.*, 2010; Little *et al.*, 2010). *Salmonella* has the physiological mechanisms that facilitate their long-term survival in low water activity products (Tamminga *et al.*, 1976; Komitopoulou and Peñaloza, 2009; Beuchat and Mann, 2010). This pathogen can persist at least

for 12 months in walnut and peanut butter (Blessington *et al.*, 2012; Nummer *et al.*, 2012; Kataoka *et al.*, 2014). Uesugi *et al.* (2006) found *Salmonella* inoculums in almonds stored at -20 and 4°C even after 550 days.

Peanut butter ($a_w \approx 0.30$) is an important confectionary product widely consumed in many countries. The manufactory process may vary depending on the end product, and may include the following steps: blending, grinding in one or two stages, pasteurizing at 71 to 77°C for 20 min (Ma *et al.*, 2009), cooling, filling, and deaerating. Contaminated ingredients, insufficient processing, or post-processing conditions may introduce *Salmonella* into peanut butter (Podolak *et al.*, 2010; Chang *et al.*, 2013).

As mentioned before in this chapter, synergistic effects of low water activity and high fat content may induce cross-tolerance in *Salmonella* against heat treatment (Podolak *et al.*, 2010; He *et al.*, 2011; ICMSE, 2011). Several studies show

the risk of permanence of this pathogen even after stronger heat treatment ($>80^{\circ}\text{C}$). Pecan nut was heat treated at 120°C for 20 min for 1 log cfu/g inactivation of *Salmonella* (Beuchat and Mann, 2011). *Salmonella* serovars could survive in peanut butter at temperatures as high as 90°C (Shachar and Yaron, 2006). It took 120 min for 7D inactivation of *Salmonella* Tennessee in peanut butter even when heated at 90°C (Ma *et al.*, 2009). According to Sirsat *et al.* (2011), a sublethal step during thermal processing, rather than reducing initial population of *Salmonella* could induce stress response mechanisms, thereby increasing pathogen resistance. This forced the Food and Drug Administration (FDA) to recommend a thermal validation process with a threshold of 5 log reduction of *Salmonella* (FDA, 2009).

Aflatoxin contamination percentage varies (may reach up to 50 %) with the type of nut, time, and place where the sample was produced (Table 27.1). In most of the cases, contamination

Table 27.1 Aflatoxin contamination in different types of nut worldwide.

Country	Nuts	Aflatoxin contamination (%)	Reference
Brazil	Peanuts	17	Gonçalez <i>et al.</i> , 2008
	Peanuts	10	Zorzete <i>et al.</i> , 2011
	Brazil nuts	11	Reis <i>et al.</i> (2012)
	Brazil nuts	38	Iamanaka <i>et al.</i> , 2014
	Cashew	35	Milhome <i>et al.</i> , 2014
Iran	Pistachio	37	Cheraghali <i>et al.</i> , 2007
South Korea	Nuts	11	Chun <i>et al.</i> , 2007
Pakistan	Almonds	30	Luttfullah and Hussain, 2011
	Walnuts	40	
	Peanuts	40	
	Pistachio	20	
	Pine nuts	20	
Saudi Arabia	Walnuts	50	El Tawila <i>et al.</i> , 2013
	Hazelnut	43	
	Pistachio	34	
	Peanuts	18	
	Almonds	17	
Turkey	Cashew	15	Hepsag <i>et al.</i> , 2014
	Pistachio	15	
	Peanuts	19	

levels higher than the maximum tolerant limit (4 or 10 µg/kg) (Codex Alimentarius, 2004, 2010) were found in less than 11% of the samples (Luttfullah and Hussain, 2011; El Tawila *et al.*, 2013; Iamanaka *et al.*, 2014; Milhome *et al.*, 2014). There are a variety of treatments reported in the literature that were used to reduce aflatoxin contamination in nuts and peanuts, such as ammonia, hydrogen peroxide, ozone, irradiation, and heat treatment (Bhat *et al.*, 2010; Leong *et al.*, 2010; Scott, 1998). However, depending on the initial aflatoxin concentration, these treatments are not able to ensure product safety. For example, 95% of peanut butter samples analyzed in Turkey were contaminated with aflatoxin B1 (Yentur *et al.*, 2006). Moreover, some of these treatments may affect the sensory characteristics of the product, thus limiting their application.

27.4 Honey

Honey is the natural sweet substance produced by honey bees (*Apis mellifera*) from the nectar, secretions, or excretions of plant-sucking insects. It mainly consists of fructose and glucose (not less than 60 g/100 g) and water (17–20 %) (Codex Alimentarius, 2001). Low a_w (<0.65), the presence of several antimicrobial substances (hydrogen peroxide, pinocembrin, lysozyme, acids, terpenes, etc.), low protein content, and high viscosity of honey do not favor microbial growth (Snowdon and Cliver, 1996). Nonetheless, honey is prone to microbial contamination both from pre- and post-harvest processing conditions. The prevalent contamination sources are nectar of flowers, pollen, dust, air, honey bee, other insects, food handlers, and equipment (Snowdon and Cliver, 1996).

A wide range of microorganisms has been isolated from honey (Snowdon and Cliver, 1996). A gradient water activity can contribute to microbial growth, especially osmophilic yeasts such as *Saccharomyces* spp. and *Zygosaccharomyces* spp. (Pitt and Hocking, 1985; Tokuoka *et al.*, 1985). These yeasts can

grow, ferment sugar, and produce alcohol and carbon dioxide. *Gluconobacter* and *Lactobacillus* are the main bacteria genus isolated from honey (Ruiz-Argueeso and Rodriguez-Navarro, 1975). *Micrococcus* and *Pseudomonas* have also been reported (Snowdon and Cliver, 1996).

Clostridium botulinum is the only microorganism of public health concern related to honey. The spores germinate in the immature infantile intestine and produce botulism neurotoxin, causing infant botulism in children less than one year of age (Arnon *et al.*, 1977). Toxins A and B are the most common toxins associated with infantile botulism. This disease was first recognized in 1976 (Midura and Arnon, 1976) and since then has been reported in several cases around the world. Usually the honey contamination rate is below 1 spore/g (Snowdon and Cliver, 1996). According to Arnon *et al.* (1979), the contamination level in samples associated with infant botulism may be as low as 5–70 spores/g. The isolation rate of *C. botulinum* spores in honey samples varies between 2 and 26% worldwide (Rall *et al.*, 2003; ICMSE, 2005; Nevas *et al.*, 2005; Kuplulu *et al.*, 2006). The processing environment plays an important role in determining microbiota of honey. For example, contaminated equipment may introduce bacteria and yeasts in extracted honey. There are only a few unit operations involved in honey processing compared to other low-moisture foods (Figure 27.1). During traditional honey processing, extracted honey is filtered to remove extraneous solids (e.g., pollens, wax). This step reduces the microbial population in honey. Compared to traditional honey processing, industrial processing is more efficient in reducing microbial count because it involves pasteurization (66–77 °C). However, *C. botulinum* spores cannot be removed or inactivated without changing the product features. The viability of *C. botulinum* spores in honey is directly related to the storage temperature. The *C. botulinum* spore count remained unchanged for 1 year at 3.9 °C and 100 days at 25 °C (Nakano *et al.*, 1989).

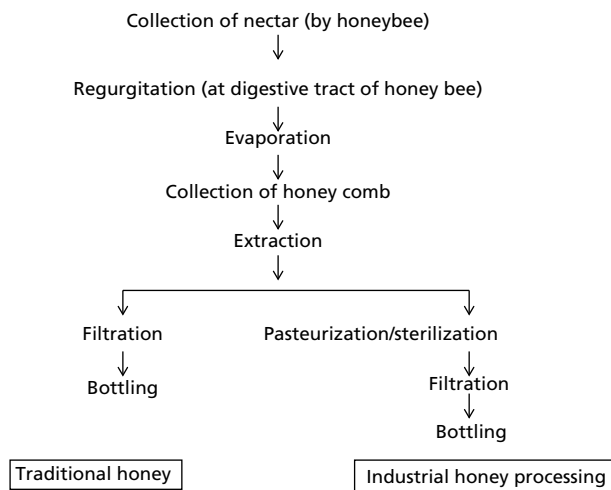


Figure 27.1 Schematic diagram of the honey manufacturing process.

27.5 Sugar

Sugar ($a_w \approx 0.45$) is commonly derived from sugar cane or sugar beet. The sugar processing from sugar cane comprises various steps such as washing, crushing, extraction, clarification, evaporation, heating (80–100°C), crystallization, centrifugation, drying, and refining. On the other hand, sugar processing from sugar beet includes storage of beets for days or months at 1.5–5°C, fluming, washing, slicing, extraction, heating (75–90°C), clarification, evaporation, filtration, crystallization, and refining (Thompson, 2009). The microbiological contamination may originate from primary processing steps, especially from soil, insects, workers, and tools, or from the processing environment. The pre-harvest microflora is comprised of *Flavobacterium*, *Lactobacillus*, *Xanthomonas*, *Arthrobacter*, *Streptomyces*, *Enterobacter*, *Pseudomonas*, *Erwinia*, *Leuconostoc*, *Corynebacterium*, spore-forming bacteria, yeasts, and molds (Bungee *et al.*, 1975; Wojtczak *et al.*, 2012). During the extraction stage, the sugar content (10–18% RDS) along with other nutrients and pH (5–5.6) of cane juice favor growth of various microorganisms. The number of bacteria in the juice ranges from 10^5 to 10^7 cfu/ml. *L. mesenteroides* and *L. dextrans* produce acids, dextran, and mucus that may lead to sugar loss up to 1.5% (Wojtczak

et al., 2012). Along with bacteria, fungi (*Penicillium* and *Aspergillus*) are capable of inverting the juice by excreting invertase enzymes. During the next processing steps, the product is subjected to successive heat treatments at 70–100°C for minutes or even hours to obtain refined sugar (Andrade and Castro, 2006). Most of the vegetative cells are destroyed, except mesophilic and thermophilic spore-forming, including *B. stearothermophilus*, *B. coagulans*, *C. thermosaccharolyticum*, and *Desulfotomaculum nigrificans* (ICMSF, 2005). These bacteria cause serious spoilage problems in the food industry, where sugar is used as an ingredient. Additionally, lactic acid bacteria such as *Leuconostoc* and *Lactobacillus* (Belamri *et al.*, 1993) can be introduced in the product by cross-contamination after thermal processing. These microorganisms have been associated with slime formation in sugar juice (Perguin, 1940). *Torulopsis*, *Zygosaccharomyces*, and *Hansenula* are yeasts often isolated in refined sugar and may cause some problems in products with high moisture (Thompson, 2009).

27.6 Syrups

Sugar syrups are classified in liquid sugar, starch-based syrup, and tree sap (maple tree – *Acer saccharum*). Each syrup has a different

processing history with individual unit operations. Liquid sugar is obtained by mixing refined sugar and water, followed by heat treatment at 120–130 °C. Glucose syrup is a concentrated aqueous solution obtained from acid or enzymatic hydrolysis of starch or inulin (ICMSE, 2011). Its content must be at least 20% dextrose equivalents and 70% total solids (Codex Alimentarius, 1994). Maple sap is composed of sucrose, glucose, fructose, organic acids, minerals, and nitrogenous and phenolic compounds (Lagacé *et al.*, 2004). It is collected from a maple tree through the tapping method. The likely sources of contamination are raw materials and the processing environment. *Pseudomonas*, *Aerobacter*, *Leuconostoc*, and *Bacillus* have been isolated from maple syrup (Morselli and Feldheim 1988). Among these, *Pseudomonas geniculata* is the predominant species. All these microorganisms grow as sap drips into the tube, bucket, or bag. These microbes involve in inversion process where sucrose breaks down to fructose and glucose (Lagacé *et al.*, 2004). Evaporated syrup is again heated at 85–87 °C to facilitate clarification and handling. Clarification combined with precipitation and filtration can further reduce the microbial load of the syrup. Although a major proportion of the microbial population is killed during syrup processing and end product characteristics (66–76° Brix, 0.70–0.88 a_w) do not support the growth of most microorganisms, syrups are less microbiologically stable than powder or granulated sugar. Variations in a_w during processing and storage contribute to microbial growth (Thompson, 2009). The most prevalent spoilage microorganisms in syrups are osmophilic yeast (*Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, and *Saccharomyces mellis*) and molds (*Penicillium* and *Aspergillus*) (Pitt and Hocking, 2009; ICMSE, 2011). To date there is no literature report about a food-borne outbreak associated with consumption of syrups. Nevertheless, *Clostridium botulinum* has already been isolated from corn syrup (ICMSE, 2005).

27.7 Conclusion

This chapter focuses on the effects of unit operations on microbiota of low moisture foods such as cocoa, chocolate, nuts, honey, sugars, and syrups. A high sugar content and low water activity of these products do not favor the proliferation of microorganisms, but heat-resistant bacteria can survive heat treatment applied to them. In fact, post-processing recontamination is the major contributor to food-borne outbreaks. Insufficient hygiene, contaminated ingredients and equipment, inadequate storage conditions, environment, and personnel may all contribute to the risk of pathogens in the final product. Therefore, implementation and adherence to good agricultural, manufacture, and hygiene practices (GAP, GMP, and GHP), including storage conditions (temperature and humidity) must be considered as priority measures in the low moisture food industry.

References

- Afoakwa, E.O., Paterson, A., Fowler, M., and Ryan, A. 2008. Flavor formation and character in cocoa and chocolate: a critical review. *Critical Reviews of Food Science Nutrition*, **48**, 840–857.
- Amezqueta, S., González-Peñas, E., Murilo, M., and de Cerain, A.L. 2005. Occurrence of ochratoxin A in cocoa beans: effect of shelling. *Food Additives and Contaminants*, **22**, 590–595.
- Andrade, S.A.C. and Castro O.S.B. 2006. *Engenharia e tecnologia açucareira*. Departamento de Engenharia Química CTG–UFPE, Pernambuco.
- Ardhana, M.M. and Fleet, G.H. 2003. The microbial ecology of cocoa bean fermentations in Indonesia. *International Journal of Food Microbiology*, **86**, 87–99.
- Arnon, S.S., Midura, T.F., Clay, S.A., Wood, R.M., and Chin, J. 1977. Infant botulism: epidemiological, clinical and laboratory aspects. *The Journal of the American Medical Association*, **237**, 1946–1951.
- Arnon, S.S., Midura, T.F., Damus, K., Thompson, B., Wood, R.M., and Chin, J. 1979. Honey and

- other environmental risk factors for infant botulism. *Journal of Pediatrics*, **94**, 331–336.
- Bansal, A., Jones, T.M., Adb, S.J., Danyluk, M.D., and Harris, L.J. 2010. Most-probable-number determination of *Salmonella* levels in naturally contaminated raw almonds using two sample preparation methods. *Journal of Food Protection*, **73**, 1986–1992.
- Barrile, J.C. and Cone, J.F. 1970. Effect of added moisture on the heat resistance of *Salmonella* Anatum in milk chocolate. *Applied Microbiology*, **19**, 177–178.
- Barrile, J.C., Ostovar, K., and Keeney, P.G. 1971. Microflora of cocoa beans before and after roasting at 150°C. *Journal of Milk and Food Technology*, **34**, 369–371.
- Beckett, S.T. 2008. In: *The Science of Chocolate*, 2nd edn. The Royal Society of Chemistry, Cambridge, UK.
- Beckett, S.T. (2009) In: *Industrial Chocolate Manufacture and Use*, 4th edn. Wiley-Blackwell, London, UK.
- Belamri, M., Douiri, K., Fakhereddine, L., and Tantaoui-Elaraki, A. 1993. Preliminary study on the saccharolytic activity of thermophilic bacteria from extraction beet juice. *International Sugar Journal*, **95**, 7–22.
- Bernardi, A.O., da Silva, D.H.L., da Silva, I.C.R.L.C.N., Duarte, E.M., and Nascimento, M.S. 2014. Pesquisa de *Salmonella* e outras enterobactérias nas etapas de pós-colheita do amendoim [Online]. Available at: <http://www.sbmicrobiologia.org.br/cdmicroal2014/resumos/R0372-1.html> (20 May 2015).
- Beuchat, L.R. and Mann, D.A. 2010. Factors affecting infiltration and survival of *Salmonella* on in-shell pecans and pecan nutmeats. *Journal Food Protection*, **73**, 1257–1268.
- Beuchat, L.R. and Mann, D.A. 2011. Inactivation of *Salmonella* on in-shell pecans during conditioning treatments preceding cracking and shell-ing. *Journal of Food Protection*, **74**, 588–602.
- Bhat, R., Rai, R.V., and Karim, A.A. 2010. Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 57–81.
- Blessington, T., Mitcham, E.J., and Harris, L.J. 2012. Survival of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on inoculated walnut kernels during storage. *Journal of Food Protection*, **75**, 245–254.
- Bonvehí, S.J. 2004. Occurrence of ochratoxin A in cocoa products and chocolate. *Journal of Agricultural and Food Chemistry*, **52**, 6347–6352.
- Brera, C., Debegriach, F., De Santis, B., Iafrate, E., Pannunzi, E., Berdini, C., Prantera, E., Gregori, E., and Miraglia, M. 2011. Ochratoxin A in cocoa and chocolate products from the Italian market: occurrence and exposure assessment. *Food Control*, **22**, 1663–1667.
- Bungee, W.M., Cole, D.E., and Nielsen, G. 1975. Microflora and invert sugars in juice from healthy tissue of stored sugar beets. *Applied Microbiology*, **29**, 780–781.
- Burnett, S.L., Gehm, E.R., Weissinger, W.R., and Beuchat, L.R. 2000. Survival of *Salmonella* in peanut butter and peanut butter spread. *Journal of Applied Microbiology*, **89**, 472–477.
- Calhoun, S., Post, L., Warren, B., Thompson, S., and Bontempo, A.R. 2013. Prevalence and concentration of *Salmonella* on raw shelled peanuts in the United States. *Journal of Food Protection*, **76**, 575–579.
- CDC (Centers for Disease Control and Prevention). 2007. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter – United States, 2006–2007. *Morbidity Mortality Weekly Report*, **56**, 521–524.
- CDC (Centers for Disease Control and Prevention). 2009. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products – United States, 2008–2009. *MMWR Morbidity and Mortality Weekly Report*, **58**, 85–90.
- CDC (Centers for Disease Control and Prevention). 2012. Investigative update: multistate outbreak of *Salmonella* Bredeney infections linked to peanut butter manufactured by Sunland, Inc (Final Update) [Online]. Available at: <http://www.cdc.gov/salmonella/bredeney-09-12/> (20 March 2015).

- CDR-HPA (Health Protection Agency, UK). 2006. National increase in human *Salmonella* Montevideo infections in England and Wales: March to June. *CDR: The Communicable Disease Report, Weekly*, **16**, 1–2.
- Chang, A.S., Sreedharan, A., and Schneider, K.R. 2013. Peanut and peanut products: a food safety perspective. *Food Control*, **32**, 296–303.
- Cheraghali, A.M., Yazdanpanah, H., Doraki, N., Abouhossain, G., *et al.* 2007. Incidence of aflatoxins in Iran pistachio nuts. *Food and Chemical Toxicology*, **45**, 812–816.
- Chun, H.S., Kim, H.J., Ok, H.E., Hwang, J., and Chung, D. 2007. Determination of aflatoxin levels in nuts and their products consumed in South Korea. *Food Chemistry*, **102**, 385–391.
- Codex Alimentarius. 1972. *Recommended international code of hygienic practice for tree nuts*, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Rome.
- Codex Alimentarius. 1994. *Sugars, cocoa products and chocolate and miscellaneous products*, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Rome.
- Codex Alimentarius. 2001. *Revised Codex Standard for Honey, Standards and Standard Methods*, Joint FAO/WHO Food Standards Program, Codex Alimentarius Commission, Rome.
- Codex Alimentarius. 2004. *CAC/RCP 55-2004, Code of practice for the prevention and reduction of aflatoxin contamination in peanuts*, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Rome.
- Codex Alimentarius. 2010. *CAC/RCP 59-2005, Code of practice for the prevention and reduction of aflatoxin contamination in tree nuts*, Joint FAO/WHO Food Standards Program, Codex Alimentarius Commission, Rome.
- Copetti, M.V., Pereira, J.L., Iamanaka, B.T., Pitt, J.I., and Taniwaki, M.H. 2010. Ochratoxigenic fungi and ochratoxin A in cocoa during farm processing. *International Journal of Food Microbiology*, **143**, 67–70.
- Copetti, M.V., Iamanaka, B.T., Pereira, J.L., Frisvad, J.C., and Taniwaki, M.H. 2011. Mycobiota of cocoa: from farm to chocolate. *Food Microbiology*, **28**, 1499–1504.
- Copetti, M.V., Iamanaka, B.T., Pereira, J.L., Lemes, D.P., Nakano, F., and Taniwaki, M.H. 2012. Co-occurrence of ochratoxin A and aflatoxins in chocolate marketed in Brazil. *Food Control*, **26**, 36–41.
- Copetti, M.V., Iamanaka, B.T., Nester, M.A., Efraim, P., and Taniwaki, M.H. 2013. Occurrence of ochratoxin A in cocoa by-products and determination of its reduction during chocolate manufacture. *Food Chemistry*, **136**, 100–104.
- Cordier, J.L. 1994. HACCP in the chocolate industry. *Food Control*, **5**, 171–175.
- Costa, A.K.F., Freire, F.C.O., Vieira, I.G.P., Andrade, J.A., and Mendes, F.N.P. 2009. Fungos associados à castanha-do-Brasil (*Bertholletia excelsa* Humb. and Bompl) e ao amendoim (*Arachis hypogaea* L.) comercializados em Fortaleza (Ceará). *Revista Ciência Agronômica*, **40**, 455–460.
- Craven, P.C., Mackel, D.C., Baine, W.B., Barker, W.H., and Gangarosa, E.J. 1975. International outbreak of *Salmonella eastbourne* infection traced to contaminated chocolate. *The Lancet*, **1**, 788–792.
- Danyluk, M.D., Jones, T.M., Abd, S.J., Schlitt-Dittrich, F., Jacobs, M., and Harris, L.J. 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. *Journal of Food Protection*, **70**, 820–827.
- D'Aoust, J.Y. 1977. *Salmonella* and the chocolate industry. A review. *Journal of Food Protection*, **40**, 718–727.
- Deering, A.J., Pruitt, R.E., Mauer, L.J., and Reuhs, B.L. 2012. Examination of the internalization of *Salmonella* serovar Typhimurium in peanut, arachis hypogaea, using immunocytochemical techniques. *Food Research International*, **45**, 1037–1043.
- El Tawila, M.M., Neamatallah, A., and Serdar, S.A. 2013. Incidence of aflatoxins in commercial nuts in the holy city of Mekkah. *Food Control*, **29**, 121–124.
- FDA (Food and Drug Administration). 2009. *Peanut Corporation of America (PCA), Blakely, GA: FDA form 483 (inspectional observations) January 9–27, 2008* [Online]. Available at: <http://www.fda.gov/ora/frequent/default.html>. (4 February 2015).

- Freire, F.C.O., Kozakiewicz, Z., and Paterson, R.R. 2000. Mycoflora and mycotoxins in Brazilian black pepper, white pepper and Brazil nuts. *Mycopathologia*, **149**, 13–19.
- Freitas-Silva, O. and Venâncio, A. 2011. Brazil nuts: benefits and risks associated with contamination by fungi and mycotoxins. *Food Research International*, **44**, 1434–1440.
- Gilmour, M. and Lindblom, M. 2008. Management of ochratoxin A in the cocoa supply chain: a summary of work by the CAOBISCO/ECA/FCC working group on ochratoxin A. In: *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade* (eds Leslie, J.F., Bandyopadhyay, R., and Visconti, A.), pp. 231–243. CABI, Wallingford.
- Gonçalves, E., Nogueira, J.H., Fonseca, H., Felicio, J.D., Pino, F.A., and Corrêa, B. 2008. Mycobiota and mycotoxins in Brazilian peanut kernels from sowing to harvest. *International Journal of Food Microbiology*, **123**, 184–190.
- He, Y., Guo, D., Yang, J., Tortorello, M.L., and Zhang, W. 2011. Survival and heat resistance of *Salmonella enterica* and *Escherichia coli* O157:H7 in peanut butter. *Applied Environmental Microbiology*, **77**, 8434–8438.
- Hepsag, F., Golge, O., and Kabak, B. 2014. Quantitation of aflatoxins in pistachios and groundnuts using HPLC-FLD method. *Food Control*, **38**, 75–81.
- Hockin, J.C., D'Aoust, J.J., Bowering, D., Jessop, J.H., Khama, B., Liar, H., and Milling, M.E. 1989. An international outbreak of *Salmonella nima* from imported chocolate. *Journal of Food Protection*, **52**, 51–54.
- Hocking, A. 1988. Moulds and yeasts associated with foods of reduced water activity: ecological considerations. In: *Food Preservation by Moisture Control* (ed. Seow, C.C.), pp. 57–71. Elsevier Applied Science Publishers, Barking, UK.
- Horn, B.W., Greene, R.L., Sorensen, R.B., Blankenship, P.D., and Dorner, J.W. 2000. Conidial movement of nontoxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in peanut fields following application to soil. *Mycopathologia*, **151**, 81–92.
- Iamanaka, B.T., Nakano, F., Lemes, D.P., Ferranti, L.S., and Taniwaki, M.H. 2014. Aflatoxin evaluation in ready-to-eat Brazil nuts using reversed-phase liquid chromatography and post-column derivatisation. *Food Additives and Contaminants*, **31**, 917–923.
- ICMSF (International Commission on Microbiological Specifications for Foods). 1996. Toxigenic fungi: *Aspergillus*. In: *Microorganisms in Foods, 5: Microbiological Specifications of Food Pathogens* (eds Roberts, T.A., et al.), pp. 347–381. Chapman & Hall, London, UK.
- ICMSF (International Commission on Microbiological Specifications for Foods). 2005. Cocoa, chocolate, and confectionery. In: *Microorganisms in Foods, 6: Microbiological Ecology of Food Commodities*, 2nd edn, pp. 467–479. Kluwer Academic/Plenum Publishers, New York, USA.
- ICMSF (International Commission on Microbiological Specifications for Foods). 2011. Cocoa, chocolate and confectionery. In: *Microorganisms in Foods, 8: Use of Data for Assessing Process Control and Product Acceptance*, pp. 241–246. Springer, New York, USA.
- Isaacs, S., Aramini, J., Ciebin, B., Farrar, J.A., et al. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *Journal of Food Protection*, **68**, 191–198.
- Kataoka, A., Enache, E., Black, D.G., Elliott, P.H., Napier, C.D., Podolak, R., and Hayman, M.M. 2014. Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and *Enterococcus faecium* in peanut paste formulations at two different levels of water activity and fat. *Journal of Food Protection*, **77**, 1252–1259.
- Komitopoulou, E. and Peñaloza, W. 2009. Fate of *Salmonella* in dry confectionery raw materials. *Journal of Applied Microbiology*, **106**, 1892–1900.
- Krapf, T. and Gantenbein-Demarchi, C. 2010. Thermal inactivation of *Salmonella* spp. during conching. *LWT – Food Science and Technology*, **43**, 720–723.
- Kumagai, S., Nakajima, M., Tabata, S., Ishikuro, E., et al. 2008. Aflatoxin and ochratoxin A contamination of retail food and intake of these mycotoxins in Japan. *Food Additives and Contaminants*, **25**, 1101–1106.

- Kuplulu, O., Goncuoglu, M., Ozdemir, H., and Koluman, A. 2006. Incidence of *Clostridium botulinum* spores in honey in Turkey. *Food Control*, **17**, 222–224.
- Lagacé, L., Pitre, M., Jacques, M., and Roy, D. 2004. Identification of the bacterial community of maple sap by using amplified ribosomal DNA (rDNA) restriction analysis and rDNA sequencing. *Applied Environmental Microbiology*, **70**, 2052–2060.
- Leong, Y.H., Ismail, N., Latif, A.A., and Ahmad, R. 2010. Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. *Food Control*, **21**, 334–338.
- Little, C.L., Rawal, N., de Pinna, E., and McLauchlin, J. 2010. Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. *Food Microbiology*, **27**, 171–174.
- Luttfullah, G. and Hussain, A. 2011. Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. *Food Control*, **22**, 426–429.
- Ma, L., Zhang, G., Gerner-Smidt, P., Mantripragada, V., Ezeoke, I., Doyle, M.P. 2009. Thermal inactivation of *Salmonella* in peanut butter. *Journal of Food Protection*, **72**, 1596–1601.
- Manda, P., Dano, D.S., Kouadio, J.H., Diakité, A., et al. 2009. Impact of industrial treatments on ochratoxin A content in artificially contaminated cocoa beans. *Food Additives and Contaminants*, **26**, 1081–1088.
- Midura, T.F. and Arnon, S.S. 1976. Infant botulism. Identification of *Clostridium botulinum* and its toxins in faeces. *Lancet*, **2**, 934–936.
- Miksch, R., Leek, J., Myoda, S., Nguyen, T., Tenney, K., Svidenko, V., Greeson, K., and Samadpour, M. 2013. Prevalence and counts of *Salmonella* and enterohemorrhagic *Escherichia coli* in raw, shelled runner peanuts. *Journal of Food Protection*, **76**, 1668–1675.
- Milhome, M.A.L., Lima, C.G., Lima, L.K., Lima, F.A.F., Sousa, D.Q.B., and Nascimento, R.F. 2014. Occurrence of aflatoxins in cashew nuts produced in northeastern Brazil. *Food Control*, **42**, 34–37.
- Morselli, M.F. and Feldheim, W. 1988. Ahornsirup – eine Uebersicht (Maple syrup – a review). *Zeitschrift fuer Lebensmitt. Untersuchung und Forschung*, **186**, 6–10.
- Mounjouenpou, P., Gueule, D., Fontana-Tachon, A., Guyot, B., Tondje, P.R., and Guiraud, J. 2008. Filamentous fungi producing ochratoxin A during cocoa processing in Cameroon. *International Journal of Food Microbiology*, **128**, 234–241.
- Nakano, H. and Sakaguchi, G. 1991. An unusually heavy contamination of honey products by *Clostridium botulinum* type F and *Bacillus alvei*. *FEMS Microbiology Letters*, **63**, 171–177.
- Nakano, H., Okabe, T., Hashimoto, H., and Sakaguchi, G. 1989. Incidence of *Clostridium botulinum* in honey of various origins. *Japanese Journal of Medical Science and Biology*, **43**, 183–195.
- Nascimento, M.S., Silva, N., Silva, I.F., Silva, J.C., Marques, É.R., and Santos, A.R.B. 2010. Enteropathogens in cocoa pre-processing. *Food Control*, **21**, 408–411.
- Nascimento, M.S., Brum, D.M., Pena, P.O., Berto, M.I., and Efraim, P. 2012. Inactivation of *Salmonella* during cocoa roasting and chocolate conching. *International Journal of Food Microbiology*, **159**, 225–229.
- Nascimento, M.S., Pena, P.O., Brum, D.M., Imazaki, F.T., Tucci, M.L., and Efraim, P. 2013. Behavior of *Salmonella* during fermentation, drying and storage of cocoa beans. *International Journal of Food Microbiology*, **167**, 363–368.
- Nevas, M., Lindstrom, M., Hautamaki, K., Puoskari, S., and Korkeala, H. 2005. Prevalence and diversity of *Clostridium botulinum* types A, B, E and F in honey produced in the Nordic countries. *International Journal of Food Microbiology*, **105**, 145–151.
- Nielsen, D.S., Teniola, O.D., Ban-koffi, L., Owusu, M., Andersson, T.S., and Holzapfel, W.H. 2007. The microbiology of Ghanaian cocoa fermentations analysed using culture-dependent and culture-independent methods. *International Journal of Food Microbiology*, **114**, 168–186.
- Nummer, B.A., Shrestha, S., and Smith, J.V. 2012. Survival of *Salmonella* in a high sugar, low water- activity, peanut butter flavored candy fondant. *Food Control*, **27**, 184–187.

- Ostovar, K. and Keeney, P.G. 1973. Isolation and characterization of microorganisms involved in the fermentation of Trinidad's cacao beans. *Journal of Food Science*, **38**, 611–617.
- Papalexandratou, Z.C.N., Camu, N., Falony, G., and De Vuyst, L. 2011. Comparison of the bacterial species diversity of spontaneous cocoa bean fermentations carried out at selected farms in Ivory Coast and Brazil. *Food Microbiology*, **28**, 964–973.
- Perquin, L.H.C. 1940. On the incidental occurrence of rod-shaped, dextran producing bacteria in a beet-sugar factory. *Antonio van Leeuwenhoek and the Journal of Microbiology and Serology*, **6**, 227–229.
- Pinkas, J.M., Battista, K., and Morille-Hinds, T. 2009. Microbiological spoilage of spices, nuts, cocoa and coffee. In: *Compendium of the Microbiological Spoilage of Foods and Beverages* (eds Sperber, W.H. and Doyle, M.P.), pp. 325–350. Springer, New York, USA.
- Pitt, J.I. and Hocking, A.D. 1985. *Fungi and Food Spoilage*. Academic Press, Sydney, NSW, Australia.
- Pitt, J.I. and Hocking, A.D. 2009. *Fungi and Food Spoilage*, 3rd edn. Springer, New York, USA.
- Podolak, R., Enache, E., Stone, W., Black, D.G., and Elliott, P.H. 2010. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection*, **73**, 1919–1936.
- Rall, V.L.M., Bombo, A.J., Lopes, T.F., Carvalho, L.R., and Silva, M.G. 2003. Honey consumption in the state of São Paulo: a risk to human health? *Anaerobe*, **9**, 299–303.
- Reis, T.A., Oliveira, T.D., Baquião, A.C., Gonçalves, S.S., Zorzete, P., and Corrêa, B. 2012. Mycobiota and mycotoxins in Brazil nut samples from different states of the Brazilian Amazon region. *International Journal of Food Microbiology*, **159**, 61–68.
- Ruiz-Argüeso, T. and Rodríguez-Navarro, A. 1975. Microbiology of ripening honey. *Journal of Applied Microbiology*, **30**, 893–896.
- Saleemullah, A.I., Khalil, I.A., and Shah, H. 2006. Aflatoxin contents of stored and artificially inoculated cereals and nuts. *Food Chemistry*, **98**, 699–703.
- Schatzki, T.F. and Ong, M.S. 2001. Dependence of aflatoxin in almonds on the type and amount of insect damage. *Journal of Agricultural and Food Chemistry*, **49**, 4513–4519.
- Schwan, R.F. and Wheals, A.E. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Food Science and Nutrition*, **44**, 205–221.
- Schwan, R.F., Rose, A.H. and Board, R.G. 1995. Microbial fermentation of cocoa beans, with emphasis on enzymatic degradation of the pulp. *Journal of Applied Bacteriology — Symposium Supplement*, **79**, 96–107.
- Scott, P.M. 1998. Industrial and farm detoxification processes for mycotoxins. *Revue de Médecine Vétérinaire*, **149**, 543–548.
- Scott, V.N., Chen, Y.U.H., Freier, T.A., Kuehm, J., et al. 2009. Control of *Salmonella* in low moisture foods. I: minimizing entry of *Salmonella* into a processing facility. *Journal of Food Protection*, **29**, 342–353.
- Shachar D. and Yaron S. 2006. Heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter. *Journal of Food Protection*, **69**, 2687–2691.
- Silva, F.V.M. and Gibbs, P.A. 2012. Thermal pasteurization requirements for the thermal inactivation of *Salmonella* in foods. *Food Research International*, **45**, 695–699.
- Sirsat, S.A., Burkholder, K.M., Muthaiyan, A., Dowd, S.E., Bhunia, A.K., and Ricke, S.C. 2011. Effect of sublethal heat stress on *Salmonella* Typhimurium virulence. *Journal of Applied Microbiology*, **110**, 813–822.
- Smedt, J.M., Chartron, S., Cordier, J.L., Graff, E., et al. 1991. Collaborative study of the International Office of Cocoa, Chocolate and Sugar Confectionery on *Salmonella* detection from cocoa and chocolate processing environmental samples. *International Journal of Food Microbiology*, **13**, 301–308.
- Snowdon, J.A. and Cliver, D.O. 1996. Microorganisms in honey. *International Journal of Food Microbiology*, **31**, 1–26.
- Tamminga, S.K., Beumer, R.R., Kampelmacher, E.H., and Leusden, F.M.V. 1976. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in chocolate. *Journal of Hygiene*, **76**, 41–47.

- Thompson, S.S., Miller, K.B., and Lopez, A.S. 2013. Cocoa and coffee. In: *Food Microbiology: Fundamentals and Frontiers* (eds Doyle, M.P. and Buchanan, R.L.), 2nd edn, pp. 881–900. ASM Press, Washington, DC, USA.
- Thompson, S. 2009. Microbiological spoilage of high-sugar products. In: *Compendium of the Microbiological Spoilage of Foods and Beverages* (eds Sperber, W.H. and Doyle, M.P.), pp. 301–324. Springer, New York, USA.
- Tokuoka, K., Ishitani, T., Goto, S., and Komagata, K. 1985. Identification of yeasts isolated from high-sugar foods. *Journal of General and Applied Microbiology*, **31**, 411–427.
- Torres-Vitela, R., Escartin, E.F., and Castillo, A. 1995. Risk of Salmonellosis associated with consumption of chocolate in Mexico. *Journal of Food Protection*, **58**, 478–481.
- Uesugi, A.R., Danyluk, M.D., and Harris, L.J. 2006. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at –20, 4, 23, and 35 °C. *Journal of Food Protection*, **69**, 1851–1857.
- Werber, D., Dreesman, J., Feil, F., Treeck, U.V., et al. 2005. International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases*, **5**, 7–17.
- Wojtczak, M., Biernasiak, J., and Papiewska, A. 2012. Evaluation of microbiological purity of raw and refined white cane sugar. *Food Control*, **25**, 136–139.
- Woodroof, J.G. 1983. In: *Peanuts: Production, Processing, Products*, 3rd edn. AVI Publishing Company, Inc., Westport, CT, USA.
- Yentur, G., Er, B., Ozkan, M.G., and Oktem, A.B. 2006. Determination of aflatoxins in peanut butter and sesame samples using high performance liquid chromatography method. *European Food Research Technology*, **224**, 167–170.
- Zorzete, P., Reis, T.A., Felício, J.D., Baquião, A.C., Makimoto, P., and Corrêa, B. 2011. Fungi, mycotoxins and phytoalexin in peanut varieties, during plant growth in the field. *Food Chemistry*, **129**, 957–964.

CHAPTER 28

Microbial ecology of wine

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28.1 Introduction

Wine quality depends on many factors, the microorganism's activity being one of the more important. A wide diversity of microorganisms, yeasts, and bacteria are involved in winemaking and, thus, determination of the composition and evolution of the different species present during this process would clearly help to increase the quality of a wine.

After confirmation of the role of microorganisms as responsible for the alcoholic fermentation by Pasteur in the nineteenth century, the predominant role of the genus *Saccharomyces*, mainly the species *S. cerevisiae* and *S. bayanus*, on the complete fermentation of the grape sugars became clear. However, nowadays it is known that the grape-wine ecological habitat has a much more complex microbial biodiversity.

Several highly specialized species of yeast and bacteria are active in different phases of the fermentation and contribute to the transformation of grape juice to wine. During winemaking, the biodiversity initially present on the grape surface and in the early stages of fermentation tends to decrease as the ethanol content, the main limiting factor during fermentation, increases. Generally, at the end of the fermentation, exclusively *S. cerevisiae* and *S. bayanus*, the best adapted species to high ethanol content in the medium, are found. Despite this, the contribution of the so-called non-*Saccharomyces* species present at the start of fermentation and their concentration variation until the final

dominance of *S. cerevisiae* can be crucial in determining the quality of the wine (Fleet, 2003; Jolly *et al.*, 2006).

Malolactic fermentation (MLF) is a biochemical transformation conducted by lactic acid bacteria (LAB), which usually takes place after alcoholic fermentation during winemaking, and is generally desirable in the production of red wines, as well as in some white wines. MLF produces a biological deacidification of wine by transforming malic acid in lactic acid, with a consequent increase in the pH; it also contributes to the microbiological stability of wine and leads to the production of many secondary compounds that induce changes in the organoleptic properties of the wine (Davis *et al.*, 1986; Lonvaud-Funel, 1999; Ugliano *et al.*, 2003). This process can be conducted by LAB belonging to the genera *Oenococcus*, *Lactobacillus*, and *Pediococcus* (Wibowo *et al.*, 1988); however, the main agent of MLF is *Oenococcus oeni*, because of its ability to grow in the particular conditions of wine, which are a high ethanol content, low pH, and the presence of SO₂ (Wibowo *et al.*, 1988; Davis *et al.*, 1988; Kunkee, 1991).

28.2 Biodiversity of grape microorganisms

Several studies over the past 10 years have shown that the biodiversity and the quantity of the microorganisms present on the surface of the grape berry is highly dependent on many

factors, including the health state of the grapes, the climate temperature, and the pesticide treatments. Recent works seems to confirm that the “terroir” idea should be extended to the micro-biological aspect, that is, that the geographical distribution of the organisms associated with grapes is not randomly dispersed but is dependent on the cultivar, the location of the vineyard, and the vintage (Bokulich *et al.*, 2014).

Recently, it has been reported that while yeast counts fluctuated between 10^2 and 10^5 in healthy grapes, it is two orders of magnitude higher in damaged berries (Barata *et al.*, 2012). The number and type of species of yeasts are strongly influenced by the grapes health, as it influences the access of microorganisms to the nutrients of the juice.

Three groups of yeast can be distinguished on the grape surfaces (Barata *et al.*, 2012):

- 1 An oligotrophic group with oxidative metabolism represented by *Basidiomycetes*, such as *Cryptococcus* spp. and *Rhodotorula* spp., which dominate the surface poor in nutrients of the sound berry.
- 2 A group of ascomycetes with an oxidative, or weakly fermentative, metabolism represented by *Metschnikowia pulcherrima* and some species of the genus *Candida*, *Hanseniaspora*, and *Pichia*, being *H. uvarum*, the yeast most frequently detected. These species increase their presence and become dominant during ripening.
- 3 A strongly fermentative group is detected on the surface of damaged berries, which, as commented above, used to have a greater number of yeast cells. Although basidiomycetes and ascomycetes oxidative yeasts are also present, usually, under this condition, the predominating species are strong fermenting yeasts, such as *Zygosaccharomyces* spp. and some species of *Candida* and *Torulaspora*. Interestingly, it has been described that some yeasts are frequently associated to fungal grape diseases. For instance, *M. pulcherrima* and *Candida zemplinina* are recurrently found in botrytized grapes and juices (Sipiczki, 2003, 2006).

S.cerevisiae, which predominates during alcoholic fermentation, is rarely found on the surface of the berry. This fact supports the view that the winery environment, and not the vineyard, represents the natural habitat of this yeast (Martini, 2003).

On the other hand, and since LAB are minor partners of the grape microbiota (the initial LAB population in wine grapes is low, around 10^2 cfu/g, Bae *et al.*, 2006), few studies have focused on the bacteria associated with grapes. For example, *Lactobacillus plantarum*, *L. casei*, *L. brevis*, *L. hilgardii*, *L. curvatus*, *L. buchneri*, *Leuconostoc dextranicum*, and *Leuconostoc mesenteroides* were inconsistently isolated from several grape varieties harvested from vineyards in Spain (Sieiro *et al.*, 1990; Suárez *et al.*, 1994), France (Lafon-Lafourcade *et al.*, 1983), and Germany (Weiller and Radler, 1970). Moreover, most studies have failed to detect *O. oeni* in grapes or vineyards (Bae *et al.*, 2006; Lafon-Lafourcade *et al.*, 1983; Renouf *et al.*, 2007), despite its frequent isolation from winery environments after fermentation (Edwards *et al.*, 1991; Garijo *et al.*, 2009).

One of the few common enological bacteria detected on grape skins is *Gluconobacter oxydans* (Renouf *et al.*, 2007), an acetic acid bacteria (AAB), an important group of bacteria in the food and beverage industry, which can oxidize ethanol to acetic acid. AAB are ubiquitous and have also been found on grapes (Bartowsky, 2008; Bartowsky and Henschke, 2008; González *et al.*, 2005; Valera *et al.*, 2011). Up to 10^6 cell/g of AAB, mainly *Acetobacter*, can be found in damaged grapes (Joyeux *et al.*, 1984).

28.3 Microorganism ecology in winemaking

The microbial composition of grape must after crushing reflect the composition on the berry surface at harvest. Later, the availability of nutrients and the high concentration of sugar promote the growth of fermentative species, while the others tend to succumb or be inactivated.

Numerous variables define the ecological mosaic of fermentation and several works have analyzed the microbial development under different fermentation conditions (Fleet, 1993; Torija *et al.*, 2001; Beltran *et al.*, 2002; Van Keulen *et al.*, 2003; Di Maro *et al.*, 2007; Bezerra-Bussoli *et al.*, 2013).

28.3.1 Yeast ecology

Taking into account a spontaneous fermentation of grapes, that is, without the massive inoculation of selected strains of *S. cerevisiae* that upsets the natural ecology in winemaking (whose advantages and disadvantages are currently subject of discussion), the following ecological phases can be outlined:

Phase 1: Crushing. The microbial composition in crushed, and eventually in pressed grapes, reflects that of the grapes they come from. Among these species, those able to grow in the grape must be able to do so because of their fermentative metabolism and are relatively few in number; the most frequently found are *M. pulcherrima*, some *Candida* species, including *C. stellata*, and *H. uvarum*. *S. cerevisiae* is usually present at very low concentrations in the must.

Phase 2: Initial phase of alcoholic fermentation. In this step the growth of non-*Saccharomyces* species is favored by their initial concentration and fermentative metabolism, and their persistence is dependent on the ethanol resistance of single species. For instances, *M. pulcherrima* succumbs above 2–3% ethanol, while *Candida* and, especially, *K. apiculata* can survive at up to 6–8%. During this stage *S. cerevisiae* grows quickly until it gradually becomes the dominant species.

Phase 3: Prevalence of *Saccharomyces*. Although some species such as *C. stellata* can resist high concentrations of ethanol, the exponential growth of *S. cerevisiae* tends to dominate the fermentation, being usually the only species detected by the end of the process. The concentration of *S. cerevisiae* can reach in the order of 10^8 in 5–6 generations, and it can complete the fermentation of grape

musts under sugar concentrations greater than 250 g/l. Even though, at this stage, a great biodiversity of *S. cerevisiae* species are present (Valero *et al.*, 2007; Schuller *et al.*, 2005), the fermentation is mainly conducted by a small number of strains. (Versavaud *et al.*, 1995).

Numerous factors can affect both the number and the charge of the species present during the winemaking process, especially among the non-*Saccharomyces* yeasts.

The harvesting system, manual or mechanical, can determine major or minor damage to the berries and modify the composition of the initial microbial load in the must, especially if the time interval between harvesting and crushing is delayed (Boulton *et al.*, 1996). Some antifungals used against *Botrytis* seem to favor the growth of *Metschnikowia pulcherrima* in must (Regueiro *et al.*, 1993). Cold settlement of must in the pre-fermentative step and low fermentation temperatures commonly represent growth advantage for non-*Saccharomyces*, such as *Candida* spp. and *Hanseniaspora uvarum*, while high temperatures promote *S. cerevisiae* growth (Hierro *et al.*, 2006).

During fermentation, ethanol concentration is the most prominent variable that determines the temporal sequence of yeast species predominance; only a few can grow above 10% v/v, such as some species of *Candida*, *Torulaspora*, *Zygosaccharomyces*, and *Schizosaccharomyces pombe*.

The sulfur dioxide content affects the amount of *Basidiomycetes* present after crushing, but has less influence on the fermentative yeasts, with *S. cerevisiae* usually being less sensitive than the non-*Saccharomyces* spp. (Rementeria *et al.*, 2003).

Another important factor is the period at which fermentation takes place. When conducted at the beginning of the harvest time the influence of the vineyard microflora is greater than at the end, since, as *S. cerevisiae* is strongly implanted in the winery (on tanks and equipment surfaces), it quickly overcomes the non-*Saccharomyces* species (Ribéreau-Gayon *et al.*, 2004).

28.3.2 LAB ecology

Grape must frequently contains bacterial species, 10^2 – 10^3 cfu/ml, of the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Oenococcus* (Costello *et al.*, 1983; Fleet *et al.*, 1984; Pardo and Zuniga, 1992; Fleet, 1993; Fugelsang and Edwards, 1997).

During the first days of alcoholic fermentation, LAB can increase to a maximum of 10^4 cfu/ml, and then decline due to the presence of SO_2 , the ethanol content, and the competition with yeasts (Fugelsang and Edwards, 1997). At the end of the alcoholic fermentation, the bacteria population increases again, up to the 10^6 cfu/ml necessary to start MLF.

A crucial factor in this phase is the pH, as it determines the species of LAB present in wine; usually *O. oeni* is the only one present in wines having pH below 3.5, while a higher pH promotes the growth of *Lactobacillus* and *Pediococcus* spp. (Davis *et al.*, 1986). In fact, a recent study by Juega *et al.* (2014) showed that bacteria isolated in Albariño and Caiño wines, with a pH of about 3.6, were *Pediococcus* spp., which successfully perform MLF on the wine without negative effects.

28.4 Microorganism ecology during aging

After alcoholic and malolactic fermentations, the reduction of nutrients and fermentable compounds, together with the racking, fining, and filtration operations and storage during the aging of wine, tend to drastically reduce the load of microorganisms in wine. After these two fermentations, with a few exceptions related to the particular typologies of some wines, any residual microorganism should be considered a contaminant and should ideally be absent. Even *S. cerevisiae* must be considered as a spoilage yeast, for example, in the elaboration of sweet dessert wines, due to its capability to ferment residual sugar and, thus, to alter the quality of the wine. Normally, in the case of dry wines with alcohol contents greater than 12–13%, few microorganisms are able to

survive and to be active. In the undesirable case of tanks not properly dried after being washed, some species of *Candida* spp. and *Pichia* spp. are able to form films that, if not eliminated, can produce high amounts of acetic acid and other substances with a negative sensorial impact. Yeast of the genera *Zygosaccharomyces*, particularly *Z. bailii* and the specie *Saccharomycodes ludwigii*, can cause refermentations in wines with residual sugar contents due to their resistance to high concentrations of ethanol and SO_2 (Loureiro and Malfeito-Ferreira, 2003).

The genus *Brettanomyces*/*Dekkera* is probably considered the most detrimental contaminant microorganism for the quality of red wine. Several studies conducted worldwide (Chatonnet *et al.*, 1995; Gerbaux *et al.*, 2000; Suárez *et al.*, 2005) have shown that the presence of this microorganism in the wine field is a stringent problem. Currently, the genus *Brettanomyces*, anamorph of *Dekkera*, includes five species, with *Brettanomyces bruxellensis* being the most common in wine (Henick-Kling *et al.*, 2000; Kurtzman *et al.*, 2011).

Brettanomyces were isolated in equipment, walls, floors, and oak barrels used in the winery. The contamination by this yeast is usually manifested after alcoholic and malolactic fermentations, mostly during aging in barrels (Chatonnet, 2000). These yeasts have the capability of growing under very wide ranges of temperature, acidity, sulfur dioxide, and ethanol (Gerós *et al.*, 2000; Silva *et al.*, 2004), frequently remaining in a latent state, ready to grow when the conditions become favorable and reaching concentrations of 10^4 – 10^5 cells/ml. From the sensorial point of view, *Brettanomyces* growth is detrimental for the wine's quality, due to the appearance of strong, unpleasant odors, described as horse sweat, band aid, or burnt plastic. These sensorial faults are mainly caused by volatile phenols produced by this yeast, starting from phenol precursors present in wine.

Moreover, poor management during bottling and storage of red wine can also give rise to spoilage by *Acetobacter pasteurianus* (Bartowsky

and Henschke, 2004, 2008), with the undesirable production of acetic acid. Du Toit *et al.* (2005) isolated AAB in tanks and barrels, thus showing that these bacteria can survive in quite low oxygen availability. Different authors (Waters *et al.*, 1996; Caloghiris *et al.*, 1997; Bartowsky and Henschke, 2008) suggested that the oxygen permeation of the natural cork can facilitate the formation of the neck ring deposit in the bottles.

In addition to AAB, some LAB can also cause spoilage in wine and give some faults to wine; for example, some species of *Pediococcus damnosus* and *Pediococcus pentosaceus* can produce exopolysaccharides, molecules implicated in ropiness (Lonvaud-Funel *et al.*, 1993; Fugelsang and Edwards, 1997), while some *Lactobacillus* can, for example, degrade the glycerol with the subsequent production of acrolein, implicated in the development of bitterness in wine.

28.5 Microbial identification by classical methods

The adoption of appropriate methods of microbial identification is essential to study the presence and evolution of the microbial species during the winemaking process. Classical identification techniques based on morphological, physiological, and biochemical essays (Barnett *et al.*, 1990; Kurtzmann *et al.*, 2011) have been largely overcome in the last two decades by molecular analysis based on the microorganism's genome. Major criticisms of the traditional methodologies are the time needed to get results and their reliability and reproducibility. In fact, phenotypical assessments based on morphological and biochemical traits are influenced by the physiological state of the cells.

However, some classical methods are still used, allowing the rapid determination of the total number of microorganisms in must and wine, and the rapid identification of some of them. Microscope observation, supported by

counting the chamber cells (e.g., the Bürker chamber) and methylene blue staining, gives rapid information on total cells, viability, and, in a few cases, allows identification of the genus. For example, genus *Hanseniaspora* and *Saccharomyces* (*Saccharomyces ludwigii*) can be easily identified by their characteristic lemon-shape morphology and distinguished by their size, with *Saccharomyces* being much larger than *Hanseniaspora* (8–10 and 15–20 µm, respectively). *Schizosaccharomyces* is also recognized due to their fission reproduction. However, overall, the other wine-related species have the anonymous ovoid shape with a budding reproduction and are indistinguishable from microscope observations. Figure 28.1 shows some species observed using the optical microscope.

Most of the bacteria grown in wine can be isolated by traditional microbiological techniques using nutrient agar media. The most common used medium for LAB is MRS (deMan Rogosa Sharpe broth), sometimes added with 20% tomato (or apple or grape) juice.

Information on the morphological and physiological characteristics and on the evolution of taxonomic and systematic information related to yeasts can be followed in the constantly updated editions of *The Yeast: A Taxonomic Study* (Kurtzman *et al.*, 2011) and for bacteria in *Bergey's Manual of Systematic Bacteriology* (Vos *et al.*, 2009).

The main characteristics of the genera (yeast and bacteria) found in wine are displayed in Table 28.1.

28.6 Microbial identification by molecular methods

In the last two decades, DNA-based diagnostic techniques have revolutionized the study of microorganisms, and new methods are continuously being developed for the molecular identification and characterization of yeast and bacteria (reviewed in Ruiz *et al.*, 2000; Pozo-Bayón *et al.*, 2009; Fernandez-Espinar *et al.*,

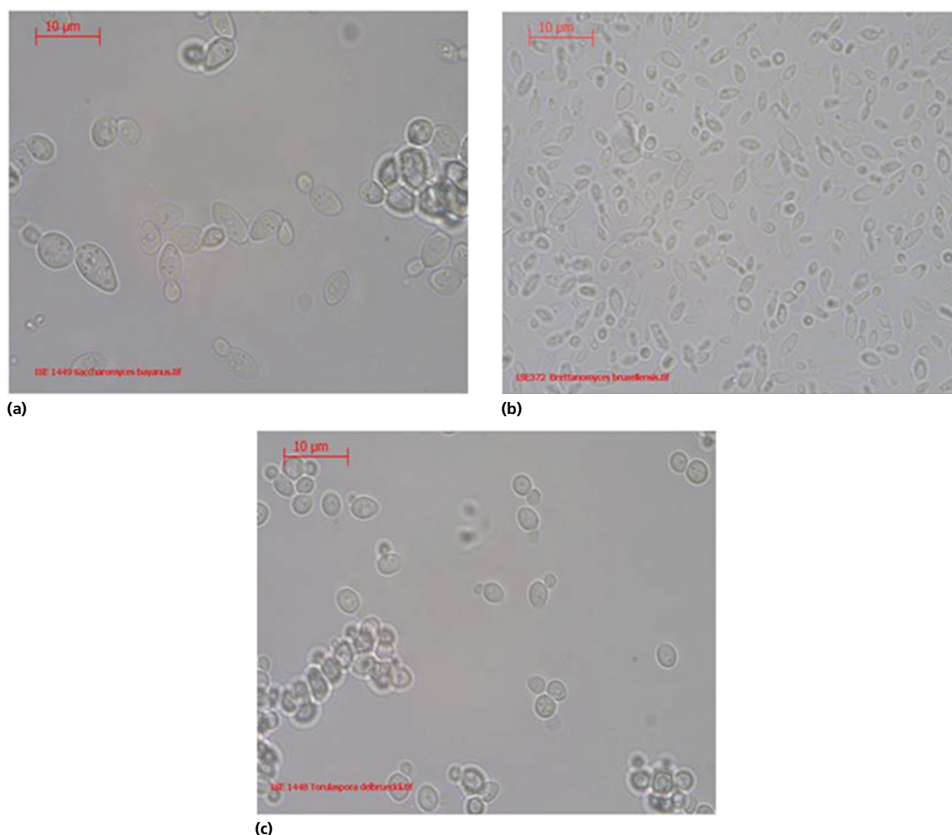


Figure 28.1 Yeast species observed under microscope: (a) *Saccharomyces bayanus*, (b) *Dekkera bruxellensis*, (c) *Torulaspora delbrueckii*.

2011; Ivey and Phister, 2011). Table 28.2 displays the main molecular identification techniques for microorganisms of wine.

Molecular methods can be direct or indirect. In the latter, the microorganisms have to be cultivated on agar media, before the species and their frequency can be subsequently determined by molecular analysis of DNA extracted from a random sample of the colonies grown. This allows an accurate determination at the species level and sometimes a subspecific characterization; however, it has the drawbacks of the time consumed and its incapability to detect viable but non-cultivable (VBNC) cells (Millet and Lonvaud-Funel, 2000).

In direct methods, molecular analysis is performed on the sample (must or wine) without

prior cultivation, reducing the time needed and allowing the detection of non-cultivable species. One drawback is the difficulties in differentiating viable from dead cells, as the target DNA (and in some case also RNA) persists after the death of the microorganisms (Hierro *et al.*, 2006).

Recently the metagenomic approach has been applied to the study of microbial communities in ecosystems, providing a great insight into the processes responsible for microbial diversity; for example, it has been shown that the microbial population is strongly related to climatic conditions, grape variety, and vineyard environmental conditions (Bokulich *et al.*, 2014). These authors concluded that there is a unique microbial pattern that influences the

Table 28.1 Main species and characteristics of must/wine related species: (A) yeasts; (B) bacteria.

(A) Yeasts				
Genus	Morphology		Cell size (µm)	Main species in wine
<i>Saccharomyces</i>	Spherical Elongated		5–10 × 5–12	<i>S. cerevisiae</i> <i>S. bayanus</i> <i>S. exiguus</i> <i>S. pastorianus</i> <i>S. paradoxus</i>
<i>Candida</i>	Ellipsoidal Elongated		2.2–3.0 × 3.0–5.2	<i>C. stellata</i> <i>C. vini</i> <i>C. vinaria</i> <i>C. membranifacies</i> <i>C. zemplinina</i>
<i>Debaryomyces</i>	Spherical Short oval		2–7 × 2.4–8.5	<i>D. hansenii</i>
<i>Hanseniaspora</i>	Lemon shape, elongated		1.5–5 × 2.5–11.5	<i>H. guilliermondii</i> <i>H. osmophila</i> <i>H. uvarum</i> <i>H. vineae</i>
<i>Kluyveromyces</i>	Ellipsoidal Spheroidal		3–6.5 × 5.5–8	<i>K. lactis</i> <i>K. marxianus</i> <i>K. thermotolerans</i>
<i>Metschnikowia</i>	Globose Elongated		2.5–5 × 4–7	<i>M. pulcherrima</i>
<i>Pichia</i>	Ovoidal Elongated		1.8–4.5 × 2.5–17	<i>P. anomala</i> <i>P. fermentans</i> <i>P. membranifaciens</i>
<i>Saccharomycodes</i>	Elongated Lemon shaped		4–7 × 8–23	<i>S. ludwigii</i>
<i>Schizosaccharomyces</i>	Globose Ellipsoidal		3–5 × 5–15–24	<i>S. pombe</i> <i>S. japonicus</i>
<i>Torulaspora</i>	Ellipsoidal		2.5–6.5 × 2.5–7	<i>T. delbrueckii</i>
<i>Zygosaccharomyces</i>	Ovoidal Ellipsoidal		3.5–7 × 5.5–14	<i>Z. bailii</i> <i>Z. bisporus</i> <i>Z. rouxii</i>
<i>Brettanomyces</i>	Ellipsoidal Elongated		2–7 × 3.5–18	<i>B. bruxellensis</i> <i>B. anomalus</i>
(B) Bacteria				
Genus	Morphology		Cell size (µm)	Main species in wine
<i>Lactobacillus</i>	Rods	Single, pair chains	0.5–0.7 × 1–10	<i>L. delbrueckii</i> <i>L. casei</i> <i>L. plantarum</i> <i>L. hilgardii</i> <i>L. brevis</i> <i>L. buchneri</i> <i>L. fermentum</i>

(Continued)

Table 28.1 (Continued)

<i>Oenococcus</i>	Coccus	Pairs chains	0.5–0.6 × 0.7–0.8	<i>O. oeni</i>
<i>Pediococcus</i>	Coccus	Tetrads pairs	0.5 × 1.1	<i>P. damnosus</i> <i>P. parvulus</i> <i>P. pentosaceus</i>
Leuconostoc	Coccus		0.5–0.7 × 0.7–1.2	<i>Leuconostoc mesenteroides</i>
Acetobacter	Ellipsoidal Rod-shaped	Single pair	0.6–1 × 2–4	<i>A. aceti</i> <i>A. pasteurianus</i>
Gluconobacter	Ovoid	Single pair	0.5–0.8 × 0.9–4.2	<i>G. oxydans</i>

Table 28.2 Molecular methods used to identify wine microorganisms.

Technique	Level of identification	Microorganism	Reference
PCR- species specific	Species	<i>Oenococcus oeni</i> <i>Saccharomyces cerevisiae</i> , <i>S. bayanus</i> <i>Zygosaccharomyces</i> <i>Brettanomyces</i>	Zapparoli <i>et al.</i> , 1998 Josepa <i>et al.</i> , 2000 Harrison <i>et al.</i> , 2011 Phister and Mills, 2003 Cocolin <i>et al.</i> , 2004
RFLP	Species Species Species	Yeasts Lactic acid bacteria Acetic acid bacteria	Esteve-Zarzoso <i>et al.</i> , 1999 Claisse <i>et al.</i> , 2007 González <i>et al.</i> , 2004
DGGE	species	yeasts lactic acid bacteria acetic acid bacteria	Cocolin <i>et al.</i> , 2001 Renouf <i>et al.</i> , 2006 Lopez <i>et al.</i> , 2003 De Vero <i>et al.</i> , 2006
PFGE	strain	<i>Oenococcus oeni</i> <i>Brettanomyces</i> <i>Saccharomyces</i>	Larisika <i>et al.</i> , 2008 Miot-Sertier and Lonvaud-Funnel, 2007 Vaughan-Martini <i>et al.</i> , 1993
microsatellite	strain strain	<i>S. cerevisiae</i> <i>S. cerevisiae</i>	Legras <i>et al.</i> , 2005 Vaudano and Garcia-Moruno, 2008
interdelta region	strain	<i>S. cerevisiae</i>	Legras and Karst, 2003
mtDNA	strain	<i>S. cerevisiae</i>	Guillamón <i>et al.</i> , 1994
RAPD	species species strain strain	<i>Lactobacillus</i> yeasts <i>Oenococcus oeni</i> <i>S. cerevisiae</i>	Du Plessis and Dicks, 1995 Quesada and Cenis, 1995 Reguant and Bordous, 2003 Xufre <i>et al.</i> , 2000

wine quality and asserts the existence of non-random “microbial terroir”.

In conclusion, yeasts and bacteria growth is characterized by specific metabolic activities, which determine the final organoleptic characteristics of

wine. Therefore, the possibility of knowing the grape and wine biodiversity can help to have better control of the fermentation processes and also offers a tool to detect unwanted yeasts or bacteria, which can depreciate the wine.

The development of molecular techniques for the identification of species and strains are providing strong support for microbiologists and winemakers.

References

- Bae, S., Fleet, G.H., and Heard, G.M. 2006. Lactic acid bacteria associated with wine grapes from several Australian vineyards. *Journal of Applied Microbiology*, **100**, 712–727.
- Barata, A., Malfeito-Ferreira, M., and Loureiro, V. 2012. The microbial ecology of wine grape berries. *International Journal of Food Microbiology*, **153**, 243–259.
- Barnett, J.A., Payne, R.W., and Yarrow, D. 1990. *Yeast: Characteristics and Identification*, 2nd edn. Cambridge University Press, London, UK.
- Bartowsky, E. 2008 Bacterial spoilage of wine and approaches to minimize it. *Letters in Applied Microbiology*, **48**, 149–156.
- Bartowsky, E.J. and Henschke, P.A. 2004. Acetic acid bacteria and wine: all is well until oxygen enters the scene. *Australia and New Zealand Grapegrowing and Winemaking*, **485a**, 86–91.
- Bartowsky, E. and Henschke, P. 2008. Acetic acid bacteria spoilage of bottled red wine – a review. *International Journal of Food Microbiology*, **125**, 60–70.
- Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamón, J.M., Rozès, N., and Mas, A. 2002. Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. *System and Applied Microbiology*, **25**, 287–293.
- Bezerra-Bussoli, C., Baffi, M.A., Gomes, E., and Da-Silva, R. 2013. Yeast diversity isolated from grape musts during spontaneous fermentation from a Brazilian winery. *Current Microbiology*, **67**, 356–361.
- Bokulich, N.A., Thorngate, J.H., Richardson, P.M., and Mills, D.A. 2014. Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences*, **111**, 139–148.
- Boulton, R.B., Singleton, V.L., Bisson, L.F., and Kunkee, R.E. (eds) 1996. *Principles and Practices of Winemaking*. Springer, New York, USA.
- Caloghiris, M., Waters, E.J., and Williams, P.J. 1997. An industry trial provides further evidence for the role of corks in oxidative spoilage of bottled wines. *Australian Journal of Grape and Wine Research*, **3**, 9–17.
- Chatonnet, P. 2000. La contamination des vins par *Brettanomyces* au cours de la vinification et de l'élevage: incidence, détection et moyen de lutte. *Revue des Oenologue*, **96**, 23–26.
- Chatonnet, P., Dubourdieu, D., and Boidron, J.N. 1995. The influence of *Brettanomyces/Dekkera* sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *American Journal of Enology and Viticulture*, **46**, 463–468.
- Claissé, O., Renouf, V., and Lonvaud-Funel, A. 2007. Differentiation of wine lactic acid bacteria species based on RFLP analysis of a partial sequence of *rpoB* gene. *Journal of Microbiological Methods*, **69**, 387–390.
- Cocolin, L., Heisey, A., and Mills, D.A. 2001. Direct identification of the indigenous yeasts in commercial wine fermentations. *American Journal of Enology and Viticulture*, **52**, 49–53.
- Cocolin, L., Rantsiou, K., Iacumin, L., Zironi, R., and Comi, G. 2004. Molecular detection and identification of *Brettanomyces/Dekkera* bruxelensis and *Brettanomyces/Dekkera* anomalus in spoiled wines. *Applied and Environmental Microbiology*, **70**, 1347–1355.
- Costello, P.J., Morrison, G.J., Lee, T.H., and Fleet, G.H. 1983 Numbers and species of lactic acid bacteria in wines during vinification. *Food Technology in Australia*, **35**, 14–18.
- Davis, C.R., Wibowo, D.J., Lee, T.H., and Fleet, G.H. 1986. Growth and metabolism of lactic acid bacteria during and after malolactic fermentation of wines at different pH. *Applied and Environmental Microbiology*, **51**, 539–545.
- Davis, C.R., Wibowo, D., Fleet, G.H., and Lee, T.H. 1988. Properties of wine lactic acid bacteria: their potential enological significance. *American Journal of Enology and Viticulture*, **39**, 137–142.
- De Vero, L., Gala, E., Gullo, M., Solieri, L., Landi, S., and Giudici, P. 2006. Application of denaturing

- gradient electrophoresis (DGGE) analysis to evaluate acetic acid bacteria in traditional balsamic vinegar. *Food Microbiology*, **23**, 809–813.
- Di Maro, E., Ercolini, D., and Coppola, S. 2007. Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. *International Journal of Food Microbiology*, **117**, 201–210.
- Du Plessis, E.M. and Dicks, L.M.T. 1995. Evaluation of random amplified polymorphic DNA (RAPD)-PCR as a method to differentiate *Lactobacillus acidophilus*, *Lactobacillus crispatus*, *Lactobacillus amylovorans*, *Lactobacillus gallinarum*, *Lactobacillus gasseri*, and *Lactobacillus johnsonii*. *Current Microbiology*, **31**, 114–118.
- Du Toit, W.J., Pretorius, I.J., and Lonvaud-Funel, A. 2005. The effect of sulphur dioxide and oxygen on the viability and culturability of a strain of *Acetobacter pasteurianus* and a strain of *Brettanomyces bruxellensis* isolated from wine. *Journal of Applied Microbiology*, **98**, 862–871.
- Edwards, C., Jensen, K., Spayd, S., and Seymour, B. 1991. Isolation and characterization of native strains of *Leuconostoc oenos* from Washington State wines. *American Journal of Enology and Viticulture*, **42**, 219–226.
- Esteve-Zarzoso, B., Belloch, C., Uruburu, F., and Querol, A. 1999. Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *International Journal of Systematic Bacteriology*, **49**, 329–337.
- Fernández-Espinar, M., Llopis, S., Querol, A., and Barrio, E. 2011. Molecular identification and characterization of wine yeasts. In: *Molecular Wine Microbiology* (eds Carrascosa, A.V., Muñoz, R., and González, R.). Academic Press, San Diego, CA, USA.
- Fleet, G.H. 1993. The microorganisms of wine-making – isolation, enumeration and identification. In: *Wine Microbiology and Biotechnology* (ed. Fleet, G.H.). Harwood Academic Publishers, Switzerland.
- Fleet, G.H. 2003. Yeast interactions and wine flavour. *International Journal of Food Microbiology*, *23rd International Specialized Symposium on Yeasts (ISSY-23)*, **86**, 11–22.
- Fleet, G.H., Lafon-Lafourcade, S., and Ribereau-Gayon, P. 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. *Applied and Environmental Microbiology*, **48**, 1034–1038.
- Fugelsang, K.C. and Edwards, C.G. 1997. *Wine Microbiology: Practical Applications and Procedures*. Springer, New York, USA.
- Garijo, P., Lopez, R., Santamaria, P., Ocon, E., Olarte, C., Sanz, S., and Gutierrez, A.R. 2009. Presence of lactic bacteria in the air of a winery during the vinification period. *International Journal of Food Microbiology*, **136**, 142.
- Gerbaux, V., Jeudy, S., and Monamy, C. 2000. Etude des phénols volatils dans les vins de Pinot noir en Bourgogne. *Bull. OIV*, **73**, 581–599.
- Gerós, H., Cássio, F., and Leão, C. 2000. Utilization and transport of acetic acid in *Dekkera anomala* and their implications on the survival of the yeast in acidic environments. *Journal of Food Protection*, **63**, 96–101.
- González, A., Hierro, N., Poblet, M., Rozès, N., Mas, A., and Guillamón, J.M. 2004. Application of molecular methods for the differentiation of acetic acid bacteria in a red wine fermentation. *Journal of Applied Microbiology*, **96**, 853–860.
- González, Á., Hierro, N., Poblet, M., Mas, A., and Guillamón, J.M. 2005. Application of molecular methods to demonstrate species and strain evolution of acetic acid bacteria population during wine production. *International Journal of Food Microbiology*, **102**, 295–304.
- Guillamón, J.M., Barrio, E., and Querol, A. 1994. Rapid characterization of four species of the *Saccharomyces sensu stricto* complex according to mitochondrial DNA patterns. *International Journal of Bacteriology*, **44**, 708–714.
- Harrison, E., Muir, A., Stratford, M., and Wheals, A. 2011. Species-specific PCR primers for the rapid identification of yeasts of the genus *Zygosaccharomyces*. *FEMS Yeast Research*, **11**, 356–365.
- Henick-Kling, T., Egli, C., Licker, J., Mitrakul, C., and Acree, T.E. 2000. *Brettanomyces* in wine. In: *Proceedings of the Fifth International*

- Symposium of Cool Climate Viticulture and Oenology*. Winetitles, Melbourne, Australia.
- Hierro, N., González, A., Mas, A., and Guillamón, J.M. 2006. Diversity and evolution of non-*Saccharomyces* yeast populations during wine fermentation: effect of grape ripeness and cold maceration. *FEMS Yeast Research*, **6**, 102–111.
- Ivey, M.L. and Phister, T.G. 2011. Detection and identification of microorganisms in wine: a review of molecular techniques. *Journal of Industrial Microbiology and Biotechnology*, **38**, 1619–1634.
- Jolly, N.P., Augustyn, O.P.H., and Pretorius, I.S. 2006. The role and use of non-*Saccharomyces* yeasts in wine production. *South African Journal of Enology and Viticulture*, **27**, 15–39.
- Josepa, S., Guillamon, J.M., and Cano, J. 2000. PCR differentiation of *Saccharomyces cerevisiae* from *Saccharomyces bayanus*/*Saccharomyces pastorianus* using specific primers. *FEMS Microbiology Letters*, **193**, 255–259.
- Joyeux, A., Lafon-Lafourcade, S., and Ribéreau-Gayon, P. 1984. Evolution of acetic acid bacteria during fermentation and storage of wine. *Applied and Environmental Microbiology*, **48**, 153–156.
- Juega, M., Costantini, A., Bonello, F., Cravero, M.-C., Martinez-Rodriguez, A.J., Carrascosa, A., and Garcia-Moruno, E. 2014. Effect of malolactic fermentation by *Pediococcus damnosus* on the composition and sensory profile of Albariño and Caiño white wines. *Journal of Applied Microbiology*, **116**, 586–595.
- Kunkee, R.E. 1991. Some roles of malic acid in the malolactic fermentation in wine making. *FEMS Microbiology Letters*, **88**, 55–72.
- Kurtzman, C., Fell, J.W., and Boekhout, T. 2011. *The Yeasts: A Taxonomic Study*. Elsevier, Burlington, MA, USA.
- Lafon-Lafourcade, S., Carre, E., and Ribéreau-Gayon, P. 1983. Occurrence of lactic acid bacteria during the different stages of vinification and conservation of wines. *Applied and Environmental Microbiology*, **46**, 874–880.
- Larisika, M., Claus, H., and König, H. 2008. Pulsed-field gel electrophoresis for the discrimination of *Oenococcus oeni* isolates from different wine-growing regions in Germany. *International Journal of Food Microbiology*, **123**, 171–176.
- Legras, J.L. and Karst, F. 2003. Optimisation of interdelta for *Saccharomyces cerevisiae* strain characterization. *FEMS Microbiology Letters*, **221**, 249–255.
- Legras, J.L., Ruh, O., Merdinoglu, D., and Karst, F. 2005. Selection of hypervariable microsatellite loci for the characterization of *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology*, **102**, 73–83.
- Lonvaud-Funel, A. 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. *Antonie Van Leeuwenhoek*, **76**, 317–331.
- Lonvaud-Funel, A., Guilloux, Y., and Joyeux, A. 1993. Isolation of a DNA probe for identification of glucan-producing *Pediococcus damnosus* in wines. *Journal of Applied Bacteriology*, **74**, 41–47.
- Lopez, I., Ruiz-Larrea, F., Cocolin, L., Orr, E., Phister, T., Marshall, M., Vander Gheynst, J., and Mills, D.A. 2003. Design and evaluation of PCR primers for analysis of bacterial populations in wine by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*, **69**, 6801–6807.
- Loureiro, V. and Malfeito-Ferreira, M. 2003. Spoilage yeasts in the wine industry. *International Journal of Food Microbiology*, **86**, 23–50.
- Martini, A. 2003. Biotechnology of natural and winery-associated strains of *Saccharomyces cerevisiae*. *The Official Journal of the Spanish Society for Microbiology*, **6**, 207–209.
- Millet, V. and Lonvaud-Funel, A. 2000. The viable but non-culturable state of wine micro-organisms during storage. *Letters in Applied Microbiology*, **30**, 136–141.
- Miot-Sertier, C. and Lonvaud-Funel, A. 2007. Development of a molecular method for the typing of *Brettanomyces bruxellensis* (*Dekkera bruxellensis*) at the strain level. *Journal of Applied Microbiology*, **102**, 555–562.
- Pardo, I. and Zuniga, M. 1992. Lactic acid bacteria in Spanish red rose and white musts and wines under cellar conditions. *Journal of Food Science*, **57**, 392–405.
- Phister, T. and Mills, D. 2003. Real-time PCR assay for detection and enumeration of *Dekkera bruxellensis* in wine. *Applied and Environmental Microbiology*, **69**, 7430–7434.

- Pozo-Bayón, M.A., Pardo, I., Ferrer, S., and Moreno-Arribas, V. 2009. Molecular approaches for the identification and characterization of oenological lactic acid bacteria. *African Journal of Biotechnology*, **8**, 3995–4001.
- Quesada, M.P. and Cenis, J.L. 1995. Use of random amplified polymorphic DNA (RAPD-PCR) in the characterization of wine yeasts. *American Journal of Enology and Viticulture*, **46**, 204–208.
- Reguant, C. and Bordons, A. 2003. Typification of *Oenococcus oeni* strains by multiplex RAPD-PCR and study of population dynamics during malolactic fermentation. *Journal of Applied Microbiology*, **95**, 344–353.
- Regueiro, L.A., Costas, C.L., and Rubio, J.E.L. 1993. Influence of viticultural and enological practices on the development of yeast populations during winemaking. *American Journal of Enology and Viticulture*, **44**, 405–408.
- Rementeria, A., Rodriguez, J.A., Cadaval, A., Amenabar, R., Muguruza, J.R., Hernando, F.L., and Sevilla, M.J. 2003. Yeast associated with spontaneous fermentations of white wines from the “Txakoli de Bizkaia” region (Basque country, north Spain). *International Journal of Food Microbiology*, **86**, 201–207.
- Renouf, V., Claisse, O., and Lonvaud-Funel, A. 2006. rpoB gene: a target for identification of LAB cocci by PCR-DGGE and melting curves analyses in real time PCR. *Journal of Microbiological Methods*, **67**, 162–170.
- Renouf, V., Claisse, O., and Lonvaud-Funel, A. 2007. Inventory and monitoring of wine microbial consortia. *Applied Microbiology and Biotechnology*, **75**, 149–164.
- Ribéreau-Gayon, P., Dubourdieu, D., Donèche B., and Lonvaud A. (eds) 2004. *Traité d’Oenologie*. Dunod, Paris, France.
- Ruiz, A., Poblet, M., Mas, A., and Guillamon, J.M. 2000. Identification of acetic acid bacteria by RFLP of PCR-amplified 16S rDNA and 16S–23S rDNA intergenic spacer. *International Journal of Systematic and Evolutionary Microbiology*, **50**, 1981–1987.
- Schuller, D., Alves, H., Dequin, S., and Casal, M. 2005. Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the Vinho Verde region of Portugal. *FEMS Microbiology and Ecology*, **51**, 167–177.
- Sieiro, C., Cansado, J., Agrelo, D., Velazquez, J.B., and Villa, T.G. 1990. Isolation and enological characterization of malolactic bacteria from the vineyards of northwestern Spain. *Applied and Environmental Microbiology*, **56**, 2936–2938.
- Silva, P., Cardoso, H., and Gerós, H. 2004. Studies on the wine spoilage capacity of *Brettanomyces/Dekkera* spp. *American Journal of Enology and Viticulture*, **55**, 65–72.
- Sipiczki, M. 2003. *Candida zemplinina* sp. Nov., an osmotolerant and psychrotolerant yeast that ferments sweet botrytized wines. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 2079–2083.
- Sipiczki, M. 2006. *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. *Applied and Environmental Microbiology*, **72**, 6716–6724.
- Suárez, J.A., Gonzalez, M.C., Callejo, J.J., Colomo, B., and González, A. 1994. Contribution to the study of varietal wines from Rioja and Navarra. I. Microbial growth trends during grape maturation. *Bulletin OIV*, **759–760**, 389–407.
- Suárez, R., Suárez-Lepe, J.A., Morata, A., and Calderón, F. 2007. The production of ethylphenols in wine by yeasts of the genera *Brettanomyces* and *Dekkera*: a review. *Food Chemistry*, **102**, 10–21.
- Torija, M.J., Rozès, N., Poblet, M., Guillamón, J.M., and Mas, A. 2001. Yeast population dynamics in spontaneous fermentations: comparison between two different wine-producing areas over a period of three years. *Antonie Van Leeuwenhoek*, **79**, 345–352.
- Ugliano, M., Genovese, A., and Moio, L. 2003. Hydrolysis of wine aroma precursors during malolactic fermentation with four commercial starter cultures of *Oenococcus oeni*. *Journal of Agriculture and Food Chemistry*, **51**, 5073–5078.
- Valera, M.J., Laich, E., González, S.S., Torija, M.J., Mateo, E., and Mas, A. 2011. Diversity of acetic acid bacteria present in healthy grapes from the Canary Islands. *International Journal of Food Microbiology*, **151**, 105–112.

- Valero, E., Cambon, B., Schuller, D., Casal, M., and Dequin, S. 2007. Biodiversity of *Saccharomyces* yeast strains from grape berries of wine-producing areas using starter commercial yeasts. *FEMS Yeast Research*, **7**, 317–329.
- Van Keulen, H., Lindmark, D.G., Zeman, K.E., and Gerlosky, W. 2003. Yeasts present during spontaneous fermentation of Lake Erie Chardonnay, Pinot Gris and Riesling. *Antonie Van Leeuwenhoek*, **83**, 149–154.
- Vaudano, E. and Garcia-Moruno, E. 2008. Discrimination of *Saccharomyces cerevisiae* wine strains using microsatellite multiplex PCR and band pattern analysis. *Food Microbiology*, **25**, 56–64.
- Vaughan-Martini, A., Martini, A. and Cardinali, G. 1993. Electrophoretic karyotyping as a taxonomic tool in the genus *Saccharomyces*. *Antonie van Leeuwenhoek*, **63**, 145–156.
- Versavaud, A., Courcoux, P., Roulland, C., Dulau, L., and Hallet, J.N. 1995. Genetic diversity and geographical distribution of wild *Saccharomyces cerevisiae* strains from the wine-producing area of Charentes, France. *Applied and Environmental Microbiology*, **61**, 3521–3529.
- Vos, P., Garrity, G., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., and Whitman, W. 2009. *The Bergey's Manual*, Vol. 3, *Firmicutes* (ed. Whitman, W.). Springer.
- Waters, E.J., Peng, Z., Pocock, K.F., and Williams, P.J. 1996. The role of corks in oxidative spoilage of white wines. *Australian Journal of Grape and Wine Research*, **2**, 191–197.
- Weiller, H.G. and Radler, F. 1970. Milchsäurebakterien aus Wein und von Rebenblättern. *Zentralbl. Bakteriologie Parasitenkunde Infektionskrankheiten Hygiene*, **124**, 707–732.
- Wibowo, D., Fleet, G.H., Lee, T.H., and Eschenbruch, R.E. 1988. Factors affecting the induction of malolactic fermentation in red wines with *Leuconostoc oenos*. *Journal of Applied Bacteriology*, **64**, 421–428.
- Xufre, A., Simoes, F., Girio, F., Clemente, A., Amaral-Collaco, M.T. 2000. Use of RAPD analysis of differentiation among six enological *Saccharomyces* sp. Strains. *Food Technology and Biotechnology*, **38**, 53–58.
- Zapparoli, G., Torriani, S., Pesente, P., and Dellaglio, F. 1998. Design and evaluation of malolactic enzyme gene targeted primers for rapid identification and detection of *Oenococcus oeni* in wine. *Letters in Applied Microbiology*, **27**, 243–246.

CHAPTER 29

Microbial diversity and ecology of bottled water

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29.1 Definitions of bottled water

The consumption of bottled water became popular over the last few decades, at least in some world regions. The image of purity and safety, good taste, potential health benefits, and convenience are probably major reasons behind the preference for bottled water. The annual consumption per capita of bottled water was estimated to be around 250 l in Mexico, 190 l in Italy, 170 l in Thailand, 165 l in United Arab Emirates, the leading countries, and 110 l in the United States of America (USA) (data from International Bottled Water Association for 2011, www.bottledwater.org) and 104 l in the European Union (EU) (data from European Federation of Bottled Water for 2012, <http://efbw.eu>).

“Bottled water” comprises different types of water, whose origin, collection system, properties, and designations may vary according to the world region and legislation. Being intended for human consumption, bottled water, supplied in sealed bottles or other containers, must be chemically and microbiologically considered safe (European Community, 1980; European Commission, 1998, 2009; Codex Alimentarius, 2001; World Health Organization, 2008; Food and Drug Administration, 2013). In the European

Union, bottled water comprises mineral, spring, and table water. The first two, mineral and spring, are natural waters originating from underground sources and in their natural state must be safe to drink, meaning that no disinfection is allowed (European Community, 1980; Codex Alimentarius, 2001). In contrast to natural water, table water may have its origin in different sources, such as surface water or the municipal supply, is often disinfected, and may be supplemented with minerals (European Commission, 1998). Most of the bottled water consumed in Europe is natural mineral or spring water (83% mineral and 14% spring, making 97% of the market volume in 2012, <http://efbw.eu>), while table water is rarely produced and sold. In the US, legislation on bottled water is integrated in the section devoted to food for human consumption, issued by the Food and Drug Administration (FDA). FDA defines eight categories of bottled water in which the addition of safe and suitable antimicrobial agents or fluoride is permitted, independently of the water source (Food and Drug Administration, 2013) (Table 29.1).

Natural mineral and spring water originate from specified underground sources, which must be protected from any kind of pollution. Therefore, bottled mineral and spring water should be produced in environmentally protected

Table 29.1 Description of bottled water types according to European Union (EU) and United States of America (US) Legislation.

	Designation	Description	Added ingredients	Legislation
EU	Mineral water	From underground water table or deposit and emerging from a spring tapped at one or more natural or bore exits Stable mineral composition No disinfection allowed	None	Directive 80/777/EEC Directive 2009/54/EC
	Spring water	From underground water table or deposit and emerging from a spring tapped at one or more natural or bore exits Mineral composition may fluctuate No disinfection allowed	None	Directive 98/83/EEC (spring water)
	Effervescent natural mineral water	Naturally carbonated – carbon dioxide content is the same as at source, with possible reintroduction from the same water table Fortified with gas from the spring – carbon dioxide content is greater than that at source Carbonated – fortified with carbon dioxide of other origin than the water table	Carbon dioxide from the same or other source	
	Table water	From surface or ground water source or municipal water supply May be disinfected	Minerals may be restored	Directive 98/83/EEC
US	Ground water	From a subsurface saturated zone that is under a pressure equal to or greater than atmospheric pressure Must not be under the direct influence of surface water	Safe/suitable antimicrobial agents Fluoride	Code of Federal Regulations (21CFR165.110)
	Mineral water	From a geologically and physically protected underground water source tapped at one or more bore holes or springs Contains not less than 250 parts per million total dissolved solids Stable mineral composition	Carbon dioxide in sparkling water	
	Spring water	From an underground formation from which water flows naturally to the surface of the Earth If external force is used, shall be from the same underground stratum as the spring and water must continue to flow naturally to the surface through the spring's natural orifice		
	Artesian water	From a well tapping a confined aquifer in which the water level stands at some height above the top of the aquifer May be collected with the assistance of external force		
	Well water	From a hole bored, drilled, or otherwise constructed in the ground which taps the water of an aquifer		
	Sparkling water	Carbon dioxide (natural or replaced after treatment) content is the same as at the source		
	Purified water	Produced by distillation, deionization, reverse osmosis, or other suitable processes		
	Sterile water	Meets the requirements of sterility tests		

areas, subjected to an effective management of water resources, including the protection of water catchment areas and of the surrounding ecosystem (Codex Alimentarius, 1985). These are essential conditions to ensure the sustainable production of water with the required properties, quality, and safety. In order to avoid adulteration of the original water, natural waters must be bottled at source, through the use of food-grade procedures and materials, and containers must protect the water quality (Codex Alimentarius, 1985). Natural waters originating from underground water tables or deposits (Table 29.1) are particularly interesting microbiological niches and will be the major focus of this review.

29.2 Characteristics of mineral and spring water

Although representing oligotrophic habitats (poor in nutrients), groundwater sources hold a rich and diversified microbiota and a complex chemical composition. The flow of water through small pores and cracks in the underground rock layers not only contributes to filtrate and to depurate water, but also to enrich it in specific minerals (Leclerc and Moreau, 2002). The mineral composition of water may be a factor of preference for consumers and is a characteristic of each mineral water, since it is stable. In opposition, the mineral composition of spring water may vary over time and the mineral composition may be omitted in the labeling information (Table 29.2). Among the minerals abundant in natural water and in which the daily dose is relevant for human physiological functions and health are Ca^{2+} , Mg^{2+} , and Na^+ . The concentration of these minerals is rather variable among different sources. For example, the concentration of these ions in 37 spring and mineral waters from the US and in 73 natural waters from the EU were, respectively, 0–310 mg/l and 4–575 mg/l of Ca^{2+} , 0–130 mg/l and 1–128 mg/l of Mg^{2+} , and 0–1095 mg/l and 1–1419 mg/l of

Na^+ , showing different degrees of mineralization (Azoulay *et al.*, 2001). Although no limit values are proposed for the concentration of Ca^{2+} , Mg^{2+} , and Na^+ , the analytical data composition on these and other water constituents should be provided to consumers through bottle labeling (Codex Alimentarius, 2001; European Commission, 2009; Food and Drug Administration, 2013)(Table 29.2). In spite of the natural origin, some water components, if present above certain threshold values, may have prejudicial effects on human health. Therefore, these values are regulated, with the characteristics and maximal levels of some water components being established by specific regulation (Codex Alimentarius, 2001; World Health Organization, 2008; Table 29.2). The existence of regulation and labeling recommendations prevent consumers from being misled and promote fair trading, while ensure the protection of consumers' health. Moreover, labeling information may either cover safety aspects or quality properties appealing for consumer preference. FDA legislation also contemplates non-compliance situations, which should be labeled as "Excessive" and the respective parameter (Table 29.2).

Chemical contaminants of anthropogenic origin are supposed to be absent in natural mineral and spring water collected from protected areas (Codex Alimentarius, 1985; Table 29.2). In contrast, when water has its origin in non-protected sources, such as surface water, the occurrence of some chemical contaminants cannot be disregarded, and the analysis of these contaminants is compulsory (Table 29.2). As for chemical components, microbiological contamination is not expected in natural water collected in protected areas. In these waters, only the autochthonous microbiota are supposed to be present (Codex Alimentarius, 2001; European Commission, 2009). Therefore, the occurrence of bacterial indicators of microbial contamination, either in the source or in bottled water, hints some environmental contamination in the source, handling problems during the bottling process, or inadequate fitted closures of

Table 29.2 Chemical and microbiological parameters and labeling indications of bottled water according to EU and US legislation (Table 29.1).

Parameter	EU		US
	Table water (*)	Mineral/spring water	Bottled water
Allowable levels of inorganic substances (mg/l)	Antimony	0.005	0.005
	Arsenic	0.01	0.01
	Barium	Not defined	2.0
	Beryllium	Not defined	0.004
	Cadmium	0.005	0.005
	Chromium	0.05	0.1
	Copper	2.0	1.0
	Cyanide	0.05	0.2
	Fluorides	1.5	0.8–2.4 (temperature; addition)
	Lead	0.01	0.005
	Manganese	0.05	Not defined
	Mercury	0.001	0.002
	Nickel	0.02	0.1
	Nitrates	50	10
	Nitrites	0.5	1
	Selenium	0.01	0.05
	Thallium	Not defined	0.002
	Volatile organic chemicals (VOCs)	0.001 (tetrachloroethene and trichloroethene)	21 contaminants defined, range: 10 (xylenes) – 0.002 (vinyl chloride)
	Pesticides and other synthetic organic chemicals	0.005 (vinyl chloride) 0.005 total pesticides 0.001 individual pesticides	30 contaminants defined, range: 0.7 (glyphosate) – 3 x 10 ⁻⁸ (dioxin)
	Other	0.2 (aluminum), 250.0 (sulfate)	0.2 (aluminum), 0.1 (silver), 250.0 (sulfate, not defined for mineral water)

(Continued)

Table 29.2 (Continued)

Microbiological	Total heterotrophs – 100 or 20 cfu per ml at 22 or 37°C, respectively Free of: • <i>Escherichia coli</i>, fecal enterococci and <i>Pseudomonas aeruginosa</i> in 250 ml • <i>Clostridium perfringens</i> (including spores) in 100 ml (if water originates from or is influenced by surface water)	Total heterotrophs – 20 or 5 cfu per ml at source and 100 or 20 cfu per ml, 12 h after bottling, at 22 or 37°C, respectively Free of: • Parasites and pathogenic microorganisms • <i>Escherichia coli</i>, fecal enterococci, and <i>Pseudomonas aeruginosa</i> in 250 ml • Sporulated sulphite-reducing anaerobes in 50 ml	Total coliforms – ≤1 unit with ≥ 2.2 MPN per 100 ml and <1 unit with ≥ 9.2 MPN per 100 ml or ≤ 1 unit – ≥ 4 cfu per 100 ml Free of: • <i>Escherichia coli</i>
Authorized/recommended labeling indications	Place of origin and source Mineral composition (not required for spring water) Treatment carried out (if any) Low mineral content (≤500 mg/l) Very low mineral content (≤50 mg/l) Rich in mineral salts (>1500 mg/l) Contains bicarbonate (>600 mg/l) Contains sulfate (>200 mg/l) Contains chloride (>200 mg/l) Contains calcium (>150 mg/l) Contains magnesium (>50 mg/l) Contains fluoride (>1 mg/l) Contains iron (bivalent iron >1 mg/l) Acidic (Free carbon dioxide > 250 mg/l) Contains sodium (>200 mg/l) Suitable for the preparation of infant food Suitable for a low-sodium diet (sodium <20 mg/l) May be laxative May be diuretic Bottled water/drinking water/artesian water/mineral water/sparkling bottled water/spring water/purified water (distilled, demineralized, deionized, reverse osmosis water) Low mineral content (<500 mg/l) High mineral content (>1500 mg/l) If it is from a community water system from a community water system /from a municipal source If quality is below the prescribed values: "Contains excessive bacteria" "Excessively turbid" "Abnormal color" "Abnormal odor" "Contains excessive _____" "Excessively radioactive"		

* Only parameters defined for the other types of water are indicated.

dispensers, permitting water adulteration or contamination (Codex Alimentarius, 1985; Table 29.2). In either case, this will represent a threat to water safety and may have adverse consequences on the consumer's health.

The occurrence of possible contaminants in bottled water, either chemical or microbiological, is regarded differently according to the EU and US definitions of bottled mineral and spring water. While in the US mineral and spring water are regulated in parallel with all other types of water, EU legislation is clear in this aspect (European Commission, 2009; Food and Drug Administration, 2013). In the EU, natural mineral and spring water reaching the consumer must retain its state at the source and may not suffer any treatment capable of altering the composition in its essential components. Exceptions are the separation of unstable or undesirable components (e.g., iron, sulfates, arsenic) by ozone enrichment, filtration, or decantation, as long as these processes do not alter the composition of the water in its essential constituents (Codex Alimentarius, 2001; European Commission, 2009). In addition, in contrast to US recommendations, which allow the addition of antimicrobial agents, EU legislation is clear about the prohibition of any kind of disinfection or addition of antimicrobial agents to mineral and spring water (European Commission, 2009; Food and Drug Administration, 2013). Because unsuitable containers may influence the water quality, these must be made of materials that ensure that all the characteristics of the mineral or spring water are retained. It is the above-described concept of a natural mineral in the EU that justifies the commonly used definition of "microbiologically wholesome water" (European Commission, 2009), which refers to both safe/healthy and entire (as it occurs in the source) water. In the US, given the legal framework for bottled water, the microbiota thriving in natural mineral and spring bottled water is not necessarily that found at the source, since microbial inactivation is not prohibited (Food and Drug Administration, 2013).

29.3 Useful methods to study bottled water microbiota

Mineral waters offer a unique habitat to microbiota, characterized by the shortage of organic nutrients, high mineral content, and restricted input of new colonizers, resulting in a particular ecosystem. In spite of these constraints for microbial development, mineral waters, mainly non-carbonated waters, are inhabited by abundant and diversified microbial communities (Hunter, 1993; Leclerc and Moreau, 2002; Otterholt and Charnock, 2011; Falcone-Dias *et al.*, 2012; França *et al.*, 2015). Most of these microorganisms are heterotrophic bacteria (Leclerc and Moreau, 2002). Although the values may vary according to the properties of the water, bottling time, and world region (also due to the allowance or not to add antimicrobial agents), a bottled mineral water contains up to 10^4 – 10^5 colony-forming units (cfu) per milliliter (Manaia *et al.*, 1990; Wilkinson and Kerr, 1998; Falcone-Dias *et al.*, 2012; Falcone-Dias and Filho, 2013; Otterholt and Charnock, 2011). These numbers are considerably lower in carbonated water (normally less than 20 cfu/ml) (Korzeniewska *et al.*, 2005; Varga, 2011). A large fraction of the water microbiota is non-culturable, at least under the conditions normally used. Cell injuries, slow growth rates, and the need for specific growth requirements are among the reasons that may impose a non-culturable state. The existence of the above-mentioned microbiological populations in different physiological and metabolic states demands the use of distinct methodological approaches. Considering the methods used to characterize bottled water microbiota, there are three main types: (i) cultivation; (ii) microscopic detection; (iii) total DNA screening (Figure 29.1).

29.3.1 Cultivation

Cultivation has been until now the golden standard for quality monitoring, aiming to enumerate all heterotrophic bacteria and to detect and enumerate specific bacterial indicators (Codex Alimentarius, 1985; Table 29.2).

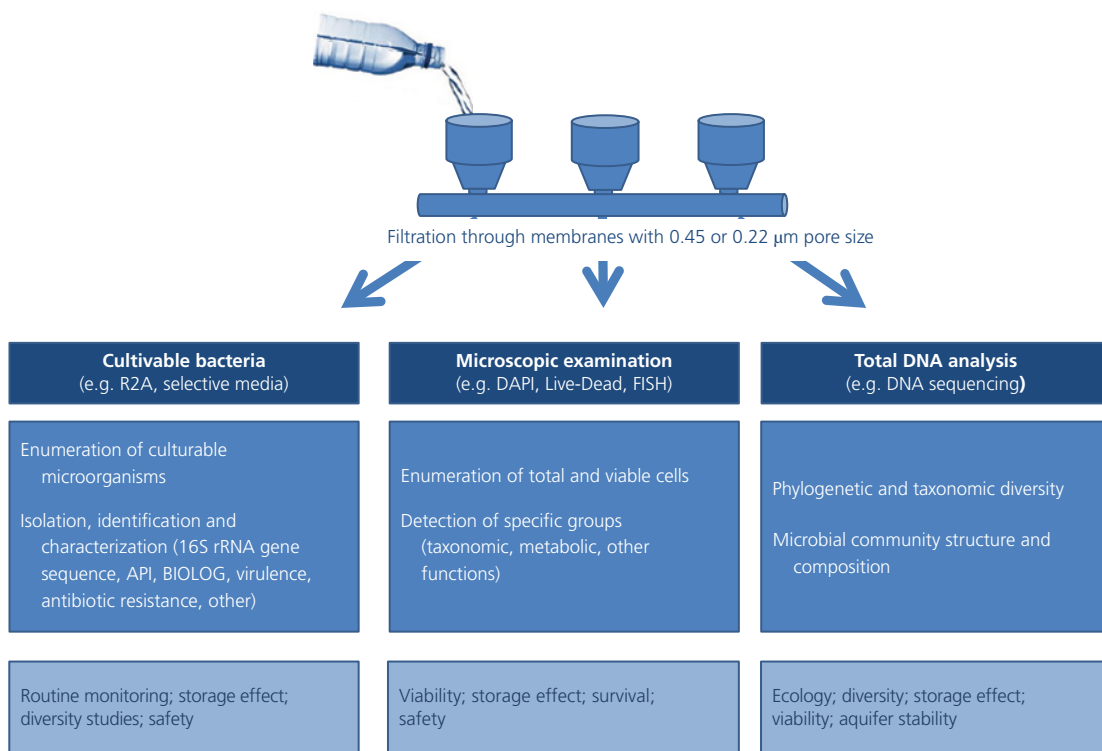


Figure 29.1 Examples of objectives and applications of approaches commonly used to characterize water microbiota based on membrane filtration methods.

However, this same approach is frequently used to survey the diversity of culturable bacteria (Manaia *et al.*, 1990; Otterholt and Charnock, 2011; Falcone-Dias *et al.*, 2012; França *et al.*, 2015). In either situation, cultivation is often used as a quantitative method, based on techniques such as spread plate, pour plate, the membrane filtration method, or, rarely nowadays, on the most probable number. The most probable number methodology, recommended by the American Public Health Association (APHA, 2005) consists of the incubation of replicated cultures across several serial dilution sets. The number of microorganisms in a sample is calculated based on the pattern of positive and negative growth results, through the use of a mathematical estimate. Despite the accuracy of this technique, it has been gradually replaced by the membrane filtration method, which is easier to perform and to interpret and with

applications in culture-independent methods. Moreover, the standardization of procedures based on the membrane filtration method offers the opportunity to produce comparable data, over different regions and time scales (International Organization for Standardization, 2001, 2002, 2003, 2008). The membrane filtration method consists of the filtration of variable volumes of water through a filtering membrane (e.g., nitro cellulose), normally with a 0.45 μm pore size. This procedure, which aims to capture the cells for further analysis, is the basis of different approaches used to characterize water microbiota (Figure 29.1). The methods described below on microscopic detection and total DNA screening are also based on a previous membrane filtration step, although the composition of the filtering membrane may differ depending on the processing required for sample analysis (DNA extraction, cell staining

and fixation, or other). A major bias of the membrane filtration procedure to capture cells is related to the incapability to retain spores and endospores, but, above all, ultramicro- or nanocells, often present in mineral waters and able to cross even 0.2 μm pores (Jones *et al.*, 1999; Leclerc and Moreau, 2002). For this reason, the use of membranes with a pore size of 0.1 or 0.2 μm may be preferred (França *et al.*, 2015).

Given the oligotrophic character of bacteria thriving in mineral water, total heterotrophic populations are preferentially recovered on nutrient-poor culture media, such as R2A or other low nutrient culture media, for example, diluted PCA (Bischofberger *et al.*, 1990; Massa *et al.*, 1998; Armas and Sutherland, 1999). Other culture media targeting microbiological indicators of water quality are often used, for example, Tergitol TTC, m-Endo Agar, or MacConkey agar for coliforms and *Escherichia coli*, *Pseudomonas* agar base, supplemented or not with cetrimide for *Pseudomonas aeruginosa*, Slanetz and Bartley or m-*Enterococcus* for enterococci, and tryptose sulfite cycloserine agar for sulfite-reducing clostridia (Marzano *et al.*, 2011; Otterholt and Charnock, 2011; Varga, 2011; Falcone-Dias *et al.*, 2012; França *et al.*, 2015). These selective and differential culture media have the advantage of offering a rapid and reliable estimate of possible contamination, with the enumeration of bacterial indicators (Table 29.2). Moreover, these and the non-selective culture media (e.g., R2A or PCA) allow the recovery of a wide range of bacteria inhabiting mineral water useful in bacterial diversity studies (Manaia *et al.*, 1990; Otterholt and Charnock, 2011; Falcone-Dias *et al.*, 2012; França *et al.*, 2015). When this is the objective, the identification is the next step. Identification can be based on biochemical tests, often available in commercial kits (e.g., API, BIOLOG) (Manaia *et al.*, 1990; Wilkinson and Kerr, 1998; Saleh *et al.*, 2008; Casanovas-Massana and Blanch, 2012), which over the last twenty years have been increasingly replaced or complemented by identifications based on the analysis of the 16S rRNA gene sequence (Otterholt and

Charnock, 2011; Casanovas-Massana and Blanch, 2012; Falcone-Dias *et al.*, 2012; França *et al.*, 2015). This was an important advance in knowledge given the recognized limitations of phenotype-based systems to identify bacteria from mineral waters (Manaia *et al.*, 1990; Morais and da Costa, 1990; Otterholt and Charnock, 2011).

29.3.2 Microscopic detection

The fraction of bacteria that can be cultured from mineral water can be as low as 0.0002% (bore-hole, see França *et al.*, 2015). Therefore, the detection and enumeration of bacteria using culture-independent approaches is mandatory if deeper insights into the water microbiota are to be achieved. The enumeration of total cells is possible through the use of DNA-intercalating agents, such as ethidium bromide, acridine orange, or DAPI (4',6-diamidino-2-phenylindole) and observation with fluorescence microscopy. These agents interact with DNA and can potentially react with viable and non-viable cells, giving an estimate of the total number of cells. However, because the distinction between alive and dead cells is important, differential staining methods have been developed. Viable cells can be specifically targeted using stains such as CTC (5-cyano-2,3-ditolyl tetrazolium) or INT (iodonitrophenyltetrazolium), which are reduced by actively respiring cells. The use of these agents in combination with DNA intercalating dyes gives an estimate of the proportion of metabolically active cells (Ferreira *et al.*, 1994; Defives *et al.*, 1999; Ramalho *et al.*, 2001). Another method, with the commercial designation "LIVE/DEAD BacLight", involves the combination of two stains that interact with nucleic acids, with only one, the propidium iodide, penetrating the cells with damaged membranes. Using fluorescence microscopy it is possible to distinguish live cells, green, from dead cells that stain red due to the reaction with both dyes simultaneously (Boulos *et al.*, 1999; Defives *et al.*, 1999; Ramalho *et al.*, 2001).

The microscopic examination allows the estimation of total cell numbers and viability,

and, combined with enumeration on non-selective culture media, also permits ratios of culturability to be established (Ferreira *et al.*, 1994; Defives *et al.*, 1999; Ramalho *et al.*, 2001; França *et al.*, 2015). These data give interesting insights into variations observed between the spring and bottled water and fluctuations occurring over time (Ferreira *et al.*, 1994; Defives *et al.*, 1999; França *et al.*, 2015). However, microscopy-based methods do not give any indication about the occurrence or variation on the abundance of specific microbial groups. The quantitative monitoring of specific microbial groups is possible through *in situ* hybridization methods, in different formats (e.g., chemiluminescent *in situ* hybridization, CISH, or fluorescent *in situ* hybridization, FISH). These methods are based on the use of taxa-specific probes, normally targeting signature sequences of the 16S rRNA gene for a given species, genus, family, or higher taxonomic ranks (Amman *et al.*, 1995; Stender *et al.*, 2000; Loy *et al.*, 2005). For example, a peptide nucleic acid probe was successfully used to detect *Pseudomonas aeruginosa* (Stender *et al.*, 2000). Multitarget surveys are also possible through the use of distinct probes that hybridize with different bacterial lineages. This kind of approach has provided interesting information about the populations responsible for bacterial growth after bottling (Loy *et al.*, 2005). However, if the study of bacterial diversity is the major aim, *in situ* hybridization methods are of limited value and other non-targeted methods must be used.

29.3.3 Total DNA screening

Non-targeted methods to assess microbial diversity gained popularity over the last decade thanks to the development of high-throughput sequencing methods, accompanied by a considerable reduction in sequencing costs. Surveys on bacterial diversity based on the 16S rRNA gene sequence analysis rely on the extraction of total DNA, PCR amplification of a region of that gene, and the generation of high numbers of reads (typically more than 2000 per sample). These reads, after organized into taxonomic

groups, normally at a level of 97% similarity, lead to taxa identification, giving a broad overview of the bacterial diversity in a sample (Vaz-Moreira *et al.*, 2011, 2013; Hansen *et al.*, 2013; França *et al.*, 2015). Total DNA screening methods based on the 16S rRNA gene sequence analysis give a higher coverage of bacterial diversity than cultivation-based methods. In a recent study characterizing the microbiota of mineral water in aquifer and bottled water, between 12 and 35% of the *Alpha*-, *Beta*- and *Gammaproteobacteria*, *Bacteroidetes*, and *Actinobacteria* were detected through both approaches, culture-dependent and total DNA analysis (França *et al.*, 2015). However, it is interesting and meaningful that not only the prevailing populations given by different methods are not fully coincident but also that some cultivable populations are not detected by culture-independent methods (Vaz-Moreira *et al.*, 2011, 2013; França *et al.*, 2015). Although both culture-dependent and culture-independent methods have recognized biases, these findings raise questions about the adequacy of the currently used microbial indicators of water quality.

Besides diversity insights, the use of next-generation sequencing methods may give important contributions to understanding the physiology and metabolism of bottled water microbiota. The analysis of the metagenome and metatranscriptome, following the extraction of total DNA or total RNA, may unveil key biochemical functions activated in the microorganisms during starvation periods or bottle adaptation stages, improving our knowledge about crucial processes underlying the microbial ecology of bottled water.

29.4 Microbiological diversity

Although there are some references to fungi, protozoa, and archaea in mineral waters (Hunter, 1993; Otterholt and Charnock, 2011; Pontara *et al.*, 2011; França *et al.*, 2015), most of the studies on the microbiota of mineral and spring water are focused on bacteria. The first

studies addressing the bacterial diversity in these habitats were based on phenotypic characteristics, often using commercial identification systems such as API or BIOLOG (Manaia *et al.*, 1990; Armas and Sutherland, 1999; Defives *et al.*, 1999). The use of genetic markers, in particular the 16S rRNA gene, on bacterial classification brought important modifications to the taxonomy and universality of bacterial identification (Janda and Abbott, 2007). In parallel, many species normally found in mineral water were reclassified, in particular former members of the genus *Pseudomonas*, nowadays included in genera such as *Brevundimonas*, *Stenotrophomonas*, *Burkholderia*, and *Ralstonia*, some of which belong to the class *Betaproteobacteria* and not to *Gammaproteobacteria*, such as the *Pseudomonas* (Euzéby, 1997). This explains, in part, why former studies, in which the identification of cultivable organisms was based on phenotypic characterization of the isolates, reported *Gammaproteobacteria* as the most abundant group of organisms thriving in non-carbonated natural mineral water. Others, also abundant, were reported as being *Alpha- and Betaproteobacteria*, *Bacteroidetes*, and *Actinobacteria* (Leclerc and da Costa, 2007).

Currently, the studies using 16S rRNA gene sequence-based identifications in natural waters, either involving culture-dependent (CD) or culture-independent (CI) methods, are scarce and from European waters (CI, Loy *et al.*, 2005; CD, Otterholt and Charnock, 2011; CD, Falcone-Dias *et al.*, 2012; CD + CI, França *et al.*, 2015) (Table 29.3). These studies suggest that each bottled mineral/spring water harbor a distinct microbiota. Moreover, it is suggested that water from the same source but collected at different dates may hold a distinct community structure (i.e., abundance of each member). Despite those variations, some taxonomic groups are prevalent in mineral/spring waters. In general, *Beta-* and *Alphaproteobacteria* followed by *Actinobacteria* are the most common bacteria inhabiting mineral/spring water, irrespective of the geographic origin (Table 29.3). Within those phyla and classes, the most

common families in spring mineral water are the *Comamonadaceae*, *Oxalobacteraceae* (*Betaproteobacteria*), *Shingomonadaceae*, *Bradyrhizobiaceae*, *Caulobacteraceae* (*Alphaproteobacteria*), *Pseudomonadaceae* (*Gammaproteobacteria*), and *Microbacteriaceae* (*Actinobacteria*), observed in bottled waters from different European countries. Within these families there are also some genera commonly found in bottled natural and spring mineral waters from different countries. Examples of these genera, which comprise some common freshwater inhabitants, are *Acidovorax*, *Hydrogenophaga*, *Variovorax*, *Curvibacter*, *Delftia*, *Rhodospirillum* (*Comamonadaceae*), *Duganella*, *Janthinobacterium* (*Oxalobacteraceae*), *Afipia*, *Bradyrhizobium* (*Bradyrhizobiaceae*), *Caulobacter* (*Caulobacteraceae*), *Sphingomonas* (*Shingomonadaceae*), *Pseudomonas* (*Pseudomonadaceae*), and *Microbacterium* (*Microbacteriaceae*). Other genera such as *Mycobacterium*, *Bacillus*, and *Staphylococcus*, although not represented by a high diversity of species, may have also a widespread occurrence in bottled natural and spring mineral water, being reported in different countries (Table 29.3).

Culture-independent methods, still uncommonly used to characterize the microbiota from bottled mineral/spring water, reveal a limited knowledge of these microbial habitats. These microbiomes, not detected by culture-dependent methods, comprise groups such as *Methylocystis*, *Hyphomicrobium*, *Limnobacter*, *Caenimonas*, *Paucibacter*, *Propionivibrio*, *Flectobacillus*, *Cloacibacterium*, *Brevibacterium*, *Alloiococcus*, and *Nitrospira*. In addition, many other bacteria unclassified above the family rank are observed to inhabit mineral waters either in the aquifer or in the bottle (Table 29.3).

One of the basic assumptions on the production of bottled natural mineral or spring water is that the microbiota of the final product has its origin exclusively in the aquifer (Codex Alimentarius, 2001; European Commission, 2009). However, studies characterizing the microbiota of natural springs used to produce bottled mineral/spring water and the relationship between both are rare, particularly if based

Table 29.3 Diversity of bacteria identified on basis of 16S rRNA gene sequence analysis in mineral or spring water, detected in the aquifer or bottled water.

Phylum	Class	Family	Genus	Source	Method	Country	Reference
Bacteria Proteobacteria	U Alpha- proteobacteria	U Bradyrhizobiaceae	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Altipia</i>	B (M, 1.5 L, PET or P); B (S, Pl)	CD	NO/PT	2; 5
			<i>Bosea</i>	B (M, 1.5 L, PET)	CD	PT	2; 3
			<i>Bradyrhizobium</i>	BH (M); B (M, 1.5 L, PET); B (M, 0.5 L, PET)	CD; CI (P/C)	DE/PT	2; 3; 4
			<i>Rhodopseudomonas</i>	B (M, Pl)	CD	NO	5
			U	S	CD	SP	1
			<i>Brevundimonas</i>	BH (M); B (M, 1.5 L, PET)	CD	PT	2; 3
			<i>Caulobacter</i>	BH (M); B (M, 1.5 L, PET); B (M, 0.5 L, PET); B (M, Pl)	CD; CI (C)	DE/NO/PT	3; 4; 5
		U Brucellaceae Caulobacteraceae	<i>Phenyllobacterium</i>	BH (M); B (M, 1.5 L, Pl)	CD	NO/PT	3; 5
			U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Methylobacterium</i>	BH (M); B (M, 1.5 L, PET); B (M, 1.5 L, Pl)	CD; CI (P)	NO/PT	3; 5
			<i>Methylocystis</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Hyphomicrobium</i>	M	CI (D)	PT	3
			<i>Mesorhizobium</i>	M; BH (M); B (M, 1.5 L, PET)	CD; CI (D)	PT	3
			U	M	CI (D)	PT	3
			<i>Rhizobium</i>	S; B (M, 1.5 L, PET)	CD	FR/SP	1; 2
			<i>Novosphingobium</i>	BH (M); B (M, 1.5 L, PET)	CD; CI (P)	PT	3
			<i>Sphingobium</i>	BH (M)	CD	PT	3
			<i>Sphingomonas</i>	BH (M); S; B (M, 1.5 L, PET or Pl); B (M, Pl)	CD	NO/PT/SP	1; 3; 5
			U	B (M, 1.5 L, Pl)	CD	NO	5
			<i>Pseudolabrys</i>	BH (M)	CD	PT	3
			"Nordella"	B (M, 1.5 L, PET)	CD	FR	2
			<i>Reyranella</i>	B (M, 1.5 L, PET)	CD	PT	3
			U	BH (M)	CD	PT	3
Proteobacteria	Beta- proteobacteria	U Xanthobacteraceae U U-Rhodospirillales U	<i>Cupriavidus</i>	M; BH (M); B (M, 1.5 L, PET)	CD; CI (D/P)	PT	3
			<i>Limnobacter</i>	B (M, 1.5 L, PET); B (M, 0.5 L, PET)	CI (C)	DE	4
			<i>Ralstonia</i>	M; BH (M); B (M, 1.5 L, PET)	CD, CI (D)	PT	2; 3
			<i>Acidovorax</i>	BH (M); S; B (M, 1.5 L, PET)	CD, CI (P)	FR/PT/SP	1; 2; 3
			<i>Caenimonas</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Curvibacter</i>	M; BH (M); B (M, 1.5 L, PET); B (M, Pl)	CD, CI (D/P)	NO/PT	2; 3; 5
			<i>Delftia</i>	S; B (M, 1.5 L, PET)	CD	PT/SP	1; 3
			<i>Hydrogenophaga</i>	B (M, 1.5 L, PET); B (M, 0.5 L, PET); B (S, Pl)	CD; CI (C)	DE/FR/NO	2; 4; 5
			<i>Paucibacter</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Pelomonas</i>	BH (M); B (M, 1.5 L, PET)	CD	PT	3
			<i>Piscinibacter</i>	B (M, 1.5 L, PET)	CD	PT	3
			<i>Polaromonas</i>	B (M, 1.5 L, PET); B (M, 0.5 L, PET); B (M, Pl); B (S/A, G)	CD; CI (C)	DE	4; 5
		Beta- proteobacteria	<i>Cupriavidus</i>	M; BH (M); B (M, 1.5 L, PET)	CD; CI (D/P)	PT	3
			<i>Limnobacter</i>	B (M, 1.5 L, PET); B (M, 0.5 L, PET)	CI (C)	DE	4
			<i>Ralstonia</i>	M; BH (M); B (M, 1.5 L, PET)	CD, CI (D)	PT	2; 3
			<i>Acidovorax</i>	BH (M); S; B (M, 1.5 L, PET)	CD, CI (P)	FR/PT/SP	1; 2; 3
			<i>Caenimonas</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Curvibacter</i>	M; BH (M); B (M, 1.5 L, PET); B (M, Pl)	CD, CI (D/P)	NO/PT	2; 3; 5
			<i>Delftia</i>	S; B (M, 1.5 L, PET)	CD	PT/SP	1; 3
			<i>Hydrogenophaga</i>	B (M, 1.5 L, PET); B (M, 0.5 L, PET); B (S, Pl)	CD; CI (C)	DE/FR/NO	2; 4; 5
			<i>Paucibacter</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			<i>Pelomonas</i>	BH (M); B (M, 1.5 L, PET)	CD	PT	3
			<i>Piscinibacter</i>	B (M, 1.5 L, PET)	CD	PT	3
			<i>Polaromonas</i>	B (M, 1.5 L, PET); B (M, 0.5 L, PET); B (M, Pl); B (S/A, G)	CD; CI (C)	DE	4; 5

Proteobacteria	Delta- proteobacteria Gamma- proteobacteria	Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum Rhodospirillum rubrum	Rhodoferrax	B (M, 1.5 L, PET or PI); B (M, PI)	CD, CI (C)	DE/NO	4; 5
			Variorax	B (M, 1.5 L, PET or PI); B (S, PI); B (M, PI); B (S/A, G)	CD	FR/NO/PT	2; 5
			Xenophilus	B (M, 1.5 L, PET)	CD	PT	3
			U	B (S, PI)	CD	NO	5
			U	M	CI (D)	PT	3
			Duganella	M; BH (M); B (M, 1.5 L, PET)	CI (D/P)	PT	3
			Herbaspirillum	BH (M); B (M, 1.5 L, PET); B (S/A, G)	CD, CI (P)	NO/PT	2; 3; 5
			Herminiimonas	B (M, 1.5 L, PET)	CD	PT	3
			Janthinobacterium	S	CD	SP	1
			Undibacterium	S; B (M, PI)	CD	NO/SP	1; 5
	Rhodocyclaceae	Rhodocyclaceae	U	BH (M); B (M, 1.5 L, PET)	CD	PT	2; 3
			Propionivibrio	M	CI (D)	PT	3
			Zoogloea	M; B (M, 1.5 L, PET)	CI (D/P)	PT	3
			Aquabacterium	B (M, 1.5 L, PET)	CD	PT	3
			Ideonella	B (M, 1.5 L, PET); B (M, 0.5 L, PET); B (M, PI)	CD; CI (C)	DE/NO	4; 5
			U	B (M, 1.5 L, PET)	CD	PT	3
			U	BH (M); B (M, 1.5 L, PET)	CD; CI (C)	DE/PT	3; 4
			U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			Aeromonas	S	CD	SP	1
			Mangrovibacter	M	CI (D)	PT	3
	Moraxellaceae	Moraxellaceae	Pantoea	BH (M)	CD	PT	3
			Acinetobacter	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			Perluclidibaca	BH (M); B (M, 1.5 L, PET)	CD, CI (P)	PT	3
			Nevskia	M; BH (M); B (M, 1.5 L, PET)	CD, CI (D)	PT	3
			U	BH (M); B (M, 1.5 L, PET)	CD	PT	3
			Pseudomonas	M	CI (D)	PT	3
			U	BH (M); S; B (M, 1.5 L, PET)	CD	FR/PT/SP	1; 2; 3
			U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			Rudaea	M	CI (D)	PT	3
			Stenotrophomonas	B (M, 1.5 L, PET)	CD	PT	3
			U	S; B (M, 1.5 L, PET)	CD	PT/SP	1; 3
Bacteroidetes	Bacteroidetes	Bacteroidetes	Flectobacillus	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			Hymenobacter	BH (M)	CD	PT	3
			U	M	CI (D)	PT	3
			Cloacibacterium	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			Flavobacterium	BH (M)	CD	PT	3
			Pedobacter	B (M, 1.5 L, PET)	CD	PT	2
			Heliomonas	BH (M)	CD	PT	3
			U	M; BH (M)	CD, CI (D)	PT	3
			U				
			U				

(Continued)

Table 29.3 (Continued)

Phylum	Class	Family	Genus	Source	Method	Country	Reference
Actinobacteria	Actinobacteria	Brevibacteriaceae	<i>Brevibacterium</i>	B (M, 1.5 L, PET)	CI (P)	PT	3
		Corynebacteriaceae	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
		Dermacoccaceae	<i>Kytococcus</i>	BH (M)	CD	PT	3
		Dietziaceae	<i>Dietzia</i>	B (M, 1.5 L, PI)	CD	NO	5
		Gaiellaceae	<i>Gaiella</i>	BH (M)	CD	PT	3
Actinobacteria	Actinobacteria	Microbacteriaceae	<i>Curtobacterium</i>	BH (M)	CD	PT	3
			<i>Leifsonia</i>	BH (M)	CD	PT	3
		Microbacteriaceae	<i>Microbacterium</i>	M; BH (M); B (M, 1.5 L, PET); B (M, PI)	CD, CI (D)	NO/PT	2; 3 ; 5
			<i>Plantibacter</i>	B (M, 1.5 L, PET)	CD	PT	3
		Micrococcaceae	<i>Arthrobacter</i>	BH (M); B (M, 1.5 L, PET)	CD	PT	2; 3
			<i>Kocuria</i>	BH (M)	CD	PT	3
		Mycobacteriaceae	<i>Mycobacterium</i>	M; BH (M); S; B (M, 1.5 L, PET)	CD, CI (D)	PT/SP	1; 3
		Nocardiaceae	<i>Nocardia</i>	BH (M)	CD	PT	3
			<i>Marmoricola</i>	BH (M)	CD	PT	3
		Propionibacteriaceae	<i>Propionibacterium</i>	M	CI (D)	PT	3
		Sporichthyaceae	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
		U	U	BH (M)	CD	PT	3
		Bacillaceae	<i>Bacillus</i>	BH (M); B (M, 1.5 L, PET); B (S, PI)	CD	FR/NO/PT	2; 3 ; 5
			<i>Lysinibacillus</i>	BH (M); B (M, 1.5 L, PET)	CD	PT	2; 3
		Carnobacteriaceae	<i>Alloiococcus</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
Elusomicrobia	Elusomicrobia	Paenibacillaceae	<i>Paenibacillus</i>	BH (M)	CD	PT	3
		Planococcaceae	<i>Chryseomicrobium</i>	M	CI (D)	PT	3
		Staphylococcaceae	<i>Staphylococcus</i>	BH (M); B (M, 1.5 L, PET)	CD	FR/PT	2; 3
		Elusomicrobiaeae	U	M	CI (D)	PT	3
		U	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
Chlorobi	Ignavibacteria	SR-FBR-L83	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
		U-Chlorobiales	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
		Deinococci	U	M	CI (D)	PT	3
Deinococcus-Thermus	Deinococcus-Thermus	Deinococcaceae	<i>Deinococcus</i>	BH (M)	CD	PT	3
Nitrospirae	Nitrospirae	Nitrospiraceae	<i>Nitrospira</i>	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
			U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
		Clade SM2F09	U	BH (M); B (M, 1.5 L, PET)	CI (P)	PT	3
		Lineage Pla4	U	B (M, 1.5 L, PET)	CI (P)	PT	3
		U	U				

U, unclassified; M, natural mineral water; S, spring water; A, artesian water; BH, bore hole; PET, polyethylene terephthalate; PI, unspecified plastic; G, glass; C, clone library; D, denaturing gradient gel electrophoresis; P, pyrosequencing. CD, culture-dependent; CI, culture-independent; DE, Germany; FR, France; NO, Norway; PT, Portugal; SP, Spain.
References: 1, Casanovas-Massana and Blanch, 2012; 2, Falcone-Dias *et al.*, 2012; 3, França *et al.*, 2015; 4, Loy *et al.*, 2005; 5, Otterholt and Charnock, 2011.

on culture-independent methods. Another issue poorly studied is the microbiological stability of the aquifer. Curiously, in contrast to the stable mineral composition attributed to mineral water, França *et al.* (2015) observed noticeable changes in the structure of the aquifer bacterial community, when the microbiota in samples collected in two different years are compared. However, the abundance of total cells and culturable bacteria, reaching maxima of 1.5×10^4 of total cells/ml and 1×10^3 cfu/ml, respectively, did not vary sharply between the two years examined (França *et al.*, 2015). In both years, these authors observed a wide diversity of bacteria in the bore-hole, with members of the families *Enterobacteriaceae* (32%) and *Pseudomonadaceae* (23%) and the phylum *Cyanobacteria* (18%) among the most abundant groups detected by 454-pyrosequencing. Other less abundant (5–10%) bacteria included unclassified *Proteobacteria*, *Deltaproteobacteria*, *Chlorobi-Ignavibacteria*, *Actinobacteria-Corinebacteriaceae*, *Paucibacter* (*Alphaproteobacteria*), and *Methylocystis* (*Betaproteobacteria/Comamonadaceae*). As expected, none of the above-mentioned organisms could be recovered by culture-dependent methods. The most abundant culturable organisms recovered from the aquifer belonged to *Alphaproteobacteria* (*Bradyrhizobium*, *Novosphingobium*, *Phenylobacterium*), *Betaproteobacteria* (*Pelomonas*, *Undibacterium*), and *Actinobacteria* (*Mycobacterium*), with relative abundance above 10%. Other minor components (5–10%) were affiliated to genera such as *Sphingomonas*, *Caulobacter*, *Cupriavidus*, and *Paenibacillus*. Although some groups present in the aquifer were observed in the bottles, others were lost, suggesting a complex rearrangement of the microbial community after bottling.

29.5 Bottling effect

Bottling creates conditions capable of promoting the growth of culturable bacteria in up to four logarithmic cycles (Bischofberger *et al.*,

1990; França *et al.*, 2015). The increase in the bacterial counts, which is intense over the first and/or second week, does not significantly revert in the subsequent storage period (Bischofberger *et al.*, 1990; Loy *et al.*, 2005; França *et al.*, 2015). The maintenance of the bacterial counts observed over the subsequent periods justifies the high numbers of colony-forming units reported in bottled mineral water obtained from the retail market (Manaia *et al.*, 1990; Falcone-Dias *et al.*, 2012; Moazeni *et al.*, 2013). As could be inferred from the previous section on microbial diversity, although the effect of bottling has been a central issue on the microbiology of bottled water, understanding of the influence of the aquifer microbiota is scant (Bischofberger *et al.*, 1990; Leclerc and Moreau, 2002; Loy *et al.*, 2005). Among the factors that promote the bacterial growth observed after bottling, the supply of dissolved organic substances and the oxygenation of water occurring during bottling are strong candidates (Bischofberger *et al.*, 1990; Leclerc and Moreau, 2002; Loy *et al.*, 2005). The supply of organic carbon, due to leaching of the bottle material, has been considered a possible factor, since in glass bottles bacterial growth after bottling is smoother than in plastic (e.g., polyethylene terephthalate, PET, or polyvinyl chloride, PVC) (Bischofberger *et al.*, 1990). However, microbial growth after bottling was reported in situations in which leaching of plastic bottles, if it occurred, did not increase the organic content to levels above the detection limit (<0.5 ppm) after one year of storage (França *et al.*, 2015). On the other hand, it has been argued that the mechanical cleaning of glass bottles could leave some residues of antimicrobial agents, capable of inhibiting bacterial growth (Bischofberger *et al.*, 1990). Other hypotheses, such as the photodegradation of dissolved organic matter occurring in the bottle and the resuscitation, meaning the reversal of non-culturability, have been advanced to explain post-bottling growth (Leclerc and Moreau, 2002). Considering different studies and evidence, it is admissible that different factors interact in order to promote

the observed growth. According to EU legislation “the revivable total colony count of a natural mineral water may only be that resulting from the normal increase in the bacterial count which it had at source” (European Commission, 2009). Indeed, studies comparing the bacterial diversity from the spring to the bottle show that the observed growth is not due to the introduction of allochthonous populations during bottling, since the microbial community is fairly preserved (Bischofberger *et al.*, 1990; Vachée *et al.*, 1997; Loy *et al.*, 2005; França *et al.*, 2015). Despite the stability of the microbial community composition, there are variations in the structure of the community and these are not independent of the bottle material (glass or plastic) and size (0.5 or 1.5 l) (Bischofberger *et al.*, 1990; Loy *et al.*, 2005). Studies on the dynamics of populations suggest that the bottling process induces a founder effect in which selected microbial populations gain advantage and switch between inactive resting cells to actively multiplying bacteria (Loy *et al.*, 2005). Ultramicrocells, which belong to taxonomic groups frequently found in water, such as *Pseudomonas*, *Flavobacterium*, and *Sphingomonas*, may be involved in this process, regaining growth capacity after bottling (Jones *et al.*, 1999; Table 29.3). The preferential growth of some bacterial groups was demonstrated by Loy *et al.* (2005) for *Hydrogenophaga* and to a minor extent for other *Betaproteobacteria* of the genera *Aquabacterium* and *Polaramonas*. This rationale justifies the increase in the cultivability ratio (cultivable/total cells) observed after bottling (Loy *et al.*, 2005; França *et al.*, 2015). In addition, it supports the resuscitation hypothesis (Leclerc and Moreau, 2002). However, the factors inducing the resuscitation of some populations are not yet understood. According to França *et al.* (2015), the changes imposed by bottling may not always be the same. Over the six months period after bottling, the community structure varied differently in independent experiments conducted in two consecutive years (França *et al.*, 2015). Although the reasons for such differences were not discussed by the

authors, the different structures of the aquifer microbial community in the two years analyzed may be part of the explanation (França *et al.*, 2015). However, interestingly, França *et al.* (2015) show that, with the exception of *Paucibacter* and unclassified *Bacteria* or *Pseudomonadaceae*, none of the most abundant groups in the borehole, detected with culture-independent methods, constituted major populations in bottled water. In opposition, *Bradyrhizobium*, *Novosphingobium*, *Cupriavidus*, *Mycobacterium*, and other organisms not detected or minor representatives in the aquifer, such as *Acidovorax*, *Caenimonas*, *Curvibacter*, *Piscinibacter*, *Duganella*, *Ralstonia*, *Ideonella*, *Flectobacillus*, *Brevibacterium*, and unclassified *Methylophilaceae*, as well as *Armatimonadetes*, were major populations in bottled water (>10%).

29.6 Microbiological contamination

Quality and safety have been major motivations behind the consumption of bottled water (Codex Alimentarius, 2001; World Health Organization, 2008). However, although bottled water is not frequently associated with acute disease episodes (Leclerc and Moreau, 2002), some occasional outbreaks have been reported in different world regions (e.g., Portugal, 1974; Germany, 2005; Spain, 2006; China, 2012). Various microbial agents were involved in these outbreaks, including bacterial indicators. Hospital-acquired wounds, and throat and lung colonization by *Pseudomonas aeruginosa* transmitted by non-carbonated mineral water was reported in a German hospital, where 15 patients in an intensive care unit suffered of wound infection or pneumonia (Eckmanns *et al.*, 2008). Recently, a gastroenteritis outbreak with a presumed origin in bottled water (the origin and type of this water was not characterized in the publication) contaminated with virulent *E. coli* was reported in a University in China (Wang *et al.*, 2012). Another gastroenteritis outbreak presumably caused by *Salmonella* Kottbus

transmitted by bottled water (the origin and type of this water was not characterized in the publication) was reported in Spain on the island of Gran Canaria (Palmera-Suárez *et al.*, 2007). The importance of the microbiological quality control and protection of water springs for bottled water production is illustrated by the epidemic of cholera in Portugal in 1974. During this epidemic, it was possible to demonstrate that the consumption of non-carbonated bottled water was significantly more common among cholera patients than among controls. In addition, *Vibrio cholerae* was isolated from the two springs that supplied mineral water to the commercial bottling plant (Blake *et al.*, 1977).

Certainly the incidence of outbreaks associated with bottled water consumption decreased with the thoughtfulness imposed by international regulations (Codex Alimentarius, 2001; World Health Organization 2008; Table 29.2). However, safety is still a major issue. Over the last decades, numerous studies dealt with the possible microbiological risks associated with bottled water. Major opportunities for the occurrence of contamination are at the source, the bottling process, or during the storage period (Codex Alimentarius, 1985; Figure 29.2). These are frequently the focus of possible contamination surveys, which are normally based

on the bacterial indicators of contamination referred to in Table 29.2.

In spite of the regional and national regulations reinforced by international recommendations (Codex Alimentarius, 2001; World Health Organization, 2008; Table 29.2), the occurrence of total coliforms, *E. coli*, enterococci or *P. aeruginosa* in bottled mineral or spring water is regularly reported. These observations have been described in 0.5, 1.5, 18.9, or 20 l bottles and the abundance of contaminants do not vary significantly during storage (Falcone-Dias and Filho, 2013). When present, contamination is observed only in a very low percentage of the examined samples (Venieri *et al.*, 2006; Varga, 2011; Falcone-Dias and Filho, 2013). This suggests that contamination has a probable origin in the bottling plant or in a deficient sanitation of dispensers and of containers that are refilled (Defives *et al.*, 1999; Zamberlan da Silva *et al.*, 2008; Marzano *et al.*, 2011; Falcone-Dias and Filho, 2013). Container reuse and dispensers both associated with 18.9 or 20 l bottles, common in public areas, represent additional and very important critical points of contamination and microbial growth due to biofilm formation (Zamberlan da Silva *et al.*, 2008; Marzano *et al.*, 2011; Falcone-Dias and Filho, 2013).

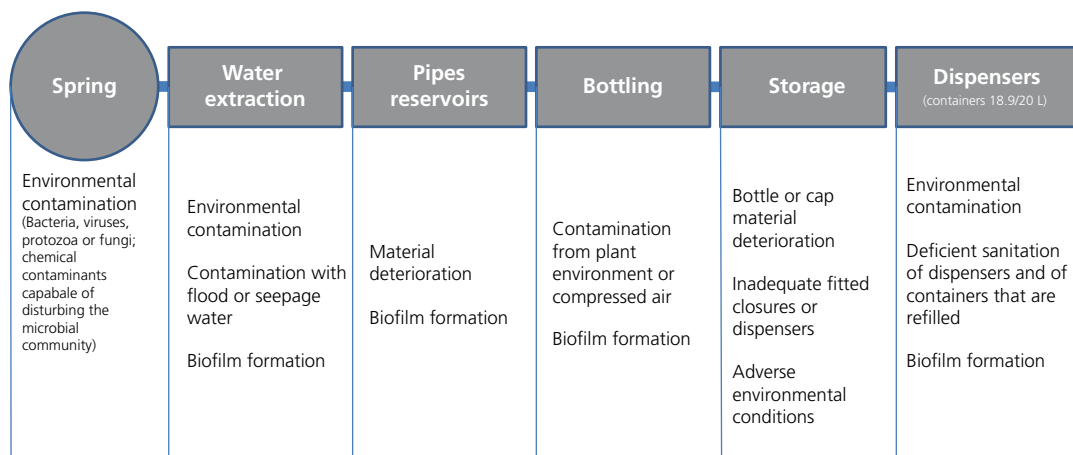


Figure 29.2 Critical points for disturbance of microbial communities or microbiological contamination, overgrowth, or decay.

These evidences demonstrate that multiple factors, either mechanical/physical, chemical, or microbiological, may favor the intake and/or the survival of microbiological contaminants. To control these factors at each stage, the implementation of hazard analysis critical control points (HACCP) systems contribute to improve water quality and consumers trust (Codex Alimentarius, 1985; Legnani *et al.*, 1999; Venieri *et al.*, 2006; Kokkinakis *et al.*, 2008). Critical points for microbiological hazards include all potential points of entrance or development of microorganisms, from the spring environment or water source to the product reaching the final consumer (Defives *et al.*, 1999; Kokkinakis *et al.*, 2008; Marzano *et al.*, 2011; Falcone-Dias and Filho, 2013) (Figure 29.2). The use of recommended hygienic practices for collecting, bottling, storage, and distribution is pivotal in the safety of bottled water (Codex Alimentarius, 1985).

When contamination or poor sanitation occurs, the fate of allochthonous bacteria entering the system will depend on several factors. The abundance of assimilable organic carbon, either supplied by sanitizers or containers migration or due to the lysis and disintegration of dead cells, may favor microbial proliferation (Defives *et al.*, 1999; Otterholt and Charnock, 2011). On the other hand, the ability to survive in the presence of low nutrient supply and to resist starvation conditions may be determinant on the success of allochthonous bacteria in water. The capacity to persist in low nutrient conditions may explain the persistent colonization of mineral water with *P. aeruginosa* and related organisms (Morais *et al.*, 1997; Legnani *et al.*, 1999; Marzano *et al.*, 2011). Also the abundance and nature of the autochthonous water microbiota is supposed to affect the fate of the allochthonous bacteria. Although the autochthonous microbiota of mineral water may have an inhibitory effect on the survival of contaminants such as *E. coli* or *P. aeruginosa* (Lucas and Ducluzeau, 1990; Moreira *et al.*, 1994), this is not a general rule. No effect or even growth promotion was reported after

intended contamination with isolates of the species *E. coli*, *Enterobacter cloacae*, or *Klebsiella pneumonia* (Moreira *et al.*, 1994). Indeed, the abundance of heterotrophic plate counts was observed to be positively correlated with the bottle survival time of *E. coli* (Otterholt and Charnock, 2011). These evidences suggest that a complex array of factors, not only chemical but mainly related with the bacterial diversity and evenness of the water microbiota, may determine the fate of a contaminant in bottled water.

29.7 A new perspective on microbiological quality and safety

Mineral and spring water holds a rich and diversified microbiota (Table 29.3). A general and pertinent assumption is that autochthonous microbiota of mineral and spring water will not represent any kind of immediate or long-term threat for human health. However, the current knowledge on mineral water microbiota and the renewed perspective of the high potential of environmental and commensal bacteria as opportunistic pathogens for humans is an invitation to reconsider the above-mentioned assumption. Mineral or spring bottled waters from different geographic areas are inhabited by both opportunistic pathogens and antibiotic-resistant bacteria (Wilkinson and Kerr, 1998; Falcone-Dias *et al.*, 2012; Vaz-Moreira *et al.*, 2014). Antibiotic resistant bacteria are known to occur in nature and pristine water habitats (Vaz-Moreira *et al.*, 2014). However, its presence in bottled water calls for some attention, since it is destined for human ingestion. Many of the mineral and spring water bacterial groups in which resistance have been observed, such as *Afipia*, *Bosea*, *Brevundimonas*, *Curvibacter*, *Ralstonia*, *Variovorax*, *Acinetobacter*, *Pantoea*, *Pseudomonas*, *Stenotrophomonas*, *Flavobacterium*, *Pedobacter*, *Arthrobacter*, *Microbacterium*, *Bacillus*, or *Staphylococcus*, have also been reported in the human microbiome, suggesting that transmission

of these bacteria from water to humans is, in principle, possible (Vaz-Moreira *et al.*, 2014; Table 29.3). On the other hand, the detection of antibiotic resistant bacteria, at densities as high as 100 cfu/ml, displaying resistance to more than 20 antibiotics belonging to eight different classes, including third-generation cephalosporins, carbapenems, and fluoroquinolones, may indicate that the risk of causing any harm to humans is not negligible (Falcone-Dias *et al.*, 2012). Most of the resistance phenotypes are probably intrinsic in these bacteria. However, their presence is not exempt from concerns for two major reasons: (i) some of these mineral and spring water bacteria are confirmed or suspected nosocomial or opportunistic pathogens; (ii) these bacteria may transfer resistance to human pathogens. Groups such as *Pseudomonas*, *Stenotrophomonas*, *Ralstonia*, *Afipia*, *Bosea*, and many others that are antibiotic resistant comprise opportunistic pathogens or persistent nosocomial agents (La Scola *et al.*, 2003; Daxboeck *et al.*, 2005; Berger *et al.*, 2006; Ryan *et al.*, 2006). Although possibly innocuous if colonizing a human, they may proliferate and gain advantage under antibiotherapy conditions, reducing the chances of therapy success. In addition, even if assuming that these will be a rare genetic event, the possibility of transfer of resistance from these bacteria to human pathogens cannot be excluded (Vaz-Moreira *et al.*, 2014). Considering the important advances on the natural microbiomes studies, mainly due to the enthusiastic use of next-generation sequencing tools, and the deep insights into the human microbiome, it is time to have a renewed perspective of valid criteria to assess bottled water quality and safety.

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References

- Amann, R.I., Ludwig, W., and Schleifer, K.-H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiology Reviews*, **59**, 143–169.
- APHA (American Public Health Association). 2005. *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.
- Armas, A.B. and Sutherland, J.P. 1999. A survey of the microbiological quality of bottled water sold in the UK and changes occurring during storage. *International Journal of Food Microbiology*, **48**, 59–65.
- Azoulay, A. Garzon, P., and Eisenberg M.J. 2001. Comparison of the mineral content of tap water and bottled waters. *Journal of General Internal Medicine*, **16**, 168–175.
- Berger, P., Papazian, L., Drancourt, M., La Scola, B., Auffray, J.-P., and Raoult, D. 2006. Ameba-associated microorganisms and diagnosis of nosocomial pneumonia. *Emerging Infectious Diseases*, **12**, 248–255.
- Bischofberger, T., Cha, S.K., Schmitt, R., Konig, B., and Schmidt-Lorenz, W. 1990. The bacterial flora of non-carbonated, natural mineral water from the springs to reservoir and glass and plastic bottles. *International Journal of Food Microbiology*, **11**, 51–71.
- Blake, P.A., Rosenberg, M.L., Florencia, J., Costa, J.B., do Prado Quintino, L., and Gangarosa, E.J. 1977. Cholera in Portugal, 1974. II. *Transmission by bottled mineral water. American Journal of Epidemiology*, **105**, 344–348.
- Boulos, L., Prévost, M., Barbeau, B., Coallier, J., and Desjardins, R. 1999. LIVE/DEAD BacLight: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *Journal of Microbiological Methods*, **37**, 77–86.
- Casanovas-Massana, A. and Blanch, A.R. 2012. Diversity of the heterotrophic microbial populations for distinguishing natural mineral waters.

- International Journal of Food Microbiology*, **153**, 38–44.
- Codex Alimentarius. 1985. Recommended international code of hygienic practice for the collecting, processing and marketing of natural mineral waters (CAC/RCP 33-1985). Available at: <http://www.codexalimentarius.net> (accessed 13.05.14).
- Codex Alimentarius. 2001. Codex standard for natural mineral waters (Codex Stan 108, 1981, rev. 1-1997). Available at: <http://www.codexalimentarius.net> (accessed 13.05.14).
- Daxboeck, F., Stadler, M., Assadian, O., Marko, E., Hirschl, A.M., and Koller, W. 2005. Characterization of clinically isolated *Ralstonia mannitolilytica* strains using random amplification of polymorphic DNA (RAPD) typing and antimicrobial sensitivity, and comparison of the classification efficacy of phenotypic and genotypic assays. *Journal of Medical Microbiology*, **54**, 55–61.
- Defives, C., Guyard, S., Oularé, M.M., Mary, P., and Hornez, J.P. 1999. Total counts, culturable and viable, and non-culturable microflora of a French mineral water: a case study. *Journal of Applied Microbiology*, **86**, 1033–1038.
- Eckmanns, T., Oppert, M., Martin, M., Amorosa, R., Zuschneid, I., Frei, U., Rüden, H., and Weist, K. 2008. An outbreak of hospital-acquired *Pseudomonas aeruginosa* infection caused by contaminated bottled water in intensive care units. *Clinical Microbiology and Infection*, **14**, 454–458.
- European Commission. 1998. Directive 98/83/EEC. On the quality of water intended for human consumption. The Council of the European Communities. *Official Journal*, **L330**, 5.12.1998.
- European Commission. 2009. Directive 2009/54/EC. On the exploitation and marketing of natural mineral waters (Recast). *Official Journal*, **L164**, 26-6-2009, pp. 0045–0058.
- European Community. 1980. Directive 80/777/EEC. On the approximation of the laws of the member states relating to the exploitation and marketing of natural mineral waters. *Official Journal of the European Communities*, **L229**, 1–10.
- Euzéby, J.P. 1997. List of bacterial names with standing in nomenclature: a folder available on the Internet. *International Journal of Systematic Bacteriology*, **147**, 590–592.
- Falcone-Dias, M.F. and Filho, A.F. 2013. Quantitative variations in heterotrophic plate count and in the presence of indicator microorganisms in bottled mineral water. *Food Control*, **31**, 90–96.
- Falcone-Dias, M.F. Vaz-Moreira, I., and Manaia, C.M. 2012. Bottled mineral water as a potential source of antibiotic resistant bacteria. *Water Research*, **46**, 3612–3622.
- Ferreira, A.-C., Morais, P.V., and da Costa, M.S. 1994. Alterations in total bacteria, iodonitrophenyltetrazolium (INT)-positive bacteria, and heterotrophic plate counts of bottled mineral water. *Canadian Journal of Microbiology*, **40**, 72–77.
- Food and Drug Administration (FDA). 2013. Beverages: Bottled water; final rule. *Federal Register*, **21**, CFR 165.110.
- França, L., López-López, A., Rosselló-Móra, R., and da Costa, M.S. 2015. Microbial diversity and dynamics of a groundwater and a still bottled natural mineral water. *Environmental Microbiology*, **17**, 577–593. doi: 10.1111/1462-2920.12430.
- Hansen, T., Skånseng, B., Hoorfar, J., and Löfström, C. 2013. Evaluation of direct 16S rDNA sequencing as a metagenomics-based approach to screening bacteria in bottled water. *Biosecurity and Bioterrorism*, **11**, S158–165.
- Hunter, P.R. 1993. The microbiology of bottled natural mineral waters. *Journal of Applied Bacteriology*, **74**, 345–352.
- International Organization for Standardization (ISO). 2001. Water quality – enumeration of culturable microorganisms and colony count by inoculation in a nutrient agar culture medium, Report 6222:2001. International Organization for Standardization, Geneva, Switzerland.
- International Organization for Standardization (ISO). 2002. Water quality – detection and enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method, Report 9308-1:2002. International Organization for Standardization, Geneva, Switzerland.

- International Organization for Standardization (ISO). 2003. Water quality – detection and enumeration of intestinal enterococci. Part 2: Membrane filtration method, Report 7899-2:2003. International Organization for Standardization, Geneva, Switzerland.
- International Organization for Standardization (ISO). 2008. Water quality – detection and enumeration of *Pseudomonas aeruginosa* – method by membrane filtration, Report 16266:2008. International Organization for Standardization, Geneva, Switzerland.
- Janda, J.M. and Abbott, S.L. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, **45**, 2761–2764.
- Jones, C.R., Chamberlain, A.H.L., and Adams, M.R. 1999. An investigation of the presence of ultramicrocells in natural mineral water. *Letters in Applied Microbiology*, **28**, 275–279.
- Kokkinakis, E.N., Fragkiadakis, G.A., and Kokkinaki, A.N. 2008. Monitoring microbiological quality of bottled water as suggested by HACCP methodology. *Food Control*, **19**, 957–961.
- Korzeniewska, E., Filipkowska, Z., Domeradzka, S., and Włodkowski, K. 2005. Microbiological quality of carbonated and non-carbonated mineral water stored at different temperatures. *Polish Journal of Microbiology*, **54**, 27–33.
- La Scola, B., Mallet, M.N., Grimont, P.A., and Raoult, D. 2003. *Bosea eneeae* sp. nov., *Bosea massiliensis* sp. nov. and *Bosea vestrisii* sp. nov., isolated from hospital water supplies, and emendation of the genus *Bosea* (Das *et al.*, 1996). *International Journal of Systematic and Evolutionary Microbiology*, **53**, 15–20.
- Leclerc, H. and da Costa, M.S. 2007. Microbiology of natural mineral waters. In: *Technology of Bottled Water* (eds Senior, D. and Dege, N.), 2nd edn, pp. 325–387. Blackwell Publishing Ltd, Oxford, UK.
- Leclerc, H. and Moreau, A. 2002. Microbiological safety of natural mineral water. *FEMS Microbiology Reviews*, **26**, 207–222.
- Legnani, P., Leoni, E., Rapuano, S., Turin, D., and Valenti, C. 1999. Survival and growth of *Pseudomonas aeruginosa* in natural mineral water: a 5-year study. *International Journal of Food Microbiology*, **53**, 153–158.
- Loy, A., Beisker, W., and Meier, H. 2005. Diversity of bacteria growing in natural mineral water after bottling. *Applied and Environmental Microbiology*, **71**, 3624–3632.
- Lucas, F. and Ducluzeau, R. 1990. Antagonistic role of various bacterial strains from the autochthonous flora of gas-free mineral water against *Escherichia coli*. *Sciences des Aliments*, **10**, 62–73.
- Manaia, C.M., Nunes, O.C., Morais, P.V., and da Costa, M.S. 1990. Heterotrophic plate counts and the isolation of bacteria from mineral water using selective and enrichment media. *Journal of Applied Bacteriology*, **69**, 871–876.
- Marzano, M.A., Ripamonti, B., and Balzaretto, C.M. 2011. Monitoring the bacteriological quality of Italian bottled spring water from dispensers. *Food Control*, **22**, 333–336.
- Massa, S., Caruso, M., Trovatelli, F., and Tosques, M. 1998. Comparison of plate count agar and R2A medium for enumeration of heterotrophic bacteria in natural mineral water. *World Journal of Microbiology and Biotechnology*, **14**, 727–730.
- Moazeni, M., Atefi, M., Ebrahimi, A., Razmjoo, P., and Vahid Dastjerdi, M. 2013. Evaluation of chemical and microbiological quality in 21 brands of Iranian bottled drinking waters in 2012: a comparison study on label and real contents. *Journal of Environmental and Public Health*, **2013**, 469590. doi: 10.1155/2013/469590.
- Morais, P.V. and da Costa, M.S. 1990. Alterations in the major heterotrophic bacterial populations isolated from a still bottled mineral water. *Journal of Applied Bacteriology*, **69**, 750–757.
- Morais, P.V., Mesquita, C., Andrade, J.L., and da Costa, M.S. 1997. Investigation of persistent colonization by *Pseudomonas aeruginosa*-like strains in a spring water bottling plant. *Applied and Environmental Microbiology*, **63**, 851–856.
- Moreira, L., Agostinho, P., Morais, P.V., and da Costa, M.S. 1994. Survival of allochthonous bacteria in still mineral water bottled in polyvinyl

- chloride (PVC) and glass. *Journal of Applied Bacteriology*, **77**, 334–339.
- Otterholt, E. and Charnock, C. 2011. Microbial quality and nutritional aspects of Norwegian brand waters. *International Journal of Food Microbiology*, **144**, 455–463.
- Palmera-Suárez, R., Garcia, P., Garcia, A., Barrasa, A., and Herrera, D. 2007. *Salmonella* Kottbus outbreak in infants in Gran Canaria (Spain), caused by bottled water, August–November 2006. *Eurosurveillance*, **12**, 3235.
- Pontara, A.V., de Oliveira, C.D., Barbosa, A.H., Dos Santos, R.A., Pires, R.H., and Martins, C.H. 2011. Microbiological monitoring of mineral water commercialized in Brazil. *Brazilian Journal of Microbiology*, **42**, 554–559.
- Ramalho, R., Cunha, J., Teixeira, P., and Gibbs, P.A. 2001. Improved methods for the enumeration of heterotrophic bacteria in bottled mineral waters. *Journal of Microbiological Methods*, **44**, 97–103.
- Ryan, M.P., Pembroke, J.T., and Adley, C.C. 2006. *Ralstonia pickettii*: a persistent Gram-negative nosocomial infectious organism. *Journal of Hospital Infection*, **62**, 278–284.
- Saleh, M.A., Abdel-Rahman, F.H., Woodard, B.B., Clark, S., Wallace, C., Aboaba, A., Zhang, W., and Nance, J.H. 2008. Chemical, microbial and physical evaluation of commercial bottled waters in greater Houston area of Texas. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering*, **43**, 335–347.
- Stender, H., Broomer, A., Oliveira, K., Perry-O'Keefe, H., Hyldig-Nielsen, J.J., Sage, A., Young, B., and Coull, J. 2000. Rapid detection, identification, and enumeration of *Pseudomonas aeruginosa* in bottled water using peptide nucleic acid probes. *Journal of Microbiological Methods*, **42**, 245–253.
- Vachée, A., Vincent, P., Struijk, C.B., Mossel, D.A.A., and Leclerc, H. 1997. A study of the fate of the autochthonous bacterial flora of still mineral waters by analysis of restriction fragment length polymorphism of genes coding for rRNA. *Systematic and Applied Microbiology*, **20**, 492–503.
- Varga, L. 2011. Bacteriological quality of bottled natural mineral waters commercialized in Hungary. *Food Control*, **22**, 591–595.
- Vaz-Moreira, I., Egas, C., Nunes, O.C., and Manaia, C.M. 2011. Culture-dependent and culture-independent diversity surveys target different bacteria – a case study in a freshwater sample. *Antonie van Leeuwenhoek Journal of Microbiology*, **100**, 245–257.
- Vaz-Moreira, I., Egas, C., Nunes, O.C., and Manaia, C.M. 2013. Bacterial diversity from the source to the tap: a comparative study based on 16S rRNA-DGGE and culture-dependent methods. *FEMS Microbiology Ecology*, **83**, 361–374.
- Vaz-Moreira, I., Nunes, O.C., and Manaia, C.M. 2014. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS Microbiology Reviews*, **38**, 761–778. doi: 10.1111/1574-6976.12062.
- Venieri, D., Vantarakis, A., Komninou, G., and Papapetropoulou, M. 2006. Microbiological evaluation of bottled non-carbonated (“still”) water from domestic brands in Greece. *International Journal of Food Microbiology*, **107**, 68–72.
- Wang, R., Cheng, H., Zong, J., Yu, P., Fu, W., Yang, F., Shi, G., and Zeng, G. 2012. An outbreak of acute gastroenteritis associated with contaminated bottled water in a university – Jiangxi, China, 2012. *Western Pacific Surveillance and Response Journal*, **3**, 20–24.
- Wilkinson, F.H. and Kerr, K.G. 1998. Bottled water as a source of multi-resistant *Stenotrophomonas* and *Pseudomonas* species for neutropenic patients. *European Journal of Cancer Care*, **7**, 12–14.
- World Health Organization (WHO). 2008. *Guidelines for Drinking-Water Quality*, 4th edn. World Health Organization, Geneva, Switzerland.
- Zamberlan da Silva, M.E., Santana, R.G., Guilhermetti, M., Filho, I.C., Endo, E.H., Ueda-Nakamura, T., Nakamura, C.V., and Dias Filho, B.P. 2008. Comparison of the bacteriological quality of tap water and bottled mineral water. *International Journal of Hygiene and Environmental Health*, **211**, 504–509.

PART IV

Closing section

CHAPTER 30

Microbial risk assessment: integrating and quantifying the impacts of food processing operations on food safety

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30.1 Introduction

Risk assessment is usually performed to assess a public health risk linked to the consumption of a potentially contaminated food by a large population, but it can also be restricted to the exposure assessment step or focused on individual risk. The population or public health risk is deduced from the distribution of the individual risk in the population resulting from the consumption of a food product contaminated by a pathogenic microorganism, and from the consumption data of the population of concern. Usually the annual risk is estimated in a country by multiplying the mean individual risk per serving by the number of servings consumed by the population in the country during one year. The individual risk is deduced from the exposure to the microbiological hazard under consideration when eating the food, and from the dose–response relationship determining the probability of infection following the consumption of contaminated servings. The exposure assessment requires information about the contamination of the food product at the time of consumption and information about the serving sizes. The final contamination level of the food under consideration depends on processes applied along the food pathway and

the evaluation of the dynamics of microbiological populations during these operations is often of primary importance when performing exposure assessment.

The food-production pathway is usually described by a succession of processing steps or modules where hazards can be introduced or eliminated, or can increase or decrease. This modeling framework was designated as the “modular process risk model” (MPRM) by Nauta (2001). Various basic processes occurring during the processing modules and affecting the fate of microorganisms along the food chain have been identified. The six basic processes proposed by Nauta (2001) were divided into two “microbial” basic processes: growth and inactivation, and four “food-handling” processes: food mixing, partitioning, removal, and cross-contamination. Numerous “farm-to-fork” models for quantitative microbial risk assessment (QMRA) have adopted the MPRM (Delhalle *et al.*, 2009; Nauta *et al.*, 2009; Billoir *et al.*, 2011; Malakar *et al.*, 2011; Daelman *et al.*, 2013; Pujol *et al.*, 2013; Rigaux *et al.*, 2013; Tenenhaus-Aziza *et al.*, 2014). This framework was also used for recently developed quantitative risk assessment tools. For instance, the process model of FDA-iRISK[®] software is based on a succession of process stages that can be associated with process

Table 30.1 Impact of basic processes on the prevalence of the microbial hazard (P) and on the microbial level (number of cells, N , or concentration of cells, C). “0” no change, “–” decrease, “+” increase (adapted from Nauta, 2001).

Processes		Discrete modeling approach			Concentration modeling approach		Unit size
		P	N_{unit}^a	N_{total}^b	P	C	
Microbial processes	Growth	0	+	+	0	+	0
	Inactivation	–	–	–	0	–	0
Food-handling processes	Mixing	+	–	0	+	–	+
	Partitioning	–	+	0	0	0	–
	Cross-contamination = redistribution	+	–	0	+	–	0
	Cross-contamination = addition	+	+	+	+	+	0
	Removal	–	0/–	–	–	0/–	0

^a N_{unit} is the number of microbial cells per food unit.

^b N_{total} is the total number of microbial cells over all food units.

types describing the impact on microbiological contamination. Although these process types are slightly different from Nauta’s basic processes, they also refer to microbial increase or decline and to food-handling processes (Chen *et al.*, 2013). Each basic process affects the prevalence of the microbial hazard, i.e., the fraction of contaminated food units and/or the microbial level in contaminated food units (Table 30.1).

The aim of this chapter is to illustrate how unitary food processing operations and successive food operations quantitatively impact exposure assessment and individual and public health risks. The second part of the chapter will be dedicated to the use of risk-based metrics in food processing.

30.2 Basic processes encountered during food processing operations

30.2.1 Microbial processes

The growth and inactivation of microbial pathogens have been studied for decades. The first mathematical models describing the inactivation of food-borne pathogens were developed more than one century ago (Mafart *et al.*,

2010) and the early works related to the heat resistance of *Clostridium botulinum* spores (Bigelow, 1921) were successfully used to ensure the safety of low-acid canned foods (Esty and Meyer, 1922). Nowadays, many inactivation models are available (Geeraerd *et al.*, 2005) and can be easily used to estimate the fate of microorganisms in contaminated food units subjected to inimical processes. The bacterial growth during food storage is one of the main factors influencing the exposure of consumers to food-borne pathogens (Nauta *et al.*, 2003; Pouillot *et al.*, 2007; Daelman *et al.*, 2013) and the risk is frequently impacted by the growth during storage in consumer fridges (Yang *et al.*, 2006; Ross *et al.*, 2009). Numerous models have therefore been developed for more than 20 years to describe the growth of food-borne pathogens and “predictive microbiology”, a specific field within microbiology, is dedicated to this modeling exercise (Ross and McMeekin, 2003).

30.2.1.1 Growth

The growth of bacterial pathogens in food will increase the number of microbial cells in initially contaminated food units but the prevalence will not be affected (Table 30.1). This increase in the level of microbial cells present in

food units is obviously accompanied by an increase in individual and public health risks.

Among the factors affecting the bacterial growth, the storage temperature and duration are the process parameters that influence the most risk linked to ready-to-eat (RTE) food products contaminated by opportunistic food-borne pathogens requiring high exposure to cause illness, such as *Listeria monocytogenes* (Pouillot *et al.*, 2009; Koutsoumanis *et al.*, 2010; Mataragas *et al.*, 2010). The effect of growth on exposure and risk is less important when foods are cooked or contaminated by more pathogenic food-borne microorganisms requiring only a low microbial dose to cause illness such as verotoxinogen *Escherichia coli* (Danyluk and Schaffner, 2011; Smith *et al.*, 2013).

Studies in predictive microbiology generally focus on the development of models to describe the lag time and the growth rate of microorganisms in food, but several authors (Pouillot *et al.*, 2009; Ellouze *et al.*, 2010; Pouillot and Lubran, 2011) have also shown that the maximum population density and bacterial competition were the most influential factors when estimating the risk of listeriosis in foods permitting the growth of the pathogen during storage.

30.2.1.2 Inactivation

The inactivation or removal of microbial pathogens induces a decrease in the number of microbial cells in contaminated food units (Table 30.1) and in risks linked to food-borne pathogens. When risk assessors choose to model change in the microbial concentration, the prevalence of contaminated food units remains unchanged, but when the QMRA model is based on the number of microbial cells per food unit, the inactivation process can result in a decrease in the prevalence (Pouillot *et al.*, 2015). Indeed, in this approach, the initially contaminated units can contain no living cells after the inactivation treatment (Membré *et al.*, 2006).

Numerous QMRA are dedicated to the efficiency assessment of cooking protocols to reduce the risk linked to contaminated foods by food-borne pathogens (Bemrah *et al.*, 2003;

Malakar *et al.*, 2011; Smith *et al.*, 2013). For instance, the cooking temperature was confirmed as being one of the main factors influencing the risk of infection by *E. coli* O157 when eating ground beef or beef cuts (Smith *et al.*, 2013; Kiermeier *et al.*, 2015).

30.2.2 Food-handling processes

30.2.2.1 Mixing

In a mixing or pooling process, food units are congregated into larger units. Some contaminated small units will be combined with uncontaminated units, resulting in an increase in prevalence and a decrease in the concentration of the microbial pathogen in large contaminated units (Table 30.1). The impact of an unequal contribution of small units has been further studied by Nauta (2005). The consequence of mixing on public health risk is uncertain because it only depends on the mean individual risk that will be affected in various ways according to the total number of microbial cells reallocated and the pathogenicity of the food-borne pathogen of concern. On the other hand, this process will tend to decrease the variability of exposure and the individual risk will be more homogeneously distributed among consumers.

This basic mixing process is commonly encountered in many food production scenarios, such as ground beef manufacturing (Smith *et al.*, 2013), milk collection from multiple farm tanks (Albert *et al.*, 2005), shredded lettuce processing (Danyluk and Schaffner, 2011), or processed food manufacturing (Daelman *et al.*, 2013).

30.2.2.2 Partitioning

Partitioning is the opposite of mixing. In this process, food units are subdivided into smaller units. When risk assessors model the concentration of the microbial pathogen assuming a homogeneous contamination, the prevalence and concentration in food units are not changed by this process. However, when the change in the number of cells per unit is modeled (Pouillot *et al.*, 2015), the prevalence may decrease and the number of microbial cells per food unit will increase

(Table 30.1). The impact of both cell clustering and unequal sized units on the reallocation of microbial cells in food units have been investigated by Nauta (2005). The effect of this process on public health risk is difficult to assess because it depends on numerous factors such as the number of microbial cells present in food units and the dose–response relationship of the pathogen under consideration, but it generally gives rise to an increasing heterogeneity in exposure with more uncontaminated food units and, inversely, more highly contaminated units increasing the variability of individual risk for consumers.

As for mixing, the partitioning process is also very common in the food industry. For instance, many processes involve the dispensation of bulk food into individual units (Malakar *et al.*, 2011; Smith *et al.*, 2013; Rigaux *et al.*, 2013), or partitioning for example in cheese molding (Sanaa *et al.*, 2004), or dicing in the diced-bacon making process (Billoir *et al.*, 2011).

30.2.2.3 Cross-contamination

Cross-contamination can refer to the transmission of microbial cells among food units. This process is described as “redistribution” (Chen *et al.*, 2013) and implies an increase in the prevalence and a decrease in the concentration of contaminated food units (Table 30.1). As in mixing, the impact of this process on public health risk is difficult to predict but we can presume that the individual risk is less heterogeneously distributed among consumers. Cross-contamination can also refer to the transmission of microbial cells from the processing environment, i.e., hands of operators, equipment, or processing premises. This process corresponds to the introduction or “addition” (Chen *et al.*, 2013) of the micro-organism in food and is characterized by an increase in the prevalence and in the microbial level in food units (Table 30.1). In this case, the individual and public health risks are increased.

Cross-contamination is the most studied food-handling process for the assessment of risk linked to the contamination of broiler meat by

Campylobacter. Numerous models have been proposed to describe the transfer of the bacterium during the processing of the broiler carcasses (Nauta *et al.*, 2009) or during the food preparation in the domestic kitchen (Mylius *et al.*, 2007; Nauta and Christensen, 2011; Gkogka *et al.*, 2013a).

30.2.2.4 Removal

Some food units can be partly or totally removed from the production process. Removal can be relevant when detection of food-borne pathogens is performed and when contaminated foods are withdrawn from the food chain. Because of the increasing sensitivity of microbiological detection methods, the highly contaminated units (or batches) may be removed more easily than units contaminated with a few microbial cells (Table 30.1). The impact of removal on public health risk is then significant since situations where the individual risk is very high will disappear, leading to a large decrease in the individual risk mean.

Kiermeier *et al.* (2015) showed that the risk of *E. coli* O157-associated illness was overestimated when the prevalence and concentration estimates of *E. coli* O157 observed in withdrawn positive batches of beef were used to assess the risk linked to the consumption of ground beef patties. Perrin *et al.* (2015) evaluated the impact of interventions such as milk farm sorting or microbiological criteria for end products on the risk of haemolytic uremic syndrome associated with the presence of enterohemorrhagic *E. coli* in raw milk soft cheeses.

30.2.3 Succession and combination of basic processes

Food safety operators are more and more aware of risk-based approaches to manage the safety of food production operations. QMRA is now widely used, not only by food authorities but also by the industry, particularly the exporting companies, which are increasingly required to undertake product risk assessments before commercializing their goods at an international level. Several examples are thus available in the

literature to describe the effect of food processing on consumer exposure to a particular hazard by consumption of a contaminated food or to illustrate the impact of a succession of unitary food processing operations on individual and/or public health risks. These examples cover a large number of foods and hazards, some of which are discussed in this section dedicated to the quantification of the impact of food processing operations on food safety through the use of microbial risk assessment. The approaches used to describe the different food processes are based on qualitative or quantitative models, static or dynamic modeling approaches, and provide deterministic or stochastic results taking into account the variability and/or the uncertainty using computation techniques such as Monte Carlo simulations or Bayesian inference. Sensitivity analysis is also used as a statistical tool to identify the most impacting steps on the final microbial concentration or risk.

30.2.3.1 Dynamics of microbial populations along food production steps

Numerous studies have been published to describe the dynamic of microorganisms along food processing steps. One example is given in the microbiological risk assessment performed by the Food Standards Australia New Zealand for raw milk cheeses (FSANZ, 2009). The purpose of this risk assessment was to use available scientific data and published models to quantify the impact of processing steps on the safety of raw milk cheeses and to rank them according to their relative importance. The authors combined information on prevalence data obtained from scientific literature or experts' opinions to the effects of the different processing technologies on selected hazards. Two different approaches were used to determine the effects of the process on selected pathogens in the various cheeses according to data available. For some categories (raw milk extra hard and Swiss-type cheeses) a qualitative approach was adopted, by assigning a certain log reduction during specific operations like

curd cooking or ripening, while quantitative approaches were developed for some other categories (raw milk Cheddar, blue, Camembert, and Feta style cheeses) for *E. coli*, *Staphylococcus aureus*, and *L. monocytogenes*.

When modeling the successive processing stages, risk assessors can choose to describe the changes in microbial concentration in food units (expressed in cfu/g) or in microbial numbers per food unit (expressed in cfu/unit). Stewart *et al.* (2003) described the evolution of the concentration of *S. aureus* during the manufacture of cream-filled baked goods. In their model, they assumed that the maximum concentration in raw materials is 10^2 cfu/g. They proposed an inactivation of 5 decimal reductions during cooking to reach a concentration after this step of 10^{-3} cfu/g. Cross-contamination can afterwards happen causing an addition of microorganisms and growth can also occur during the storage of cooked cream filling, leading to an increase in the concentration of 5 logarithms and a maximum concentration of 10^2 cfu/g in the filling of the baked goods. Finally, growth can take place during the storage and distribution of the finished products and a maximum concentration of 10^4 cfu/g is reached when cream-filled baked cakes are eaten. The main advantage of this approach lies in its simplicity when combining successive food processing stages. Unfortunately, in some cases, this approach can lead to erroneous assessments. Pouillot *et al.* (2015) showed that this method could provide a considerable overestimation of the risk, especially when a large microbial inactivation is followed by a growth step. This phenomenon is linked to the fact that the prevalence is not affected by microbial inactivation in this modeling approach only based on the concentration.

The "gold-standard", but more complex, method consists in modeling the prevalence and the number of microbial cells per contaminated food unit along the food pathway. Pouillot *et al.* (2015) showed that an alternative and simpler method, consisting in modeling the expected number (and not the variability distribution) of

cells per food unit, gave similar estimates of the average individual risk per serving and then of the public annual health risk.

Most of the recently published exposure or risk assessment studies are based on this discrete modeling approach. For instance, Billoir *et al.* (2011) used this approach to model the fate of *L. monocytogenes* during the manufacture of diced bacon. This process consists in the following production steps: brining and tumbling, steaming, dicing, and packaging. In their study, batches of 1000 breasts were initially contaminated by approximately 3000 *L. monocytogenes* cells. The initial prevalence for the breasts was 95% while 50% of the breasts contained less than 3 *L. monocytogenes* cells and 100% less than 12 cells. After brining and tumbling leading to cross-contamination with redistribution of bacterial cells, the maximum contamination of breasts was 9 cells. The following steaming step induced inactivation, leading to 50% of breasts contaminated by less than 2 cells and 100% contained less than 6 cells. Dicing was characterized by a partitioning of each breast in approximately 2000 dices. The authors estimated also that the dicing machine could contaminate dices and that cross-contamination could then occur during this step. The prevalence for dices was 1% and the maximum contamination was 2 cells per contaminated dice. Finally, approximately 60 dices were grouped together to form each packaging unit; this mixing process led to an increase in prevalence with 5% of units being contaminated and the maximum contamination was 2 cells per packaging unit.

In another study, Malakar *et al.* (2011) assessed the risk linked to non-proteolytic *C. botulinum* in dairy-based desserts. They assumed that each batch of raw material mix contains a variable spore load. A pasteurization process is then applied leading to the thermal inactivation and reduction or, in most cases, elimination of the spore population in the batch. Following heat treatment the batch was partitioned into sealed individual units. Spore loads in individual units were very low and 98% of contaminated

units contained only one spore. Units were then stored at refrigeration temperatures where germination and growth of spores can take place.

Daelman *et al.* (2013) proposed a generic model for *Bacillus cereus* in refrigerated and processed foods of extended durability (REPFED). The production process was described by six successive steps from the initial handling of raw materials to the final storage (Table 30.2). This comprehensive model showed the evolution of the prevalence according to microbial (mainly thermal inactivation) and food-handling processes encountered (mainly partitioning and cross-contamination). The contamination level was also assessed during the food manufacturing steps. This level was mainly affected by microbial processes (growth and inactivation), while food-handling processes affected only marginally the number of *B. cereus* in contaminated units. These drivers were also crucial for “risky” packages containing more than 10^5 *B. cereus*/g (Table 30.2).

30.2.3.2 Sensitivity analysis

The modular process risk modeling approach is a convenient way to identify the production steps influencing the most exposure or risks linked to food contamination. Sensitivity analysis can also be used to identify factors or assumptions having a little or great impact on the output of the QMRA (Augustin, 2011).

Billoir *et al.* (2011) used sensitivity analysis to assess the impact of uncontrolled and difficult to estimate parameters of their model linked to the cross-contamination of *L. monocytogenes* occurring during tumbling and dicing of bacon. Their objective was to check whether the lack of knowledge about the values of these parameters was critical for the use of the model.

Malakar *et al.* (2011) showed that the probability of contamination of batches of dairy-based desserts by non-proteolytic *C. botulinum* after the pasteurization step was most sensitive to the thermal resistance of spores under consideration (*D*-values). They also observed that the probability of germination and growth of spores

Table 30.2 Dynamics of the *B. cereus* population during the REPFED production process. Impact of production steps on the prevalence (*P*), on the microbial level (N_{unit} , number of cells per food unit), and on food unit size. “=” no change, “-” decrease, “+” increase (adapted from Daelman *et al.*, 2013).

Production steps	Basic processes	<i>B. cereus</i> physiological state	<i>P</i>	N_{unit} (log ₁₀ cfu/g)		Unit size
				50th/99th ^a	% > 10 ⁵ cfu/g	
Initial contamination		Spores	96.9%	-1.5/4.0	0.1%	400 kg
Raw materials handling	Cross-contamination	Spores	+	-1.6/3.9	0.1%	= 400 kg
Cooking	Inactivation	Spores	-	-2.6/3.0	0.003%	= 400 kg
Intermediate storage	Growth	Spores	- ^b	-2.9/2.7	0.002%	= 400 kg
		Vegetative cells	+	-2.1/3.7	0.04%	
Assembly and packaging	Partitionning	Spores	-	-1.1/3.3	0.002%	- 150 g
	Mixing	Spores	+	-1.5/3.2	0.002%	+ 450 g
Pasteurization	Cross-contamination	Spores	+	-1.4/3.2	0.002%	= 450 g
Storage in retail	Inactivation	Spores	-	-1.7/2.4	0.001%	= 450 g
Storage in domestic fridge	Growth	Spores and cells	=	-1.7/2.5	0.03%	= 450 g
	Growth	Spores and cells	=	-1.7/5.6	0.55%	= 450 g

^a 50th/99th: median and 99th percentiles.

^b Germination.

during the refrigerated storage was most sensitive to the highest temperatures experienced during this step.

Daelman *et al.* (2013) showed that for REPFED contaminated by *B. cereus*, the storage in the domestic fridge was the main step influencing the exposure of consumers to “risky” packages. The error on the growth rate and lag time models as well as variability in the *D*-value were also important variables underlying the significance to obtain more accurate estimates and the relevance of biological variability for this food-borne pathogen. In addition, they observed that cross-contamination during packaging, initial contamination of raw materials, and retail temperature were also important factors.

30.2.3.3 Software

QMRA usually requires the development of complex mathematical models taking into account variability and uncertainty of inputs. Risk assessors generally develop their own models implemented in dedicated software such as @Risk® (Palisade Corporation, New York, USA) or *mc2d* package (Pouillot and Delignette-Muller, 2010) in R® (The R Core Team) to run simulations and perform Monte Carlo integrations. Besides these software primarily fitted for professional risk assessors, software designed for non-specialist users such as food business operators or food safety authorities were also developed. They describe specific or generic processes from the raw material to the end product (exposure assessment modules) where input is sometimes implemented in a dose–response model assessing the risk of illness (risk assessment modules).

The oldest one is probably Sym’Previus® (www.symprevius.eu) where a “hazard analysis – critical control point (HACCP) module” allows users to estimate the dynamics of food-borne pathogens and spoilage microorganisms during food processing operations. This module is mainly dedicated to food business operators to identify operations of their

manufacturing process that most affects the microbial fate. A simple deterministic modeling approach is used describing the evolution of the microbial concentration along the food process. It is well suited to assess the impact of operations associated with microbial processes (growth and inactivation) but food-handling processes are not included. The Dairy Product Safety Predictor (www.aqr.maisondulait.fr) also allows to calculate exposure or risk assessment for specific pathogens in a selection of dairy products. Users (mainly food business operators) can also adapt the provided models to their own processes (Tenenhaus-Aziza and Ellouze, 2015).

MicroHibro® (www.microhibro.com) was developed more recently and allows a combination in an elegant and relevant way of basic processes in relation to a database of predictive models. The process module of the FDA-iRISK® software (<https://irisk.foodrisk.org>) is also based on a succession of process stages associated with microbial or food-handling processes (Chen *et al.*, 2013). The user has to implement the relevant input parameters, i.e., initial contamination, log increase or decrease, and unit sizes, and the software automatically runs the model with Monte Carlo simulations designated to address variability of input parameters.

30.3 Risk-based objectives for each processing operation

The safety of food processes relies on the application of good manufacturing and good hygiene practices (GMP/GHP) and on a food management system such as the HACCP program. However, the level of safety that these food safety systems are expected to deliver has rarely been defined in quantitative terms (Buchanan and Whiting, 1998; Van Schothorst *et al.*, 2009) even though this possibility was pointed out more than 15 years ago (Buchanan, 1995; Mayes, 1998). A convenient way to relate

food safety management and food processing operations to risk is to use the so-called “risk-based metrics”. These metrics are first defined in this section and then two strategies of setting process characteristics to reach a defined risk level are presented.

30.3.1 Risk-based metrics

30.3.1.1 Appropriate level of protection

The agreement on sanitary and phytosanitary (SPS) measures (WTO, 1995) points out that Member States have the right to adopt SPS measures to achieve their self-determined health protection level. This level is defined as “the Appropriate Level of Protection” (ALOP), and it is estimated for the country establishing a sanitary measure to protect the lives or the health of humans (also applicable for animals or plants) within its territory. In the context of food safety, an ALOP is a statement of the degree of public health protection that is to be achieved by the food safety systems implemented in a country. It is typically expressed in terms relevant to public health such as a number of cases per 100 000 population per year. Typically, an ALOP would be articulated as a statement related to the disease burden associated with a particular hazard–food combination and its consumption in a country (FAO/WHO, 2002). It can also be used in the context of continual improvement in relationship to disease reduction.

30.3.1.2 Food safety objective

The ALOP is mainly used by policy-makers with the aim of informing the public. The ALOP may not be the most adequate concept to develop and implant the necessary control measures throughout the food chain (Havelaar *et al.*, 2004). The terms in which the ALOP is expressed do not afford a tangible criterion that the industry or the other operators of the food chain can use for food safety management (Gorris, 2005). In this context, the ICMSF (2002) proposed the creation of a new concept, the “Food Safety Objective” (FSO). The ICMSF (2002) defines FSO as “the maximum frequency

and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the ALOP”. The FSO is more straightforward and easier to understand for industrials and other operators of the food chain. The FSO can thus be used as a starting criterion for selecting and developing their control measures.

30.3.1.3 Performance Objective/Performance Criterion

Two other risk-based metrics were further defined as proposed by FAO/WHO (2004), the “Performance Objective” (PO), defined as “the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption, that provides or contributes to an FSO or ALOP, as applicable”, and the “Performance Criterion” (PC), defined as “the effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO”.

30.3.1.4 Process and product criteria

Performance objective and performance criterion must also be translated to terms and concepts that food operators can introduce in their food management system, the so-called “process criteria” and “product criteria”. Van Schothorst (2002) defined these criteria as the control parameters (such as time, temperature, water activity, pH, etc.) that can be applied at a specific step to reach an efficiency criteria. In an HACCP context these would correspond with the critical control limits of a process and are defined as the parameters of a food product that are essential to ensure that an FSO will be reached (Van Schothorst, 2002, 2005). This set of objectives, criteria, and limits can be considered in the HACCP programs and GMP/GHP guides to finally achieve in this way an FSO (Van Schothorst, 2005). The sequential nature of the food process steps and the links with above-mentioned food safety metrics are shown in Figure 30.1.

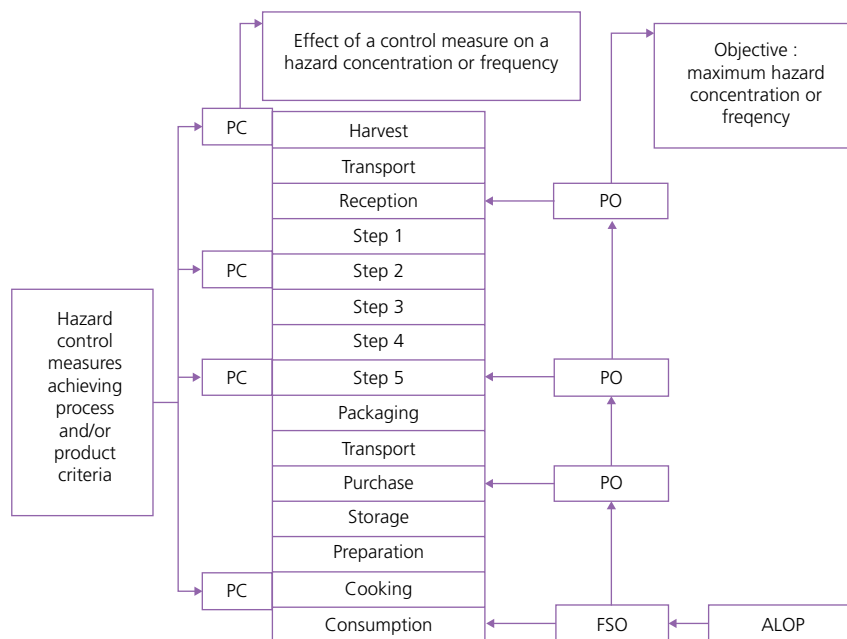


Figure 30.1 Risk management metrics in the food production chain. ALOP: Appropriate Level of Protection, FSO: Food Safety Objective, PO: Performance Objective, PC: Performance Criterion.

30.3.2 Implementation of process criteria based on food safety metrics with the ICMSF approach

In 2002, the ICMSF proposed that FSOs can be used to derive PCs for control measures in the food chain. Control measures are the actions used to prevent, eliminate, or reduce a food safety hazard to an ALOP. When establishing a PC, consideration should be paid to the initial level of a hazard and the likely changes occurring during production, distribution, storage, preparation, and use of the food. The ICMSF summarizes the situation with the following equation:

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

where H_0 is the initial level of hazard, ΣR represents the cumulative decreases in level and ΣI the cumulative increases in level. All of them are expressed in $\log_{10}$.

30.3.2.1 Point estimate approach

With this approach every term of the ICMSF equation is deterministic. Stewart *et al.* (2003) used this approach to set criteria related to *S. aureus* in cream-filled baked goods. They had set a goal to never exceed the concentration of 10^4 cfu/g throughout the manufacturing process and during storage ($FSO = 4$). The initial contamination of the filling mix linked to food handlers or contaminated equipment and environment was assumed inferior or equal to 10^2 cfu/g ($H_0 = 2$). During cooking of the cream, the authors proposed a PC of 5 decimal reductions in the concentration of *S. aureus* ($R = 5$). The process criterion (time/temperature of the heat treatment) to comply with this PC should be defined and validated by the manufacturer (for example, the authors proposed cooking until the internal temperature is 82°C). After the baking step, recontamination and growth of the microorganism can be prevented through

the application of GHP and by applying an adequate storage temperature. Despite the application of these preventive measures, the authors estimated that the population of *S. aureus* can reach a concentration of 10^2 cfu/g corresponding to an increase of the concentration for this step ($I=5$). During storage of the pastry before consumption, it is necessary to prevent the increase in the concentration of *S. aureus*. Returning to the ICMSF formula, we see that to comply with $2 - 5 + 5 + I(\text{storage}) \leq 4$, $I(\text{storage})$ must be inferior or equal to 2. This PC will be achieved by defining appropriate process criteria during storage (adequate temperature and/or shelf life) and/or product criteria (addition of preservatives to cream).

Szabo *et al.* (2003) used also this approach to evaluate the effectiveness of antimicrobial washing on *L. monocytogenes* under simulated fresh pre-cut washing conditions and evaluated the growth potential of this pathogen on the product that is packaged and then stored at either 4 or 8°C for two weeks. The results were used to demonstrate how the FSO for *L. monocytogenes* could be met in fresh pre-cut lettuce by the application, among others, of performance and process criteria. A combination of measures that control *L. monocytogenes* in the product is necessary in order to meet an FSO of $2 \log_{10}$ cfu/g of the hazard at the point of consumption. They include (i) control of the initial contamination level present in the whole lettuce, (ii) prevention of contamination during processing, (iii) reduction with washing, and (iv) control of the increase in levels by good temperature control throughout the distribution chain or shelf life duration. They showed that if the increase in concentration due to growth of viable cells of *L. monocytogenes* remaining after washing is assumed to be as high as $I = 2.7 \log_{10}$ cfu/g and the initial level of contamination on whole lettuce is $H_0 = 0.1 \log_{10}$ cfu/g, then a PC of at least $R = 0.8 \log_{10}$ reduction is required to meet the FSO.

30.3.2.2 Approach taking into account variability

In the deterministic approach, the value used to characterize a particular step of the food chain needs to be representative of this step. A PC that would have been established with mean values would imply that when the PC is met, compliance with the FSO for products would be met in 50% of cases (Zwietering *et al.*, 2010). In this context, worst-case contamination levels, increases, and reductions may be used to ensure the compliance of FSO in the deterministic approach of the ICMSF formula, but this may lead to an overly conservative PC. Rather than worst-case values, for establishing the time of a heat treatment process step, Membré *et al.* (2007) used a percentile of the distribution of the PC to verify compliance with the FSO.

Membré *et al.* (2007) first introduced variability in the ICMSF formula, where, for example, for H_0 , a distribution of values replaced “the highest values found in the literature” used in the deterministic approach. If at least one on the left term of the ICMSF formula is stochastic, the result is a probability to exceed the FSO. As previously noticed by Havelaar *et al.* (2004), Membré *et al.* (2007) confirmed that in the context of the stochastic approach, the definition of FSO is no longer valid as there will always be a probability to exceed the FSO. Zwietering *et al.* (2010) further illustrated the use of variability in the ICMSF formula by introducing variability in the approach of Szabo *et al.* (2003). A normal distribution was used for ΣI with a mean equal to the value used in the deterministic context, i.e., $2.7 \log_{10}$, and a standard deviation of $0.59 \log_{10}$. They illustrated the consequence on PC determination for the pre-cut washing step in the RTE salad. As in Membré *et al.* (2007), this example showed that the probability of compliance to the FSO (also called the level of performance) should be set to determine the PC.

Assuming that normal distributions could characterize all steps of the food chain, i.e., terms of the ICMSF formula, Zwietering *et al.*

(2010) further demonstrated that the ability to meet the FSO (at the same level of performance) can be reached by different means, by reducing the variability of inputs or changing their mean values. As all inputs are assumed to follow normal distributions, it is not necessary to carry out simulation to verify the performance level associated with a process criterion; it can be calculated straightforwardly (Figure 30.2). An Excel spreadsheet implanting the approach is available at http://www.icmsf.org/main/software_downloads.html.

30.3.3 Implementation of process criteria with quantitative risk assessment models

QMRA is increasingly used for the setting of risk-based metrics, such as FSOs and POs (Manfreda and De Cesare, 2014). Although numerous examples of a QMRA approach for setting food safety metrics in the food chain can be found, many difficulties are still there and need to be solved for increasing the application to a real situation at the country or industry levels.

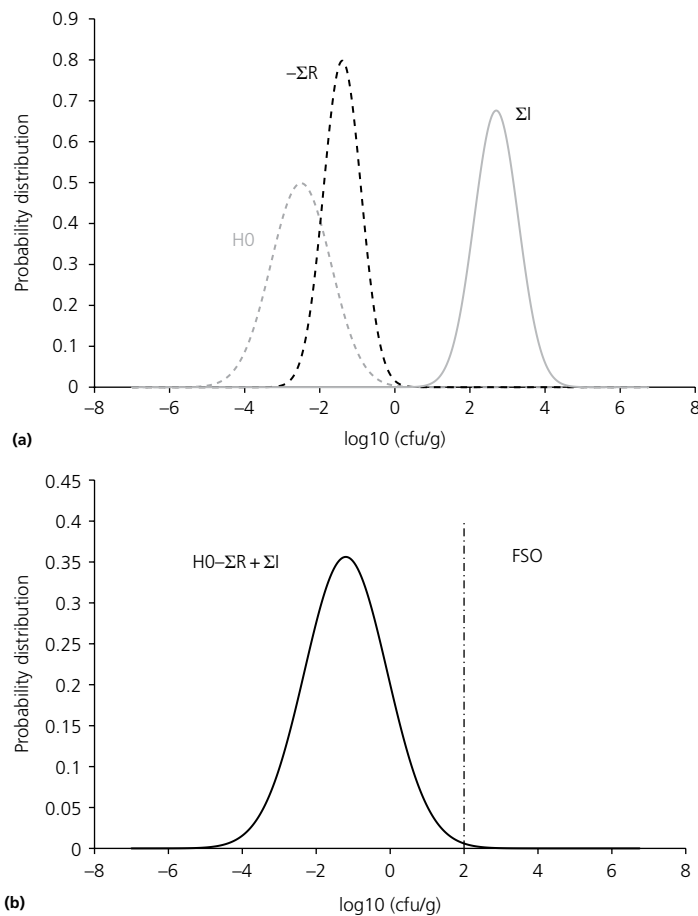


Figure 30.2 Example of the use of the ICMSF formula to determine the process criterion of pre-cut washing for *Listeria monocytogenes* in a stochastic context. (a) Probability distribution of the initial level (H_0) reduction (ΣR) and increase (ΣI) of *L. monocytogenes* in lettuce packages and (b) the resulting distribution (adapted from Zwietering *et al.*, 2010).

30.3.3.1 Possible approaches and examples of the use of QMRA to determine FSO and other metrics

One of the first examples of setting POs was proposed by Tuominen *et al.* (2007). The authors evaluated different POs for *Salmonella* for the beef chain to achieve a defined ALOP with the help of a QMRA. Van der Fels-Klerx *et al.* (2008) demonstrated how POs for *Salmonella* at various points in the broiler supply chain can be estimated, starting from the pre-set level of the PO in finished products.

Gkogka *et al.* (2013a, 2013b) recently defined two different quantitative risk assessment approaches to derive potential ALOP and risk-based metrics: a “top-down” approach, where the starting point is based on epidemiological data, and a “bottom-up” approach, based on contamination evolution in food supply chain data. In the “top-down” approach a stochastic model is built to convert epidemiological data into an ALOP, from which the FSO was derived through a series of estimates at different steps. The “bottom-up” approach starts with the occurrence of the pathogen in the food chain prior to consumption to derive the population-level impact on public health. A stochastic risk assessment model is built to convert the available data on prevalence and concentration of the pathogen in deli meats at retail into an ALOP value through a series of estimation steps. They used the two approaches to derive potential ALOP and FSO for *Salmonella* in chicken meat (Gkogka *et al.*, 2013a) and for *L. monocytogenes* in RTE meats (Gkogka *et al.*, 2013b). Using the top-down and the bottom-up approaches in parallel was found to be of great interest for validation purposes and for additional insights on input parameters influencing the results.

30.3.3.2 Difficulties met for setting FSO, PO, or other metrics in a QMRA context

A few papers introduced and illustrated the methodology, the difficulties, and some solutions to set food-based metrics with QMRA

(Havelaar *et al.*, 2004; Rieu *et al.*, 2007; Gallagher *et al.*, 2013). Some issues have been recurrently associated with the methods for setting FSO, PO, or other metrics with QMRA. They concern a top-down approach as well as uncertainty management.

It is not straightforward to get back from a given objective (e.g., FSO) “up” to operational values (e.g., critical limits, process, and product criteria). The estimation of FSO from ALOP has been initially proposed to be done by reverse engineering of the dose–response equation. Yet this approach should not be used as there is not a single combination of prevalence and concentration that will provide an ALOP (Havelaar *et al.*, 2004; Rieu *et al.*, 2007). Therefore, rather than a single concentration for FSO, different equivalent combinations of prevalence in products and dose ingested should be proposed.

Taking into account both uncertainty and variability is highly recommended in QMRA. Gallagher *et al.* (2013) recently illustrated how to use a second-order Monte Carlo model to examine the uncertainties around the risk management metrics. Their example considered *L. monocytogenes* in RTE meats. The objective was to set a PO that helps estimate the risk of illness per serving in deli meats. The authors indicated that failure to quantify accurately the uncertainties can lead to improper and too conservative POs.

30.4 Conclusion

QMRA is a convenient way to integrate and quantify the impacts of food processing operations on food safety. Quantitative models are relevant to provide deterministic or stochastic results allowing the identification of important food operations in the food-production pathway. The implementation of risk-based management options and performance criteria requires the variability and uncertainty of input parameters and models to be addressed. In this context, the derivation of performance criteria

for unitary food operations can be complicated by the high degree of freedom of the problem and by too large uncertainties leading to too much conservative criteria.

References

- Albert, I., Pouillot, R., and Denis, J.B. 2005. Stochastically modeling *Listeria monocytogenes* growth in farm tank milk. *Risk Analysis*, **25**, 1171–1185.
- Augustin, J.-C. 2011. Global sensitivity analysis applied to food safety risk assessment. *Electronic Journal of Applied Statistical Analysis*, **4**, 255–264.
- Bemrah, N., Bergis, H., Colmin, C., Beaufort, A., et al. 2003. Quantitative risk assessment of human salmonellosis from the consumption of a turkey product in collective catering establishments. *International Journal of Food Microbiology*, **80**, 17–30.
- Bigelow, W.D. 1921 Logarithmic nature of thermal death time curves. *Journal of Infectious Diseases*, **29**, 528.
- Billoir, E., Denis, J.-B., Commeau, N., Cornu, M., and Zuliani, V. 2011. Probabilistic modeling of the fate of *Listeria monocytogenes* in diced bacon during the manufacturing process. *Risk Analysis*, **31**, 237–254.
- Buchanan, R.L. 1995. The role of microbiological criteria and risk assessment in HACCP. *Food Microbiology*, **12**, 421–424.
- Buchanan, R.L. and Whiting, R.C. 1998. Risk assessment: a means for linking HACCP plans and public health. *Journal of Food Protection*, **61**, 1531–1534.
- Chen, Y., Dennis, S.B., Hartnett, E., Paoli, G., Pouillot, R., Ruthman, T., and Wilson, M. 2013. FDA-iRISK-A comparative risk assessment system for evaluating and ranking food-hazard pairs: case studies on microbial hazards. *Journal of Food Protection*, **76**, 376–385.
- Daelman, J., Membré, J.-M., Jacxsens, L., Vermeulen, A., Devlieghere, F., and Uyttendaele, M. 2013. A quantitative microbiological exposure assessment model for *Bacillus cereus* in REPFEDs. *International Journal of Food Microbiology*, **166**, 433–449.
- Danyluk, M.D. and Schaffner, D.W. 2011. Quantitative assessment of the microbial risk of leafy greens from farm to consumption: preliminary framework, data, and risk estimates. *Journal of Food Protection*, **74**, 700–708.
- Delhalle, L., Saegerman, C., Messens, W., Farnir, F., Korsak, N., Van Der Stede, Y., and Daube, G. 2009. Assessing interventions by quantitative risk assessment tools to reduce the risk of human salmonellosis from fresh minced pork meat in Belgium. *Journal of Food Protection*, **72**, 2252–2263.
- Ellouze, M., Gauchi, J.-P., and Augustin, J.-C. 2010. Global sensitivity analysis applied to a contamination assessment model of *Listeria monocytogenes* in cold smoked salmon at consumption. *Risk Analysis*, **30**, 841–852.
- Esty, J.R. and Meyer, K.F. 1922. The heat resistance of the spores of *Bacillus botulinus* and allied anaerobes. XI. *Journal of Infectious Diseases*, **31**, 650–663.
- FAO/WHO (Food Agriculture Organization/World Health Organization). 2002. Proposed draft principles and guidelines for incorporating microbiological risk assessment in the development of food safety standards, guidelines and related text. Report of a joint FAO/WHO consultation, Kiel, 18–22 March 2002. Available at: www.fao.org/docrep/006/y4302e/y4302e00.htm.
- FAO/WHO (Food Agriculture Organization/World Health Organization). (2004. Definitions on food safety objective, performance objective and performance criterion. *Joint FAO/WHO food standards programme. Codex committee on food hygiene. Thirty-sixth session*. FAO/WHO, Rome and Geneva.
- FSANZ. 2009. Microbiological Risk Assessment of Raw Milk Cheese. <http://www.foodstandards.gov.au/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD3%20Cheese%20Risk%20Assessment.pdf>
- Gallagher, D., Ebel, E.D., Gallagher, O., LaBarre, D., et al. 2013. Characterizing uncertainty when evaluating risk management metrics: risk assessment modeling of *Listeria monocytogenes* contamination in ready-to-eat deli meats. *International Journal of Food Microbiology*, **162**, 266–275.

- Geeraerd, A.H., Valdramidis, V.P., and van Impe, J.F. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, **102**, 95–105.
- Gkogka, E., Reij, M.W., Gorris, L.G.M., and Zwietering, M.H. 2013a. Risk assessment strategies as a tool in the application of the Appropriate Level of Protection (ALOP) and Food Safety Objective (FSO) by risk managers. *International Journal of Food Microbiology*, **167**, 8–28.
- Gkogka, E., Reij, M.W., Gorris, L.G.M., and Zwietering, M.H. 2013b. The application of the Appropriate Level of Protection (ALOP) and Food Safety Objective (FSO) concepts in food safety management, using *Listeria monocytogenes* in deli meats as a case study. *Food Control*, **29**, 382–393.
- Gorris, L.G. 2005. Food safety objective: an integral part of food chain management. *Food Control*, **16**, 801–809.
- Havelaar, A.H., Nauta, M.J., and Jansen, J.T. 2004. Fine-tuning Food Safety Objectives and risk assessment. *International Journal of Food Microbiology*, **93**, 11–29.
- ICMSF (International Commission on the Microbiological Specifications of Foods). 2002. *Microorganisms in Foods 7. Microbiological Testing in Food Safety Management*. Kluwer/Plenum, New York, USA.
- Kiermeier, A., Jenson, I., and Sumner, J. 2015. Risk assessment of *Escherichia coli* O157 illness from consumption of hamburgers in the United States made from Australian manufacturing beef. *Risk Analysis*, **35**, 77–89.
- Koutsoumanis, K., Pavlis, A., Nychas, G.-J.E., and Xanthiakos, K. 2010. Probabilistic model for *Listeria monocytogenes* growth during distribution, retail storage, and domestic storage of pasteurized milk. *Applied and Environmental Microbiology*, **76**, 2181–2191.
- Mafart, P., Leguerinel, I., Couvert, O., and Coroller, L. 2010. Quantification of spore resistance for assessment and optimization of heating processes: A never-ending story. *Food Microbiology*, **27**, 568–572.
- Malakar, P.K., Barker, G.C., and Peck, M.W. 2011. Quantitative risk assessment for hazards that arise from non-proteolytic *Clostridium botulinum* in minimally processed chilled dairy-based foods. *Food Microbiology*, **28**, 321–330.
- Manfreda, G. and De Cesare, A. 2014. The challenge of defining risk-based metrics to improve food safety: inputs from the BASELINE project. *International Journal of Food Microbiology*, **184**, 2–7.
- Mataragas, M., Zwietering, M.H., Skandamis, P.N., and Drosinos, E.H. 2010. Quantitative microbiological risk assessment as a tool to obtain useful information for risk managers – specific application to *Listeria monocytogenes* and ready-to-eat meat products. *International Journal of Food Microbiology*, **141**, S170–S179.
- Mayes, T. 1998. Risk analysis in HACCP: burden or benefit? *Food Control*, **9**, 171–176.
- Membré, J.-M., Amézquita, A., Bassett, J., Giavedoni, P., Blackburn, C.W., and Gorris, L.G.M. 2006. A probabilistic modeling approach in thermal inactivation: estimation of postprocess *Bacillus cereus* spore prevalence and concentration. *Journal of Food Protection*, **69**, 118–129.
- Membré, J.M., Bassett, J., and Gorris, L.G. 2007. Applying the food safety objective and related standards to thermal inactivation of *Salmonella* in poultry meat. *Journal of Food Protection*, **70**, 2036–2044.
- Mylius, S.D., Nauta, M.J., and Havelaar, A.H. 2007. Cross-contamination during food preparation: a mechanistic model applied to chicken-borne *Campylobacter*. *Risk Analysis*, **27**, 803–813.
- Nauta, M.J. 2001. A modular process risk model structure for quantitative microbiological risk assessment and its application in an exposure assessment of *Bacillus cereus* in a REPFED, RIVM Report 149106 007.
- Nauta, M.J. 2005. Microbiological risk assessment models for partitioning and mixing during food handling. *International Journal of Food Microbiology*, **100**, 311–322.
- Nauta, M. and Christensen, B. 2011. The impact of consumer phase models in microbial risk analysis. *Risk Analysis*, **31**, 255–265.

- Nauta, M.J., Litman, S., Barker, G.C., and Carlin, F. 2003. A retail and consumer phase model for exposure assessment of *Bacillus cereus*. *International Journal of Food Microbiology*, **83**, 205–218.
- Nauta, M., Hill, A., Rosenquist, H., Brynestad, S., et al. 2009. A comparison of risk assessments on *Campylobacter* in broiler meat. *International Journal of Food Microbiology*, **129**, 107–123.
- Perrin, F., Tenenhaus-Aziza, F., Michel, V., Miszczycha, S., Bel, N., and Sanaa, M. 2015. Quantitative risk assessment of haemolytic and uremic syndrome linked to O157:H7 and non-O157:H7 shiga-toxin producing *Escherichia coli* strains in raw milk soft cheeses. *Risk Analysis*, **35**, 109–128.
- Pouillot, R. and Delignette-Muller, M.L. 2010. Evaluating variability and uncertainty separately in microbial quantitative risk assessment using two R packages. *International Journal of Food Microbiology*, **142**, 330–340.
- Pouillot, R. and Lubran, M.B. 2011. Predictive microbiology models vs. modeling microbial growth within *Listeria monocytogenes* risk assessment: what parameters matter and why. *Food Microbiology*, **28**, 720–726.
- Pouillot, R., Miconnet, N., Afchain, A.-L., Delignette-Muller, M.L., Beaufort, A., Rosso, L., Denis, J.-B., and Cornu, M. 2007. Quantitative risk assessment of *Listeria monocytogenes* in French cold-smoked salmon: I. Quantitative exposure assessment. *Risk Analysis*, **27**, 683–700.
- Pouillot, R., Goulet, V., Delignette-Muller, M.L., Mahé, A., and Cornu, M. 2009. Quantitative risk assessment of *Listeria monocytogenes* in French cold-smoked salmon: II. Risk characterization. *Risk Analysis*, **29**, 806–819.
- Pouillot, R., Chen, Y., and Hoelzer, K. 2015. Modeling number of bacteria per food unit in comparison to bacterial concentration in quantitative risk assessment: impact on risk estimates. *Food Microbiology*, **45**, 245–253.
- Pujol, L., Albert, I., Johnson, N.B., and Membré, J.-M. 2013. Potential application of quantitative microbiological risk assessment techniques to an aseptic-UHT process in the food industry. *International Journal of Food Microbiology*, **162**, 283–296.
- Rieu, E., Duhem, K., Vindel, E., and Sanaa, M. 2007. Food safety objectives should integrate the variability of the concentration of pathogen. *Risk Analysis*, **27**, 373–386.
- Rigaux, C., Ancelet, S., Carlin, F., Nguyen-thé, C., and Albert, I. 2013. Inferring an augmented Bayesian network to confront a complex quantitative microbial risk assessment model with durability studies: application to *Bacillus cereus* on a courgette purée production chain. *Risk Analysis*, **33**, 877–892.
- Ross, T. and McMeekin, T.A. 2003. Modeling microbial growth within food safety risk assessments. *Risk Analysis*, **23**, 179–197.
- Ross, T., Rasmussen, S., Fazil, A., Paoli, G., and Sumner, J. 2009. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia. *International Journal of Food Microbiology*, **131**, 128–137.
- Sanaa, M., Coroller, L., and Cerf, O. 2004. Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. *Risk Analysis*, **24**, 389–399.
- Smith, B.A., Fazil, A., and Lammerding, A.M. 2013. A risk assessment model for *Escherichia coli* O157:H7 in ground beef and beef cuts in Canada: evaluating the effects of interventions. *Food Control*, **29**, 364–381.
- Stewart, C.M., Cole, M.B., and Schaffner, D.W. 2003. Managing the risk of staphylococcal food poisoning from cream-filled baked goods to meet a food safety objective. *Journal of Food Protection*, **66**, 1310–1325.
- Szabo, E.A., Simons, L., Coventry, M.J., and Cole, M.B. 2003. Assessment of control measures to achieve a food safety objective of less than 100 CFU of *Listeria monocytogenes* per gram at the point of consumption for fresh precut iceberg lettuce. *Journal of Food Protection*, **66**, 256–264.
- Tenenhaus-Aziza, F. and Ellouze, M. 2015. Software for predictive microbiology and risk assessment: a description and comparison of tools presented at the ICPMF8 Software Fair. *Food Microbiology*, **45**, 290–299.
- Tenenhaus-Aziza, F., Daudin, J.-J., Maffre, A., and Sanaa, M. 2014. Risk-based approach for microbiological food safety management in the dairy

- industry: the case of *Listeria monocytogenes* in soft cheese made from pasteurized milk. *Risk Analysis*, **34**, 56–74.
- Tuominen, P., Ranta, J., and Maijala, R. 2007. Studying the effects of POs and MCs on the *Salmonella* ALOP with a quantitative risk assessment model for beef production. *International Journal of Food Microbiology*, **118**, 35–51.
- Van der Fels-Klerx, H.J., Tromp, S., Rijgersberg, H., and Van Asselt, E.D. 2008. Application of a transmission model to estimate performance objectives for *Salmonella* in the broiler supply chain. *International Journal of Food Microbiology*, **128**, 22–27.
- Van Schothorst, M. 2002. Microbiological risk assessment in food processing. In: *Implementing the Results of a Microbiological Risk Assessment: Pathogens Risk Management*, p. 188. Woodhead, Cambridge, UK.
- Van Schothorst, M. 2005. A proposed framework for the use of FSOs. *Food Control*, **16**, 811–816.
- Van Schothorst, M., Zwietering, M.H., Ross, T., Buchanan, R.L., and Cole, M.B. 2009. Relating microbiological criteria to food safety objectives and performance objectives. *Food Control*, **20**, 967–979.
- WTO. 1995. The WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement). http://www.wto.org/english/tratop_e/sps_e/spsagr_e.htm.
- Yang, H., Mokhtari, A., Jaykus, L.-A., Morales, R.A., Cates, S.C., and Cowen, P. 2006. Consumer phase risk assessment for *Listeria monocytogenes* in deli meats. *Risk Analysis*, **26**, 89–103.
- Zwietering, M.H., Stewart, C.M., and Whiting, R.C. 2010. Validation of control measures in a food chain using the FSO concept. *Food Control*, **21**, 1716–1722.

CHAPTER 31

Quorum sensing and microbial ecology of foods

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31.1 Introduction

Bacteria communicate with each other via small diffusible chemical signal molecules termed autoinducers (AIs). As in higher organisms, the information supplied by these molecules is essential for coordinating the activities of large groups of cells. Cell-to-cell communication involves the production, release, and subsequent detection and response to AIs through a process called quorum sensing (QS) (Fuqua *et al.*, 1994), which allows bacteria to monitor the environment for the presence of other bacteria and to alter their behavior on a population-wide scale in response to changes in the number and/or species present in a microbial community (Waters and Bassler, 2005). QS was first described in the regulation of bioluminescence in *Vibrio fischeri* and *Vibrio harveyi* (Nealson *et al.*, 1970; Nealson and Hastings, 1979), and since then it has been shown to be a widespread mechanism of gene regulation in bacteria.

The microbial QS signal molecules identified so far can be divided into four broad groups: (i) *N*-acyl homoserine lactones (AHLs), known as autoinducer-1 (AI-1), which are produced and used by Gram-negative bacteria primarily for intraspecies communication (Whitehead *et al.*, 2001); (ii) a furanosyl borate diester, known as autoinducer-2 (AI-2), which is produced by both Gram-positive and Gram-negative bacteria and thought to serve as a universal signal for inter- and intraspecies

communications (Schauder *et al.*, 2001); (iii) autoinducer-3 (AI-3) of unknown structure, which is present in *Escherichia coli* O157:H7 and cross-talks with the mammalian hormone epinephrine (Sperandio *et al.*, 2003); and (iv) autoinducing peptides (AIPs), which are produced and used by Gram-positive bacteria (Lyon and Novick, 2004). In parallel with the previously mentioned QS signals, other molecules have also been described. For instance, 2-heptyl-3-hydroxy-4-quinolone is an intracellular signal molecule that has been found in *Pseudomonas aeruginosa* (Pesci *et al.*, 1999; Wade *et al.*, 2005). Diketopiperazines, which are small and diffusible molecules, were also found to be involved in QS systems (Holden *et al.*, 1999). They have high biological and pharmacological effects on cells of higher organisms, suggesting their role in bacterial conversation with plant and animal cells rather than with other bacteria. The compound *cis*-11-methyl-2-dodecenoic acid is a cell-to-cell communication signal involved in the regulation of virulence gene expression and conserved in both prokaryotes and eukaryotes (Wang *et al.*, 2004). Finally, CAI-1, of unknown chemical nature, is proposed to be responsible for *Vibrio*-specific signaling (Henke and Bassler, 2004).

Recent studies show that highly specific as well as universal QS languages exist that enable bacteria to communicate within and between species (Schauder and Bassler, 2001). Many

Gram-negative bacteria synthesize AHLs with *N*-acyl side chains ranging from 4 to 18 carbons and with an oxo-, hydroxy-, or non-substituent at the C3 position. AHLs are generally capable of diffusing across the bacterial membrane, but long-chain AHLs seem to be actively transported in and out of the cells through efflux and influx mechanisms (Whitehead *et al.*, 2001). Gram-negative bacteria appear to communicate through the standard signaling circuit LuxI/LuxR (AHL/transcriptional regulator) (Swift *et al.*, 2001). A number of factors such as temperature, pH, NaCl, growth media, and growth phase may influence AHL amounts, length of the *N*-acyl side chain and of the substitutions on the side chain of carbons of *N*-acyl side chains (Atkinson *et al.*, 1999; Geisenberger *et al.*, 2000; Gonzalez *et al.*, 2001; Lithgow *et al.*, 2001; Byers *et al.*, 2002; Ravn *et al.*, 2003).

AI-2 is a unique, universal signal that can be used by more than 70 species of both Gram-negative and Gram-positive bacteria. AI-2 is produced from *S*-adenosyl-methionine (SAM) in three enzymatic steps. Utilization of SAM as a methyl donor in cellular processes yields *S*-adenosyl-homocysteine (SAH). Afterwards, Pfs enzyme converts SAH to *S*-ribosyl-homocysteine (SRH). Finally, the LuxS protein converts SRH to homocysteine and the AI-2 precursor 2,4-dihydroxy-2-methyldihydro-3-furanone (DHMF), which spontaneously rearranges into various furanones, including AI-2 furanone of unknown structure (Federle and Bassler, 2003). It has been revealed that AI-2 production is influenced by temperature and growth medium (Cloak *et al.*, 2002). Intracellular metabolism and stress conditions can alter the AI-2 production pattern in *E. coli* K-12 (DeLisa *et al.*, 2001), whereas food additives (e.g., sodium benzoate, sodium nitrate), fatty acids (medium- and long-chain) isolated from meat products, and glucose may influence negatively AI-2 production (Lu *et al.*, 2004; Turovskiy and Chikindas, 2006; Widmer *et al.*, 2007; Soni *et al.*, 2008).

As mentioned above, Gram-positive bacteria also communicate using AIPs (Gobbetti *et al.*, 2007) and have evolved a basic communication

mechanism that is different from that used by Gram-negative bacteria. AIPs range from 5 to 26 amino acid residues in length, are linear or cyclic, and are characterized by high stability, specificity, and diversity (Dunny and Leonard, 1997).

31.2 Quorum sensing and microbial behavior

In the context of quorum sensing, the responses of bacterial cells to threshold autoinducer concentrations result in modulation of various aspects of microbial behavior. Since the first descriptions of autoinduction (Gray *et al.*, 1994; Hwang *et al.*, 1994; Pearson *et al.*, 1995), numerous cellular functions and bacterial traits have been associated with QS. In addition to bioluminescence, transfer of conjugative plasmids, and virulence, examples of such traits include symbiosis, competence, secretion of nutrient-sequestering compounds, synthesis of antimicrobial peptides, synthesis of biosurfactants, sporulation, and biofilm formation (Nadell *et al.*, 2008; Annous *et al.*, 2009). However, the discussion in this section will focus on the involvement of QS in three main bacterial phenotypes seemingly pertinent to microbial ecology of foods, namely, growth, biofilm formation, and stress resistance.

31.2.1 Growth

Among the first studies indicating a role of QS in modulation of bacterial growth was that of Sitnikov *et al.* (1996); these researchers demonstrated that genes encoding functions required for cell division in *E. coli* are regulated by two promoters, P1 and P2, with the first one being *rpoS*-stimulated and the second being regulated by SdiA (member of the LuxR subfamily of transcriptional activators) whose activity is potentiated by AHLs. It has been hypothesized that this bimodal control mechanism may comprise a fail-safe system allowing for gene transcription under a variety of different environmental and physiological conditions

(Sitnikov *et al.*, 1996). In general, it has been suggested that autoinducers function as probes or proxies, providing bacterial cells with information about the diffusive and mixing properties of their immediate environment (Redfield, 2002; Keller and Surette, 2006). In this context, assessing, either directly or indirectly, the changes occurring over time in the surrounding environment of bacteria is expected to provide an insight into the role of these compounds in food microbial ecology (Nychas *et al.*, 2009).

Regarding the growth phenotype of bacterial populations, there are some *in vitro* data suggesting a considerable effect of QS compounds on the growth kinetic parameters of food spoilage bacteria. Circumstantial evidence that *Pseudomonas fluorescens* growth is susceptible to QS was provided by Whan *et al.* (2000), who reported that addition of two signaling molecules (i.e., L-homoserine lactone and alpha-amino-gamma-butyrolactone) caused a significant reduction in both lag phase duration and exponential growth rate of two strains of the organism. Similar results with regard to the lag phase but conflicting regarding the growth rate were documented by Dunstall *et al.* (2005); when nine proprietary homoserine lactone compounds were screened for their effect on the growth kinetic parameters of three *P. fluorescens* strains, two of them (i.e., *N*-benzoyloxycarbonyl-L-homoserine lactone and *N*-3-oxyhexanoyl-DL-homoserine lactone) were found to significantly reduce the lag phase duration and increase the exponential growth rate of the tested strains. In a subsequent study, evaluating the effect of cell-free meat extract (CFME) derived from spoiled meat and containing QS compounds, it was shown that the growth rate of *P. fluorescens* and *Serratia marcescens*, recorded as the maximum slope rate of conductance changes, was enhanced in samples supplemented with CFME compared to control samples (Nychas *et al.*, 2009). Although the disparity of the findings regarding the effect of signaling molecules on growth rate can be, at least partly, attributed to strain or methodology

differences, further studies need to be conducted in order for such an effect to be clarified.

In addition to the above-mentioned *in vitro* data, data of bacterial growth in actual foods, albeit limited, are also available (Christensen *et al.*, 2003; Rasch *et al.*, 2005; Wevers *et al.*, 2009). Wevers *et al.* (2009) reported that QS affects spoilage of carrot slices by *Serratia plymuthica* by promoting colonization and mucoid bacterial outgrowth accompanied with tissue browning; an AHL-negative mutant was severely attenuated in its ability to cause spoilage, while complementation with synthetic signal molecules restored this wild-type phenotype. When trying to further characterize and explain their observations, these investigators concluded that, although butanediol fermentation is likely to play a role, molecular ecological studies need to be conducted in order to unravel the complex interactions underlying microbial food spoilage (Wevers *et al.*, 2009).

With reference to spore-forming bacteria, there is scientific evidence supporting the fact that in addition to vegetative cell division and population-level growth, QS compounds may also be involved in spore germination. L-alanine and mucopeptides are examples of compounds released by germinating spores that could serve as potential signals allowing communication between germinated and dormant *Bacillus* and/or *Clostridium* spores (Dodatko *et al.*, 2009; Peck, 2009). Although the identity of the associated signaling molecule could not be firmly established, the results of Zhao *et al.* (2005) clearly demonstrated that *Clostridium botulinum* cell-free supernatant sampled at the end of the lag phase triggered more rapid spore germination compared to the control. Such observations are indicative of the subordinate behavior of bacterial spores, providing a potential explanation for the inocula size effects recorded in challenge studies. In a relatively recent study, phase-contrast microscopy coupled with image analysis was used to investigate whether spores of non-proteolytic *C. botulinum* can interact and influence germination of their

neighbors; the results of this study certainly showed that the total number of spores had a significantly greater effect on germination probability and time-to-germination compared to the corresponding effect of local spore density (i.e., proximity to neighboring spores) (Webb *et al.*, 2012).

31.2.2 Biofilm formation

Biofilms, the structured communities of bacterial cells enclosed in a self-produced extracellular polymeric matrix adherent to inert or living surfaces, are a fundamental aspect of bacterial ecology and biology (López *et al.*, 2010; Wong and O'Toole, 2011; Steenackers *et al.*, 2012). Constituting an environmental adaptation strategy, biofilm formation confers protection to bacterial cells against a wide range of environmental challenges (Smirnova *et al.*, 2010; Nilsson *et al.*, 2011). Hence, biofilm control and eradication on both biotic and abiotic surfaces can be challenging, while biofilms formed on food-processing equipment and other food contact surfaces are very likely to serve as a persistent reservoir of microbial contamination, resulting in food spoilage or compromising food safety and human health (Iibuchi *et al.*, 2010; Van Houdt and Michiels, 2010).

Although a significant amount of research data indicate the contribution of QS systems to biofilm formation by many food-borne bacterial species, the findings of other studies have been inconclusive, with the exact linkage between these two important aspects of microbial social behavior still remaining to be determined (Parsek and Greenberg, 2005; Annous *et al.*, 2009; Skandamis and Nychas, 2012). The first indication of a connection between QS and biofilm formation was reported for *P. aeruginosa* in 1998 (Davies *et al.*, 1998), and, since then, a growing body of scientific evidence demonstrates the involvement of QS systems in all phases of biofilm formation including cell attachment, biofilm maturation, and dispersal (Stanley and Lazazzera, 2004; Parsek and Greenberg, 2005; Skandamis and Nychas, 2012).

Events involved in biofilm formation that have been implicated as likely to be under QS control include primarily secretion of extracellular polymeric substances (EPSs) and swarming motility (i.e., flagella-dependent movement of bacterial cells in the presence of extracellular slime allowing spreading over a surface) (Hammer and Bassler, 2003; Parsek and Greenberg, 2005; Hardie and Heurlier, 2008; Nadell *et al.*, 2008; Annous *et al.*, 2009). With particular reference to EPS secretion, *P. aeruginosa* appears to activate EPS production at high cell densities (Sakuragi and Kolter, 2007), while for other bacterial species, such as *Vibrio cholerae*, EPS secretion is initiated upon attachment to a surface and loss of flagellar activity, and halted when high cell densities (i.e., QS threshold) are attained (Hammer and Bassler, 2003). In an attempt to explain such variability, Nadell *et al.* (2008) investigated the evolutionary competition between strains differing in their EPS production and QS phenotypes, and concluded that QS enables bacteria to turn on and off the secretion of EPS, depending on the bacterial species, strain, and prevailing conditions. In agreement with the findings of other investigators (Xavier and Foster, 2007), these results suggest that such versatility in QS control of EPS production allows bacteria to enhance their competitive ability against other species and strains within biofilms (Nadell *et al.*, 2008). Additional biofilm-related events that have been associated with QS include the formation of channels and pillar-like structures (which ensure efficient nutrient delivery to bacterial cells within biofilms) via regulation of surfactant production (Stanley and Lazazzera, 2004) and regulation of iron acquisition systems (Parsek and Greenberg, 2005).

Despite the fact that nearly all classes of signaling molecules have been identified as components of biofilm physiology, their precise role in biofilm formation is still not clear. Activity of AHLs in naturally occurring biofilms was first reported by McLean *et al.* (1997), who employed a cross-feeding assay, using an *Agrobacterium tumefaciens* strain as an AHL-responsive

reporter, and demonstrated the presence of AHL in aquatic biofilms (growing on submerged stones) and biofilm extracts. Similarly, in a subsequent study, Stickler *et al.* (1998) demonstrated, both *in vitro* and *in vivo*, the production of AHLs by *P. aeruginosa* biofilms in a clinical setting. Data supporting an essential role of AHL-dependent QS in biofilm development have also been reported for *Aeromonas hydrophila* (Lynch *et al.*, 2002). With regard to AI-2, which serves as a universal signal for inter- and intraspecies communication and has a wide range of regulatory functions in both Gram-positive and Gram-negative bacteria, various and often conflicting findings have been reported. Although *luxS*, the AI-2 synthase gene, clearly contributes to biofilm development in some bacteria (Đapa *et al.*, 2013), inconsistent research findings prevent attribution of this phenotype to AI-2 production in all cases (Hardie and Heurlier, 2008). Indeed, both positive (e.g., in *E. coli*, *Salmonella enterica*, and *Campylobacter jejuni*) and negative (e.g., in *Bacillus cereus*, *Helicobacter pylori*, and *Mycobacterium avium*) effects of AI-2 or AI-2-like compounds on biofilm formation have been documented (Auger *et al.*, 2006; Gonzalez-Barrios *et al.*, 2006; Geier *et al.*, 2008; Annous *et al.*, 2009; Vidal *et al.*, 2011; Steenackers *et al.*, 2012), while the absence of any considerable effect has also been observed (Yoon and Sofos, 2008; Annous *et al.*, 2009). For instance, although there are research data demonstrating that a mutation in the *luxS* gene of *Staphylococcus aureus* had no effect on biofilm formation (Doherty *et al.*, 2006), in a study undertaken by Yu *et al.* (2012) it was shown that mutation of this gene in a *S. aureus* strain resulted in increased (compared to the wild-type strain) biofilm formation both *in vitro* (static, flowing, and anaerobic conditions) and *in vivo* (murine model). According to Yoon and Sofos (2008), AI-2-based QS activity of *S. enterica* and *E. coli* O157:H7 strains did not influence biofilm formation on food contact surfaces under monoculture conditions. Nonetheless, given that both of these pathogens possess SdiA,

which responds to AHLs produced by other bacterial species, it is important that the effect of AHLs likely to be produced in multiculture environments is also studied. With particular reference to *S. enterica*, there is evidence of a potential indirect effect of SdiA on the expression and assembly of plasmid-encoded fimbriae, and, thus, on biofilm formation, while a likely association of AI-3 signaling with biofilm formation has also been described (Bearson and Bearson, 2008; Steenackers *et al.*, 2012).

It becomes evident that there is a significant amount of information that still remains to be unraveled regarding the role of QS in biofilm formation, and there are certain things that need to be considered when designing and conducting research towards this direction. In order for experimental artefacts to be avoided, the applied laboratory conditions should approximate, to the greatest possible extent, the conditions encountered in food-processing environments and/or clinical settings (Parsek and Greenberg, 2005). Furthermore, since certain bacterial phenotypes, including biofilm formation, may be due to metabolic rather than signaling effects, it is really important that such effects are separated and distinctively evaluated (McDougald *et al.*, 2003; Hardie and Heurlier, 2008; Annous *et al.*, 2009; Niu *et al.*, 2013). A characteristic relevant example is that of LuxS/AI-2 for which it is critical to separate the metabolic role of the enzyme from the QS activity of the signaling molecule. For this purpose, experiments must include complementation with both *luxS* gene and purified AI-2 (Hardie and Heurlier, 2008; Annous *et al.*, 2009). Indeed, as supported by recent research findings, in addition to the effect of AI-2 on *E. coli* biofilm development, LuxS is also capable of altering biofilm structure through AI-2-independent metabolic effects, with the metabolic intermediate SAM appearing to be important in this latter pathway (Niu *et al.*, 2013).

In general, the study of biofilms, referred to as “biofilmology”, still holds a substantial research potential within the next decade, and the cooperation of various scientific disciplines

has been acknowledged as of vital importance towards this direction (Karunakaran *et al.*, 2011; Wong and O'Toole, 2011). More specifically, additional studies under controlled *in vitro* conditions, assessing the effects of specific QS compounds in monoculture or composite cultures on the dynamics of biofilms, need to be carried out if the role of QS signals in different stages of biofilm formation is to be ascertained (Skandamis and Nychas, 2012). Full understanding of QS in bacterial biofilms will also require consideration of evolutionary competition both within and among these social groups; the ecological and evolutionary processes driving QS, biofilm formation, and, in particular, their interaction need to be disentangled and individually assessed and characterized (Nadell *et al.*, 2008). Finally, it has been opined that another key research challenge is the determination of the functional consequences of QS in a biofilm community, and in this context information that needs to be obtained refers to (i) the diffusion ability of signal molecules within a biofilm; (ii) the rate at which biofilm cells produce signal molecules; and (iii) the location in a biofilm at which QS is first induced (Parsek and Greenberg, 2005).

31.2.3 Stress resistance

Bacteria are commonly exposed to environmental stresses, both in their natural habitats as well as during exposure to host immune responses or interventions/processes aiming at their inactivation. From a food microbiology perspective, the term "stress" usually refers to a physical, chemical, or nutritional condition which, by being insufficiently severe to assure bacterial death, results in sublethally injured bacteria (Wesche *et al.*, 2009). Assessing and characterizing the involvement of QS in the stress resistance of food-borne spoilage or pathogenic bacteria is expected to improve our understanding of their survival strategies and, thus, facilitate the design of effective control measures.

The alternative sigma factor RpoS, regulator of the general stress response in many bacteria,

also controls the expression of QS-controlled genes in some bacterial species such as *P. aeruginosa*, an event that provides a first line of evidence of the interrelation between signaling systems and stress resistance (Schuster *et al.*, 2004). The stress resistance phenotypes most commonly associated with QS signaling systems are starvation and oxidative stress resistance (Hassett *et al.*, 1999; McDougald *et al.*, 2003; Kolling and Mathews, 2007; Geier *et al.*, 2008; Goldová *et al.*, 2011). As shown by McDougald *et al.* (2003), AI-2-like signals produced by certain *Vibrio* spp. are capable of inducing the environmentally relevant phenotypes of starvation adaptation and oxidative stress resistance at an interspecies level. Research data referring to the emerging opportunistic pathogen *Mycobacterium avium* indicate that AI-2 does not per se act as a QS signal with regard to biofilm formation; instead, AI-2 most likely exerts effects on environmental stresses of biotic or abiotic origin, and particularly on oxidative stress response, with the latter, in turn, triggering biofilm development (Geier *et al.*, 2008). A potential association of QS with bacterial stress resistance is also supported by the observed induction of *sdiA* upon exposure of *S. enterica* to sudden pH reductions, and the subsequent enhanced resistance of the organism to bile (Rychlik and Barrow 2005). Additional stress resistance phenotypes that have been positively associated with QS systems include resistance of *P. aeruginosa* biofilms to sodium dodecyl sulfate (Davies *et al.*, 1998) and hydrogen peroxide (Hassett *et al.*, 1999), and resistance of *E. coli* O157:H7 to sodium hypochlorite (Kolling and Mathews, 2007), while a potential QS regulation of copper toxicity in *P. aeruginosa* has also been described (Thaden *et al.*, 2010). Finally, as suggested by preliminary research findings (Moker *et al.*, 2010; Jubair *et al.*, 2012), QS may play a role in the shift of certain bacterial species to the so-called "persister" phenotype (i.e., viability for extended periods of time under stress conditions).

In some cases, no clear association of signaling molecules with bacterial stress resistance

could be established. In a study assessing the ability of *Listeria monocytogenes* to acquire resistance to nisin and lactic acid, it was concluded that the pathogen's resistance to these antimicrobials was not mediated through AI-2-dependent QS pathways (Turovskiy *et al.*, 2007). However, the researchers presumed that the acid tolerance exhibited by the organism was somewhat triggered by a pheromone molecule other than AI-2. Similarly, in a subsequent study, Yoon and Sofos (2010) reported that AI-2-based QS did not play a major role in the heat and acid adaptation/resistance of *S. enterica*. Overall, given the frequently inconclusive outcome of research studies and the complexity of resistance mechanisms, it is conspicuous that further studies are needed if the explicit role of QS in bacterial responses to food-related stresses is to be confirmed and defined.

31.3 Quorum sensing and food ecology

Food microbial ecology is related to the analysis of bacterial communication in different food ecosystems (Smith *et al.*, 2004; Ammor *et al.*, 2008). An up-to-date overview of the studies regarding the presence of AHLs and AI-2 signals in foods of animal (i.e., meat, poultry, dairy products, and seafood) and plant (i.e., fresh produce) origin, as well as the contribution of certain members of the food microbial community on the detected signals, is presented in Table 31.1. Most of these findings are associated with high populations of Gram-negative bacteria, mainly *Pseudomonas* spp. and genera of the Enterobacteriaceae family, which possess AHL-based systems and have been extensively associated with food spoilage (Gram *et al.*, 2002; Nychas *et al.*, 2008). In a recently published work, the presence of AHLs was observed in minced beef stored at various temperatures, both aerobically and under modified atmospheres, and in the presence of both Enterobacteriaceae and pseudomonads at

populations ranging from 10^7 to 10^9 cfu/g; on the other hand, no signal molecules were detected in minced beef stored under modified atmospheres in the presence of volatile compounds of oregano essential oil, where both of these bacterial groups failed to grow in high numbers (Blana and Nychas, 2014).

The most commonly found AHL in foods is C6-3-oxo-HSL, which appears to play a role in regulating the expression of LipB, required for the lipolytic and proteolytic activities in *Serratia proteomaculans* (Bruhn *et al.*, 2004), phenotypes potentially associated with spoilage of different foods and linked to QS regulation in several bacteria (Jay *et al.*, 2003; Liu *et al.*, 2006). In contrast, C8-3-oxo-HSL, which has also been frequently detected in foods (Ravn *et al.*, 2003; Tan *et al.*, 2014), and usually produced by plant-associated bacteria (Cha *et al.*, 1998; Elasmri *et al.*, 2001), has been linked to virulence genes in the opportunistic human pathogen *P. aeruginosa* and the fish pathogen *Yersinia ruckeri* (Pearson *et al.*, 1994; Kastbjerg *et al.*, 2007).

With regard to the potential contribution of Gram-positive bacteria in the production of AI-2, the role of *Brochothrix thermosphacta* and lactic acid bacteria in the spoilage process should be definitely taken into account (Nychas *et al.*, 2008). As demonstrated by the findings of a recent study, in which meat extracts were prepared and lactic acid bacteria were isolated during storage of meat at different temperatures, a low AI-2 activity was observed in meat extracts compared to the corresponding activity for the dominant lactic acid bacteria (Blana *et al.*, 2011). Such findings do raise questions related to the contribution of these signals in the selection of specific bacterial strains to the microbial succession and, consequently, to spoilage (e.g., the rate or type of spoilage) during storage.

Although the presence of QS signals has been indicated in many food products, the extrapolation of *in vitro* findings to real food ecosystems is still difficult and questionable. The current knowledge of the QS effect in the food sector needs to be expanded beyond the state of the art. More specifically, the experimental

Table 31.1 Quorum sensing signals detected in foods.

Product and/or isolates	Source/ Growth ^a	QS signal response ^b	Reference
<i>Meat and poultry</i>			
Enterobacteriaceae	Chill-stored vacuum-packed meat/ –	C6-HSL or C6-3-oxo-HSL	Gram <i>et al.</i> , 1999
Meat extracts/ Enterobacteriaceae	Chill-stored vacuum-packed beef/ –	C6-3-oxo-HSL, C6-HSL	Bruhn <i>et al.</i> , 2004
Pseudomonads and Enterobacteriaceae	Aerobically chilled-stored ground beef and chicken breast fillets/ –	Medium- and long-side chain AHLs	Liu <i>et al.</i> , 2006
<i>Pseudomonas fragi</i>	Fresh and spoiled meat/ –	Borated AI-2	Ferrocino <i>et al.</i> , 2009
<i>Pseudomonas aeruginosa</i>	–/ Growth and biofilm formation in the presence of chicken broth containing AHLs	C4-HSL, C6-HSL, C8-HSL, and C12-HSL	Zhang <i>et al.</i> , 2014
Lactic acid bacteria	Minced beef packaged under modified atmospheres at 0, 5, 10, and 15°C/ –	AI-2 activity	Blana <i>et al.</i> , 2011
<i>Campylobacter coli</i> , <i>C. jejuni</i> , <i>Salmonella</i> Typhimurium and <i>Escherichia coli</i> O157:H7	–/ Chicken broth at 4, 25, and 37°C	AI-2	Cloak <i>et al.</i> , 2002
<i>Escherichia coli</i> O157:H7	–/ Raw ground beef, pork, chicken and turkey broths	AI-2	Silagyi <i>et al.</i> , 2009
<i>Escherichia coli</i> O157:H7 survival and virulence gene expression	–/ Presence of AI-2 and ground beef extracts	Borated AI-2	Soni <i>et al.</i> , 2008
<i>Aeromonas hydrophila</i>	Beef/ –	C4-HSL	Medina-Martinez <i>et al.</i> , 2006
<i>Yersinia enterocolitica</i>	–/ Fresh beef and pork extracts	C6-3-oxo-HSL, C6-HSL	Medina-Martinez <i>et al.</i> , 2007
Beef steak, beef patties, chicken breast, and turkey patties	–/ –	AI-2 activity	Lu <i>et al.</i> , 2004
Aerobically chill-stored ground beef	–/ –	C4-HSL, C6-3-oxo-HSL, and undefined AHLs	Liu <i>et al.</i> , 2006
Fatty acids, as inhibitors to AI-2	Derived from poultry meat/ –	Borated AI-2	Widmer <i>et al.</i> , 2007
Fatty acids, as inhibitors to AI-2	Derived from ground beef/ –	Borated AI-2	Soni <i>et al.</i> , 2008
Meat extracts	Minced pork stored aerobically at 5 and 20°C/ –	Short-, medium-, and long-side chain AHLs, AI-2	Nychas <i>et al.</i> , 2009
Meat extracts	Minced beef stored at 0, 5, 10, and 15°C under different packaging conditions/ –	C6-3-oxo-HSL and low AI-2 activity	Blana and Nychas, 2014

(Continued)

Table 31.1 (Continued)

Product and/or isolates	Source/ Growth ^a	QS signal response ^b	Reference
<i>Dairy products</i>			
<i>Hafnia alvei</i> (planktonic and sessile cells)	Raw milk/ –	AHLs with up to six carbon atoms	Viana <i>et al.</i> , 2009
Gram-negative psychrotrophic bacteria	Cooled raw milk/ –	Broad range of AHLs	Pinto <i>et al.</i> , 2007
<i>Pseudomonas fluorescens</i>	Refrigerated raw milk/ Presence of AHLs	C4-, C6-, C7-, C8-, C10-, C12-, C14-, Z-HSL, and C6-3-oxo-HSL	Dunstall <i>et al.</i> , 2005
<i>Campylobacter coli</i> , <i>C. jejuni</i> , <i>Salmonella</i> Typhimurium, and <i>Escherichia coli</i> O157:H7	–/ Milk at 4, 25, and 37°C	AI-2	Cloak <i>et al.</i> , 2002
<i>Yersinia enterocolitica</i>	–/ Milk agar	Response of biosensor detecting broad range of AHLs	Medina-Martinez <i>et al.</i> , 2007
<i>Arthrobacter nicotianae</i> , <i>Corynebacterium ammoniagenes</i> , <i>Corynebacterium casei</i> , <i>Microbacterium barkeri</i> , <i>Microbacterium gubbeenense</i> , and <i>Staphylococcus equorum</i> subsp. <i>linens</i>	Smear surface of ripened cheeses/ –	AI-2 activity	Gori <i>et al.</i> , 2011
Milk samples (whole and organic)	–/ –	AI-2 activity	Lu <i>et al.</i> , 2004
Raw milk aerobically chill-stored	–/ –	C6-3-oxo-HSL, C12-HSL, and undefined AHLs	Liu <i>et al.</i> , 2006
<i>Seafood</i>			
Fish extracts/ Enterobacteriaceae	Cold-smoked salmon/ –	Undefined AHLs/ C6-HSL or C6-3-oxo-HSL	Gram <i>et al.</i> , 1999
<i>Serratia proteomaculans</i>	Cold-smoked salmon/ Influence of food preservative parameters	C6-3-oxo-HSL	Ravn <i>et al.</i> , 2003
<i>Hafnia alvei</i>	Vacuum packed, refrigerated spherical fish paste/ –	C6-3-oxo-HSL and C8-3-oxo-HSL	Tan <i>et al.</i> , 2014
<i>Enterobacter agglomerans</i>	Cold-smoked salmon/ Influence of food preservative parameters	C6-3-oxo-HSL and C8-3-oxo-HSL	Ravn <i>et al.</i> , 2003
<i>Aeromonas hydrophila</i>	–/ Simulated shrimp and dory fish agars	C4-HSL	Medina-Martinez <i>et al.</i> , 2006
<i>Yersinia enterocolitica</i>	–/ Fish extract	C6-3-oxo-HSL, C6-HSL	Medina-Martinez <i>et al.</i> , 2007

Frozen fish samples (red drum)	–/–	AI-2 activity	Lu et al., 2004
Aerobically chill-stored fish filelets	–/–	Predominantly medium-side chain AHLs	Liu et al., 2006
Fish infected with the AHL producer <i>Vibrio anguillarum</i>	–/–	C10-3-oxo-HSL and C6-3-hydroxy-HSL	Buchholtz et al., 2006
Morbund fish infected with the AHL producer <i>Yersinia ruckeri</i>	–/–	C8-3-oxo-HSL	Kastbjerg et al., 2007
<i>Fresh produce</i> Bacteria representing the genera <i>Agrobacterium</i> , <i>Erwinia</i> , <i>Pantoea</i> , <i>Pseudomonas</i> , <i>Ralstonia</i> , <i>Rhizobium</i> and <i>Xanthomonas</i>	Plant-associated bacterial/–	Broad range of AHLs	Cha et al., 1998
Enterobacteriaceae, pseudomonads, and Vibrionaceae strains	Commercial bean sprouts/–	C6-3-oxo-HSL, C10-3-oxo-HSL, C10-3-hydroxy-HSL, C4-HSL, C6-HSL, C8-HSL, C10-HSL, and undefined AHLs	Rasch et al., 2005
Bean sprouts	Stored at 5 °C/–	Broad range of AHLs	Gram et al., 2002
Bean sprouts	–/–	C6-3-oxo-HSL	Rasch et al., 2005
Salad tomatoes, whole raw carrots, and cantaloupes	–/–	AI-2 activity	Lu et al., 2004
<i>Yersinia enterocolitica</i>	–/Lettuce and cucumber extracts	C6-3-oxo-HSL, C6-HSL	Medina-Martinez et al., 2006
<i>Yersinia enterocolitica</i>	–/Mexican salad, lettuce salad, cucumber, and soya extracts agar	Response of biosensor detecting broad range of AHLs	Medina-Martinez et al., 2007
<i>Aeromonas hydrophila</i>	–/Simulated broccoli, parsley, and spinach agars	C4-HSL	Medina-Martinez et al., 2006
<i>Escherichia coli</i> O157:H7	–/Cantaloupe, lettuce, alfalfa sprouts, and spinach broths	AI-2	Silagyi et al., 2009
<i>Enterobacter asburiae</i>	Batavia lettuce/–	C4-HSL and C6-HSL	Lau et al., 2013

^a–, not determined or not applied in the study.

^bHSL, homoserine lactone; AI-2, autoinducer-2; AHLs, *N*-acyl homoserine lactones.

setups in future studies should focus on the spatial heterogeneity of a food system where cells exist as planktonic, sessile, immobilized, or constrained forms and sensing occurs in diverse communities under dynamic environments. Also, the potential variability caused by QS at the single-cell level, which may elucidate the true heterogeneity of a population, and the boundaries of the applied quantitative and qualitative methods used to estimate QS signals should be taken into account (Skandamis and Nychas, 2012).

31.4 Quorum quenching

Unknown mechanisms of cell-to-cell signaling make use of compounds that antagonize quorum sensors in order to control or inhibit the QS-regulated behaviors of spoilage and/or

pathogenic bacteria without affecting the bacterial growth (Smith *et al.*, 2004; Ammor *et al.*, 2008). The application of these compounds, usually referred as quorum sensing inhibitors (QSIs), has been extensively investigated at the clinical level, while their use in food preservation and food safety is limited. Alternative strategies to modulate pathogen virulence and as an intervention for preventing or delaying food spoilage have been also proposed using QS signals in order to generate false sensing and confuse bacteria (Skandamis and Nychas, 2012). Such approaches are referred to as QS inhibition or quorum quenching. The possible AHL degradation mechanisms and QSIs are depicted in Figure 31.1. Halogenated furanones, AHL analogs, AHL-lactonases and -acylases, 4-nitro-pyridine-*N*-oxide, patulin, penicillic acid, vanilla, garlic and methanolic

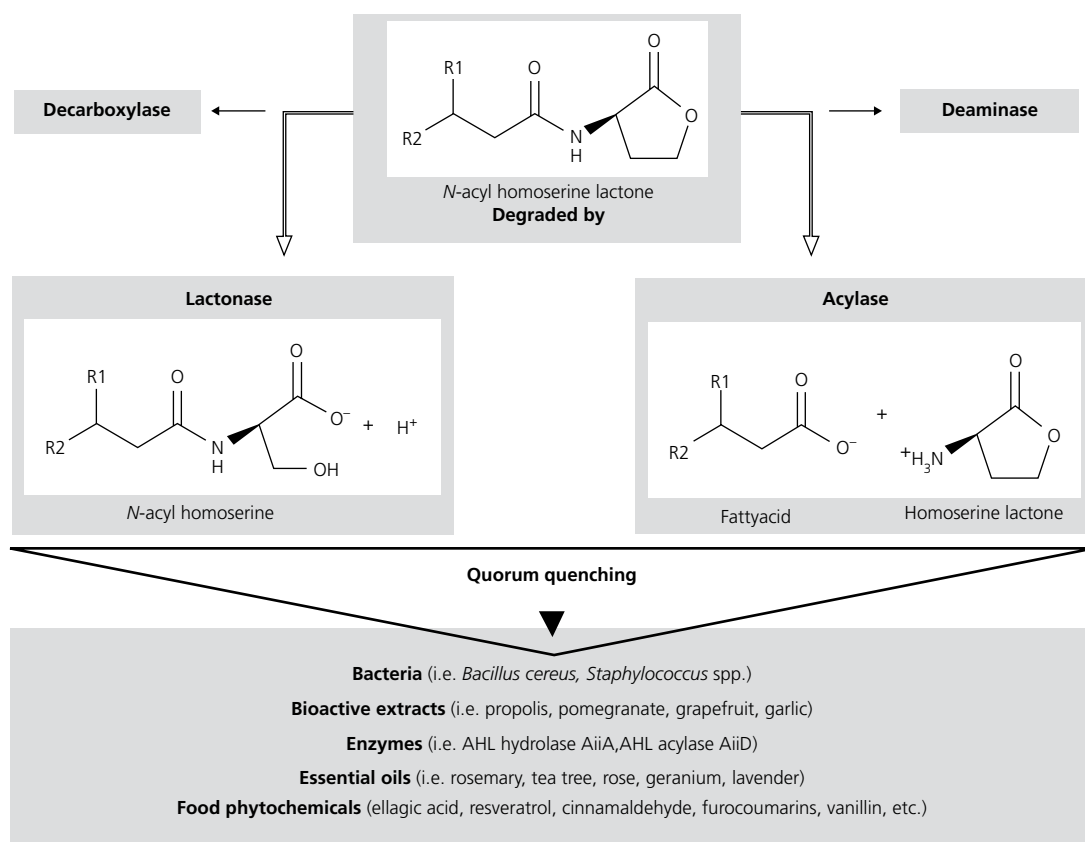


Figure 31.1 N-acyl homoserine lactone degradation mechanisms and categories of potential quorum quenching compounds.

extracts, cinnamaldehyde, and ascorbic acid are among the compounds that have been described as QSIs (Galloway *et al.*, 2012; Hong *et al.*, 2012; Kalia, 2013; Koh *et al.*, 2013; Nazzaro *et al.*, 2013). Inhibitory compounds that may mask the AI-2 QS effect have been reported in foods of animal origin (Lu *et al.*, 2004; Soni *et al.*, 2008).

References

- Ammor, M.S., Michaelidis, C., and Nychas, G.-J.E. 2008. Insights into the role of quorum sensing in food spoilage. *Journal of Food Protection*, **71**, 1510–1525.
- Annous, B.A., Frataamico, P.M., and Smith, J.L. 2009. Quorum sensing in biofilms: why bacteria behave the way they do. *Journal of Food Science*, **74**, R24–R37.
- Atkinson, S., Throup, J.P., Stewart, G.S., and Williams, P. 1999. A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. *Molecular Microbiology*, **33**, 1267–1277.
- Auger, S., Krin, E., Aymerich, S., and Gohar, M. 2006. Autoinducer 2 affects biofilm formation by *Bacillus cereus*. *Applied and Environmental Microbiology*, **72**, 937–941.
- Bearson, B.L. and Bearson, S.M. 2008. The role of the QseC quorum-sensing sensor kinase in colonization and norepinephrine-enhanced motility of *Salmonella enterica* serovar Typhimurium. *Microbial Pathogenesis*, **44**, 271–278.
- Blana, V.A. and Nychas, G.-J.E. 2014. Presence of quorum sensing signal molecules in minced beef stored under various temperature and packaging conditions. *International Journal of Food Microbiology*, **173**, 1–8.
- Blana, V.A., Doulgeraki, A.I., and Nychas, G.-J.E. 2011. Autoinducer-2-like activity in lactic acid bacteria isolated from minced beef packaged under modified atmospheres. *Journal of Food Protection*, **74**, 631–635.
- Bruhn, J.B., Christensen, A.B., Flodgaard, L.R., *et al.* 2004. Presence of acylated homoserine lactones (AHLs) and AHL-producing bacteria in meat and potential role of AHL in spoilage of meat. *Applied and Environmental Microbiology*, **70**, 4293–4302.
- Buchholtz, C., Nielsen, K.F., Milton, D.L., Larsen, J.L., and Gram, L. 2006. Profiling of acylated homoserine lactones of *Vibrio anguillarum* *in vitro* and *in vivo*: influence of growth conditions and serotype. *Systematic and Applied Microbiology*, **29**, 433–445.
- Byers, J.T., Lucas, C., Salmond, G.P., and Welch, M. 2002. Nonenzymatic turnover of an *Erwinia carotovora* quorum-sensing signaling molecule. *Journal of Bacteriology*, **184**, 1163–1171.
- Cha, C., Gao, P., Chen, Y.-C., Shaw, P.D., and Farrand, S.K. 1998. Production of acyl-homoserine lactone quorum-sensing signals by Gram-negative plant-associated bacteria. *Molecular Plant–Microbe Interactions*, **11**, 1119–1129.
- Christensen, A.B., Riedel, K., Eberl, L., *et al.* (2003) Quorum-sensing-directed protein expression in *Serratia proteamaculans* B5a. *Microbiology*, **149**, 471–483.
- Cloak, O.M., Solow, B.T., Briggs, C.E., Chen, C.Y., and Frataamico, P.M. 2002. Quorum sensing and production of autoinducer-2 in *Campylobacter* spp., *Escherichia coli* O157:H7, and *Salmonella enterica* serovar Typhimurium in foods. *Applied and Environmental Microbiology*, **68**, 4666–4671.
- Dapa, T., Leuzzi, R., Ng, Y.K., *et al.* 2013. Multiple factors modulate biofilm formation by the anaerobic pathogen *Clostridium difficile*. *Journal of Bacteriology*, **195**, 545–555.
- Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., and Greenberg, E.P. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science*, **280**, 295–298.
- DeLisa, M.P., Valdes, J.J., and Bentley, W.E. 2001. Mapping stress-induced changes in autoinducer AI-2 production in chemostat-cultivated *Escherichia coli* K-12. *Journal of Bacteriology*, **183**, 2918–2928.
- Dodatko, T., Akoachere, M., Muehlbauer, S.M., *et al.* 2009. *Bacillus cereus* spores release alanine that synergizes with inosine to promote germination. *PLoS One*, **4**, e6398.
- Doherty, N., Holden, M.T.G., Qazi, S.N., Williams, P., and Winzer, K. 2006. Functional analysis of

- luxS* in *Staphylococcus aureus* reveals a role in metabolism but not quorum sensing. *Journal of Bacteriology*, **188**, 2885–2897.
- Dunny, G.M. and Leonard, B.A. 1997. Cell–cell communication in Gram-positive bacteria. *Annual Review of Microbiology*, **51**, 527–564.
- Dunstall, G., Rowe, M.T., Wisdom, G.B., and Kilpatrick, D. 2005. Effect of quorum sensing agents on the growth kinetics of *Pseudomonas* spp. of raw milk origin. *Journal of Dairy Research*, **72**, 276–280.
- Elasri, M., Delorme, S., Lemanceau, P., et al. 2001. Acyl-homoserine lactone production is more common among plant-associated *Pseudomonas* spp. than among soilborne *Pseudomonas* spp. *Applied and Environmental Microbiology*, **67**, 1198–1209.
- Federle, M.J. and Bassler, B.L. 2003. Interspecies communication in bacteria. *Journal of Clinical Investigation*, **112**, 1291–1299.
- Ferrocino, I., Ercolini, D., Villani, F., Moorhead, S.M., and Griffiths, M.W. 2009. *Pseudomonas fragi* strains isolated from meat do not produce *N*-acyl homoserine lactones as signal molecules. *Journal of Food Protection*, **72**, 2597–2601.
- Fuqua, W.C., Winans, S.C., and Greenberg, E.P. 1994. Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. *Journal of Bacteriology*, **176**, 269–275.
- Galloway, W.R.J.D., Hodgkinson, J.T., Bowden, S., Welch, M., and Spring, D.R. 2012. Applications of small molecule activators and inhibitors of quorum sensing in Gram-negative bacteria. *Trends in Microbiology*, **20**, 449–458.
- Geier, H., Mostowy, S., Cangelosi, G.A., Behr, M.A., and Ford, T.E. 2008. Autoinducer-2 triggers the oxidative stress response in *Mycobacterium avium*, leading to biofilm formation. *Applied and Environmental Microbiology*, **74**, 1798–1804.
- Geisenberger, O., Givskov, M., Riedel, K., Hoiby, N., TummLer, B., and Eberl, L. 2000. Production of *N*-acyl-L-homoserine lactones by *P. aeruginosa* isolates from chronic lung infections associated with cystic fibrosis. *FEMS Microbiology Letters*, **184**, 273–278.
- Gobbetti, M., De Angelis, M., Di Cagno, R., Minervini, F., and Limitone, A. 2007. Cell–cell communication in food related bacteria. *International Journal of Food Microbiology*, **120**, 34–45.
- Goldová, J., Ulrych, A., Hercík, K., and Branny, P. 2011. A eukaryotic-type signalling system of *Pseudomonas aeruginosa* contributes to oxidative stress resistance, intracellular survival and virulence. *BMC Genomics*, **12**, 437–458.
- Gonzalez, R.H., Nusblat, A., and Nudel, B.C. 2001. Detection and characterization of quorum sensing signal molecules in *Acinetobacter* strains. *Microbiological Research*, **155**, 271–277.
- Gonzalez-Barrios, A.F., Zuo, R., Hashimoto, Y., Yang, L., Bentley, W.E., and Wood, T.K. 2006. Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, b3022). *Journal of Bacteriology*, **188**, 305–316.
- Gori, K., Moslehi-Jenabian, S., Purrotti, M., and Jespersen, L. 2011. Autoinducer-2 activity produced by bacteria found in smear of surface ripened cheeses. *International Dairy Journal*, **21**, 48–53.
- Gram, L., Christensen, A.B., Ravn, L., Molin, S., and Givskov, M. 1999. Production of acylated homoserine lactones by psychrotrophic members of the *Enterobacteriaceae* isolated from foods. *Applied and Environmental Microbiology*, **65**, 3458–3463.
- Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., and Givskov, M. 2002. Food spoilage — interactions between food spoilage bacteria. *International Journal of Food Microbiology*, **78**, 79–97.
- Gray, K.M., Passador, L., Iglewski, B.H., and Greenberg, E.P. 1994. Interchangeability and specificity of components from the quorum-sensing regulatory systems of *Vibrio fischeri* and *Pseudomonas aeruginosa*. *Journal of Bacteriology*, **176**, 3076–3080.
- Hammer, B.K. and Bassler, B.L. 2003. Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Molecular Microbiology*, **50**, 101–114.
- Hardie, K.R. and Heurlier, K. 2008. Establishing bacterial communities by “word of mouth”: LuxS and autoinducer 2 in biofilm development. *Nature Reviews Microbiology*, **6**, 635–643.

- Hassett, D.J., Ma, J.-F., Elkins, J.G., *et al.* (1999) Quorum sensing in *Pseudomonas aeruginosa* controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Molecular Microbiology*, **34**, 1082–1093.
- Henke, J.M. and Bassler, B.L. 2004. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *Journal of Bacteriology*, **186**, 6902–6914.
- Holden, M.T., Ram Chhabra, S., de Nys, R., *et al.* 1999. Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. *Molecular Microbiology*, **33**, 1254–1266.
- Hong, K.-W., Koh, C.-L., Sam, C.-K., Yin, W.-F., and Chan, K.-G. (2012) Quorum quenching revisited – from signal decays to signalling confusion. *Sensors*, **12**, 4661–4696.
- Hwang, I., Li, P.L., Zhang, L., *et al.* 1994. TraI, a LuxI homologue, is responsible for production of conjugation factor, the Ti plasmid *N*-acylhomoserine lactone autoinducer. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 4639–4643.
- Iibuchi, R., Hara-Kudo, Y., Hasegawa, A., and Kumagai, S. 2010. Survival of *Salmonella* on a polypropylene surface under dry conditions in relation to biofilm-formation capability. *Journal of Food Protection*, **73**, 1506–1510.
- Jay, J.M., Vilai, J.P., and Hughes, M.E. 2003. Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5–7 °C. *International Journal of Food Microbiology*, **81**, 105–111.
- Jubair, M., Morris Jr, J.G., and Ali, A. 2012. Survival of *Vibrio cholerae* in nutrient-poor environments is associated with a novel “persister” phenotype. *PLoS One*, **7**, e45187.
- Kalia V.C. 2013. Quorum sensing inhibitors: an overview. *Biotechnology Advances*, **31**, 224–245.
- Karunakaran, E., Mukherjee, J., Ramalingam, B., and Biggs, C.A. 2011 “Biofilmology”: a multidisciplinary review of the study of microbial biofilms. *Applied Microbiology and Biotechnology*, **90**, 1869–1881.
- Kastbjerg, V.G., Nielsen, K.F., Dalsgaard, I., *et al.* 2007. Profiling acylated homoserine lactones in *Yersinia ruckeri* and influence of exogenous acyl homoserine lactones and known quorum-sensing inhibitors on protease production. *Journal of Applied Microbiology*, **102**, 363–374.
- Keller, L. and Surette, M. 2006. Communication in bacteria: an ecological and evolutionary perspective. *Nature Reviews Microbiology*, **4**, 249–258.
- Koh, C.-L., Sam, C.-K., Yin, W.-F., *et al.* 2013. Plant-derived natural products as sources of anti-quorum sensing compounds. *Sensors*, **13**, 6217–6228.
- Kolling, G.L. and Matthews, K.R. 2007. Influence of enteric bacteria conditioned media on recovery of *Escherichia coli* O157:H7 exposed to starvation and sodium hypochlorite. *Journal of Applied Microbiology*, **103**, 1435–1441.
- Lau, Y.Y., Sulaiman, J., Chen, J.W., Yin, W.-F., and Chan, K.-G. 2013. Quorum sensing activity of *Enterobacter asburiae* isolated from lettuce leaves. *Sensors*, **13**, 14189–14199.
- Lithgow, J.K., Danino, V.E., Jones, J., and Downie, J.A. 2001. Analysis of *N*-acyl homoserine-lactone quorum-sensing molecules made by different strains and biovars of *Rhizobium leguminosarum* containing different symbiotic plasmids. *Plant and Soil*, **232**, 3–12.
- Liu, M., Gray, J.M., and Griffiths, M.W. 2006. Occurrence of proteolytic activity and *N*-acyl-homoserine lactone signals in the spoilage of aerobically chill-stored proteinaceous raw foods. *Journal of Food Protection*, **69**, 2729–2737.
- López, D., Vlamakis, H., and Kolter, R. 2010. Biofilms. *Cold Spring Harbor Perspectives in Biology*, **2**, a000398.
- Lu, L., Hume, M.E., and Pillai, S.D. 2004. Autoinducer-2-like activity associated with foods and its interaction with food additives. *Journal of Food Protection*, **67**, 1457–1462.
- Lynch, M.J., Swift, S., Kirke, D.F., Keevil, C.W., Dodd, C.E.R., and Williams, P. 2002. The regulation of biofilm development by quorum sensing in *Aeromonas hydrophila*. *Environmental Microbiology*, **4**, 18–28.
- Lyon, G.J. and Novick, R.P. 2004. Peptide signaling in *Staphylococcus aureus* and other Gram-positive bacteria. *Peptides*, **25**, 1389–1403.

- McDougald, D., Srinivasan, S., Rice, S.A., and Kjelleberg, S. 2003. Signal-mediated cross-talk regulates stress adaptation in *Vibrio* species. *Microbiology*, **149**, 1923–1933.
- McLean, R.J.C., Whiteley, M., Stickler, D.J., and Fuqua, W.C. 1997. Evidence of autoinducer activity in naturally occurring biofilms. *FEMS Microbiology Letters*, **154**, 259–263.
- Medina-Martínez, M.S., Uyttendaele, M., Demolder, V., and Debevere, J. 2006. Influence of food system conditions on *N*-acyl-L-homoserine lactone production by *Aeromonas* spp. *International Journal of Food Microbiology*, **112**, 244–252.
- Medina-Martínez, M.S., Uyttendaele, M., Meireman, S., and Debevere, J. 2007. Relevance of *N*-acyl-L-homoserine lactone production by *Yersinia enterocolitica* in fresh foods. *Journal of Applied Microbiology*, **102**, 1150–1158.
- Moker, N., Dean, C.R., and Tao, J. 2010. *Pseudomonas aeruginosa* increases formation of multidrug-tolerant persister cells in response to quorum-sensing signalling molecules. *Journal of Bacteriology*, **192**, 1946.
- Nadell, C.D., Xavier, J.B., Levin, S.A., and Foster, K.R. 2008. The evolution of quorum sensing in bacterial biofilms. *PLoS Biology*, **6**, 171–179.
- Nazzaro, F., Fratianni, F., and Coppola, R. 2013. Quorum sensing and phytochemicals. *International Journal of Molecular Sciences*, **14**, 12607–12619.
- Nealson, K.H. and Hastings, J.W. 1979. Bacterial bioluminescence: its control and ecological significance. *Microbiology Reviews*, **43**, 496–518.
- Nealson, K.H., Platt, T., and Hastings, J.W. 1970. Cellular control of the synthesis and activity of the bacterial luminescence system. *Journal of Bacteriology*, **104**, 313–322.
- Nilsson, R.E., Ross, T., and Bowman, J.P. 2011. Variability in biofilm production by *Listeria monocytogenes* correlated to strain origin and growth conditions. *International Journal of Food Microbiology*, **150**, 14–24.
- Niu, C., Robbins, C.M., Pittman, K.J., et al. 2013. LuxS influences *Escherichia coli* biofilm formation through autoinducer-2-dependent and autoinducer-2-independent modalities. *FEMS Microbiology Ecology*, **83**, 778–791.
- Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., and Koutsoumanis, K. 2008. Meat spoilage during distribution. *Meat Science*, **78**, 77–89.
- Nychas, G.-J.E., Dourou, D., Skandamis, P., Koutsoumanis, K., Baranyi, J., and Sofos, J. 2009. Effect of microbial cell-free meat extract on the growth of spoilage bacteria. *Journal of Applied Microbiology*, **107**, 1819–1829.
- Parsek, M.R. and Greenberg, E.P. 2005. Sociomicrobiology: the connections between quorum sensing and biofilms. *TRENDS in Microbiology*, **13**, 27–33.
- Pearson, J.P., Gray, K.M., Passador, L., et al. 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 197–201.
- Pearson, J.P., Passador, L., Iglewski, B.H., and Greenberg, E.P. 1995. A second *N*-acylhomoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*, **92**, 1490–1494.
- Peck, M.W. 2009. Biology and genomic analysis of *Clostridium botulinum*. *Advances in Microbial Physiology*, **55**, 183–265.
- Pesci, E.C., Milbank, J.B., Pearson, J.P., et al. 1999. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of American – Physical Sciences*, **96**, 112129–11234.
- Pinto, U.M., Viana, E.S., Martins, M.L., and Vanetti, M.C.D. 2007. Detection of acylated homoserine lactones in Gram-negative proteolytic psychrotrophic bacteria isolated from cooled raw milk. *Food Control*, **18**, 1322–1327.
- Rasch, M., Andersen, J.B., Nielsen, K.F., et al. 2005. Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts. *Applied and Environmental Microbiology*, **71**, 3321–3330.
- Ravn, L.F., Christensen, A.B., Molin, S., Givskov, M., and Gram, L. 2003. Influence of food preservation parameters and associated microbiota on production rate, profile and stability of acylated homoserine lactones from food-derived

- Enterobacteriaceae. *International Journal of Food Microbiology*, **84**, 145–156.
- Redfield, R.J. 2002. Is quorum sensing a side effect of diffusion sensing? *TRENDS in Microbiology*, **10**, 365–370.
- Rychlik, I. and Barrow, P.A. 2005. *Salmonella* stress management and its relevance to behaviour during intestinal colonization and infection. *FEMS Microbiology Reviews*, **29**, 1021–1040.
- Sakuragi, Y. and Kolter, R. 2007. Quorum-sensing regulation of the biofilm matrix genes (*pel*) of *Pseudomonas aeruginosa*. *Journal of Bacteriology*, **189**, 5383–5386.
- Schauder, S. and Bassler, B.L. 2001. The languages of bacteria. *Genes and Development*, **15**, 1468–1480.
- Schauder, S., Shokat, K., Surette, M.G., and Bassler, B.L. 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Molecular Microbiology*, **41**, 463–476.
- Schuster, M., Hawkins, A.C., Harwood, C.S., and Greenberg, E.P. 2004. The *Pseudomonas aeruginosa* RpoS regulon and its relationship to quorum sensing. *Molecular Microbiology*, **51**, 973–985.
- Silagyi, K., Kim, S.-H., Lo, Y.M., and Wei, C.-I. 2009. Production of biofilm and quorum sensing by *Escherichia coli* O157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat deli, and produce products. *Food Microbiology*, **26**, 514–519.
- Sitnikov, D.M., Schineller, J.B., and Baldwin, T.O. 1996. Control of cell division in *Escherichia coli*: regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 336–341.
- Skandamis, P.N. and Nychas G.-J.E. 2012. Quorum sensing in the context of food microbiology. *Applied and Environmental Microbiology*, **78**, 5473–5482.
- Smirnova, T.A., Didenko, L.V., Azizbekyan, R.R., and Romanova, Y.M. 2010. Structural and functional characteristics of bacterial biofilms. *Microbiology*, **79**, 413–423.
- Smith, J.L., Fratamico, P.M., and Novak, J.S. 2004. Quorum sensing: a primer for food microbiologists. *Journal of Food Protection*, **67**, 1053–1070.
- Soni, K.A., Jesudhasan, P., Cepeda, M., et al. 2008. Identification of ground beef derived fatty acid inhibitors of autoinducer-2 based cell signaling. *Journal of Food Protection*, **71**, 134–138.
- Sperandio, V., Torres, A.G., Jarvis, B., Nataro, J.P., and Kaper, J.B. 2003. Bacteria-host communication: the language of hormones. *Proceedings of the National Academy of Sciences of the United States of America – Physical Sciences*, **100**, 8951–8956.
- Stanley, N.R. and Lazazzera, B.A. 2004. Environmental signals and regulatory pathways that influence biofilm formation. *Molecular Microbiology*, **52**, 917–924.
- Steenackers, H., Hermans, K., Vanderleyden, J., and De Keersmaecker, S.C.J. 2012. *Salmonella* biofilms: an overview on occurrence, structure, regulation and eradication. *Food Research International*, **45**, 502–531.
- Stickler, D.J., Morris, N.S., McLean, R.J.C., and Fuqua, C. 1998. Biofilms on indwelling urethral catheters produce quorum-sensing signal molecules in situ and *in vitro*. *Applied and Environmental Microbiology*, **64**, 3486–3490.
- Swift, S., Downie, J.A., Whitehead, N.A., Barnard, A.M., Salmond, G.P., and Williams, P. 2001. Quorum sensing as a population-density-dependent determinant of bacterial physiology. *Advances in Microbial Physiology*, **45**, 199–270.
- Tan, J.-Y., Yin, W.-F., and Chan, K.-G. 2014. Quorum sensing activity of *Hafnia alvei* isolated from packed food. *Sensors*, **14**, 6788–6796.
- Thaden, J.T., Lory, S., and Gardner, T.S. 2010. Quorum-sensing regulation of a copper toxicity system in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, **192**, 2557–2568.
- Turovskiy, Y. and Chikindas, M.L. 2006. Autoinducer-2 bioassay is a qualitative, not quantitative method influenced by glucose. *Journal of Microbiological Methods*, **66**, 497–503.
- Turovskiy, Y., Rosenberg, L., and Chikindas, M.L. 2007. Autoinducer-2-mediated quorum sensing is not involved in *Listeria monocytogenes*' adaptive responses to the food preservatives lactic acid and nisin. *Journal of Food Safety*, **27**, 386–399.

- Van Houdt, R. and Michiels, C.W. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. *Journal of Applied Microbiology*, **109**, 1117–1131.
- Viana, E.S., Campos, M.E.M., Ponce, A.R., Mantovani, H.C., and Vanetti, M.C.D. 2009. Biofilm formation and acyl homoserine lactone production in *Hafnia alvei* isolated from raw milk. *Biological Research*, **42**, 427–436.
- Vidal, J.E., Ludewick, H.P., Knuckel, R.M., Zahner, D., and Klugman, K.P. 2011. The LuxS-dependent quorum-sensing system regulates early biofilm formation by *Streptococcus pneumoniae* strain D39. *Infection and Immunity*, **79**, 4050–4060.
- Wade, D.S., Calfee, M.W., Rocha, E.R., et al. 2005. Regulation of *Pseudomonas* quinolone signal synthesis in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, **187**, 4372–4380.
- Wang, L.H., He, Y., Gao, Y., et al. 2004. A bacterial cell–cell communication signal with cross-kingdom structural analogues. *Molecular Microbiology*, **51**, 903–912.
- Waters, C.M. and Bassler, B.L. 2005. Quorum sensing: cell-to-cell communication in bacteria. *Annual Review of Cell and Developmental Biology*, **21**, 319–346.
- Webb, M.D., Stringer, S.C., Le Marc, Y., Baranyi, J., and Peck, M.W. 2012. Does proximity to neighbours affect germination of spores of non-proteolytic *Clostridium botulinum*? *Food Microbiology*, **32**, 104–109.
- Wesche, A.M., Gurtler, J.B., Marks, B.P., and Ryser, E.T. 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *Journal of Food Protection*, **72**, 1121–1138.
- Wevers, E., Moons, P., Van Houdt, R., Lurquin, I., Aertsen, A., and Michiels, C.W. 2009. Quorum sensing and butanediol fermentation affect colonization and spoilage of carrot slices by *Serratia plymuthica*. *International Journal of Food Microbiology*, **134**, 63–69.
- Whan, L., Dunstall, G., and Rowe, M.T. 2000. A study of the growth kinetics of two pseudomonads from pasteurized milk and the possible role of quorum sensing. *Milchwissenschaft*, **55**, 371–373.
- Whitehead, N.A., Barnard, A.M., Slater, H., Simpson, N.J., and Salmond, G.P. 2001. Quorum-sensing in Gram-negative bacteria. *FEMS Microbiology Reviews*, **25**, 365–404.
- Widmer, K.W., Soni, K.A., Hume, M.E., Beier, R.C., Jesudhasan, P., and Pillai, S.D. 2007. Identification of poultry meat-derived fatty acids functioning as quorum sensing signal inhibitors to auto-inducer-2 (AI-2). *Journal of Food Science*, **72**, M363–M368.
- Wong, G.C.L. and O'Toole, G.A. 2011. All together now: integrating biofilm research across disciplines. *MRS Bulletin*, **36**, 339–342.
- Xavier, J.B. and Foster, K.R. 2007. Cooperation and conflict in microbial biofilms. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 876–881.
- Yoon, Y. and Sofos, J.N. 2008. Autoinducer-2 activity of Gram-negative foodborne pathogenic bacteria and its influence on biofilm formation. *Journal of Food Science*, **73**, M140–M147.
- Yoon, Y. and Sofos, J.N. 2010. Absence of association of autoinducer-2-based quorum sensing with heat and acid resistance of *Salmonella*. *Journal of Food Science*, **75**, M444–M448.
- Yu, D., Zhao, L., Xue, T., and Sun, B. 2012. *Staphylococcus aureus* autoinducer-2 quorum sensing decreases biofilm formation in an *icaR*-dependent manner. *BMC Microbiology*, **12**, 288–300.
- Zhang, Q.Q., Ye, K.P., Wang, H.H., Xiao, H.M., Xu, X.L., and Zhou, G.H. 2014. Inhibition of biofilm formation of *Pseudomonas aeruginosa* by an acylated homoserine lactones-containing culture extract. *LWT – Food Science and Technology*, **57**, 230–235.
- Zhao, L., Montville, T.J., and Schaffner, D.W. 2005. Evidence for quorum sensing in *Clostridium botulinum* 56A. *Letters in Applied Microbiology*, **42**, 54–58.

CHAPTER 32

Heterogeneity in *Bacillus subtilis* spore germination and outgrowth: an area of key challenges for “omics” in food microbiology

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32.1 Bacterial spores in the food industry

Food industries are concerned with spore-forming bacteria such as *Bacillus* and *Clostridium* spp. The organisms cause immense problems because they are resistant to many preservation processes that are commonly used. This allows spores and thus their parent vegetative cells to survive commonly applied food processing and conservation methods. Therefore food industries must invest huge amounts in food quality control to reduce spore-related problems and ensure a microbiologically safe supply of high-quality food products to consumers. Currently, the food industry aims for less intense, more fit-for-purpose preservation methods. These should fulfill consumer demands for healthy nutritious and natural food products whilst maintaining the required microbiological stability levels. It is known that dry and wet heat induce damage via different mechanisms. On the one hand, dry heat primarily causes DNA damage (Setlow, 2006), while, on the other hand, wet heat causes protein denaturation in a rather non-specific way (Coleman *et al.*, 2007). Similar to these two thermal treatments preservation strategies often have different molecular bases.

This characteristic has been exploited in the application of what has been coined as “the hurdle preservation concept”. This preservation technology generally consists of the combination of more than one antimicrobial strategy such as the use of low concentrations of salt or acidification with specific weak organic acids often along with the application of a mild heat regime. The most naturally occurring and common weak organic acid used in food industries are sorbic acid, propionic acid, lactic acid, and acetic acid (Beales, 2004; Theron and Lues, 2007). The antimicrobial activity of these acids in aqueous solution is pH dependent, with the maximum effect occurring at low pH, where the undissociated state of the acid is favored (Piper *et al.*, 2001). The undissociated acid molecules are lipophilic and will penetrate plasma membranes, thus entering the cells. Inside the cell the acid encounters an internal pH, which is nearly neutral. As the pK_a values of many weak acid preserving acids are between 4.2 and 4.9, inside the cells a rapid dissociation of acid molecules into charged protons and anions takes place. These charged compounds cannot subsequently diffuse back across the plasma membrane. As a result, intracellular acidification of the cell cytosol, resulting from the accumulation

of protons, takes place and inhibits key metabolic activities involved in glycolysis (Krebs *et al.*, 1983) thereby perturbing the ATP yield of cellular metabolism (Van Beilen *et al.*, 2014). The mode of action of antimicrobial compounds is nowadays generally studied using some aspect of omics technology (Brul *et al.*, 2012), an example of which is the study of the response to acetic acid and sorbic acid by Ter Beek *et al.* (2014). Importantly, in most instances significant numbers of cells are used for array or, currently more and more, RNA-seq analysis. In food microbiology the presence of high numbers of spoilage organisms is, however, not very common practice. Initially anywhere between 1 and 10^3 cells per gram or milliliter may be present, which means that individual heterogeneity in behavior becomes a major determinant in predicting the occurrence of unwanted microbial growth (Smelt *et al.*, 2008, 2013). The ability to analyze genomics data at single cell level is what sets a major challenge to the scientific community at large and the food microbiology community in particular. In this chapter, we will discuss these aspects in state of the art microbial analysis of relevance to food microbiology. We will use as model organism *Bacillus subtilis*, an aerobic spore-forming bacterium with (many) sequenced genome(s).

32.2 The *Bacillus* genus

The *Bacillus* genus consists of Gram-positive, rod-shaped bacteria. The bacteria are ubiquitously present and inhabit water, soil, and live on as well as inside plants and animals (Jensen *et al.*, 2003; Nicholson, 2002; Tam *et al.*, 2006; Vilain *et al.*, 2006). Due to their ubiquitous occurrence, among all *Bacillus* species, *B. subtilis* is the most studied. It is the model organism for Gram-positive bacteria. The genome of *B. subtilis* was one of the first bacterial genomes to be sequenced. The organism exhibits natural competence (incorporates exogenous DNA, thus allowing for genetic modification) (Kunst *et al.*, 1997; Moszer, 1998). *Bacillus* forms

endospores that enables it to survive under unfavorable environmental conditions (Gould, 2006; Setlow, 2003, 2006). The position of the endospore differs among different bacterial species. For example, bacteria having terminal, subterminal, or centrally placed endospores are *Clostridium tetani*, *Bacillus subtilis*, and *Bacillus cereus*, respectively. Bacterial spores are phase bright, oval, dormant, metabolically inactive, stress-resistant structures formed when the cells are exposed to unfavorable conditions. The process through which they are formed is called sporulation.

32.3 Sporulation cycle

The process of spore formation has been studied in detail by using *B. subtilis* as the model organism. Figure 32.1 shows the sporulation cycle in detail. It is divided into seven different stages. The vegetative cells are considered to be in Stage 0. These undergo an unequal cell division to form a small forespore (also known as the prespore), which develops into the spore, and a large mother cell, which is necessary to sustain spore formation after which it ultimately lyses (programmed cell death). The next phase, Stage I, is the condensation stage, where the two genomes of the vegetative cell (post-asymmetric cell division) fuse to form a single axial filament of chromatin. At Stage II a septum is formed by membrane invagination that takes place at one pole of the mother cell. Soon after the division, distinct programs of gene expression are initiated in the mother cell and forespore, which are directed by sporulation-specific RNA polymerase σ factors, σF in the forespore and σE in the mother cell. At Stage III, a protoplast is formed within the mother cell. After division, the mother cell engulfs the forespore, resulting in the double-membrane enveloped spore structure. On completion of the engulfment, σG becomes active in the forespore and σK in the mother cell (Figure 32.2). Next, Stage IV is the stage where the deposition of the primordial germ cell wall and cortex between the membranes of

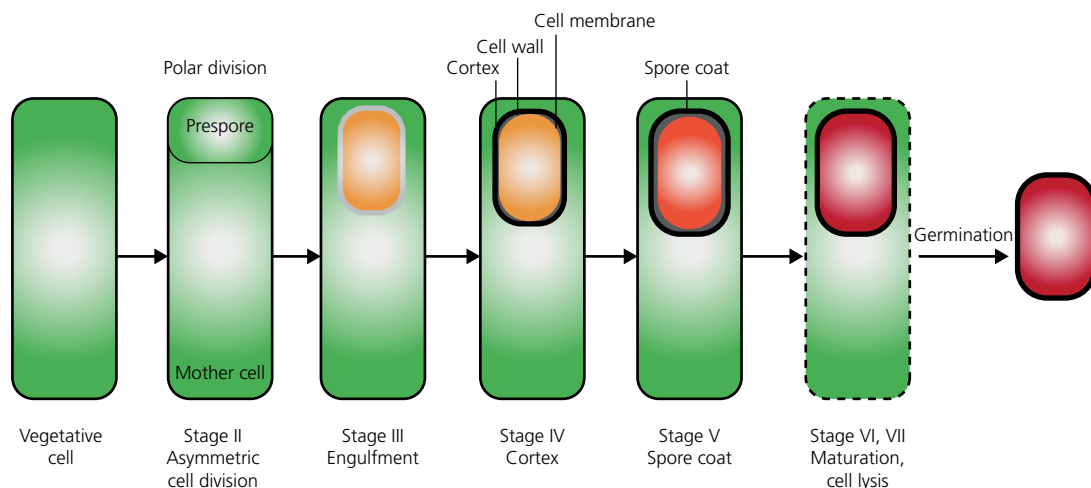


Figure 32.1 The sporulation cycle of spore-forming bacteria (adapted from Errington, 2003).

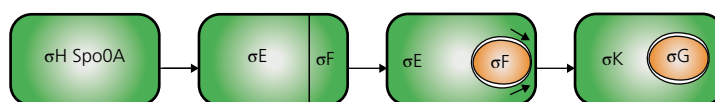


Figure 32.2 Gene regulation during sporulation (adapted from Piggot and Hilbert, 2004).

the spore protoplast occurs (Errington, 2003). In Stage V the spore coat deposition around the cortex takes place and at Stage VI the “maturation” of the spore occurs, upon which the spore develops its characteristic resistance properties. Finally, during Stage VII, the mother cell lyses and releases the completed spore (Piggot and Hilbert, 2004; Stephens, 1998). After the release, the spore undergoes further maturation with a final increase in (thermal) stress resistance (Abhyankar *et al.*, 2015).

32.4 Endospore structure and its resistance

The environmental resistance properties of spores are generally considered to be due to their well-assembled structure. The spore is made up of different layers, i.e., exosporium, coat, cortex, and core (Figure 32.3). Exosporium is the outer layer that lies on top of the spore coat (Driks, 1999; Henriques and Moran, 2007).

It is composed of a low-density network of glycosylated proteins. The network is loosely attached to the outer coat. The exact function of the exosporium is, however, not known (Driks, 1999; Henriques and Moran, 2007; Waller *et al.*, 2004). This layer is optional and present in, for example, *B. cereus* but not in *B. subtilis*. The spore coat is present around the outer membrane. It is made up of proteins and works like a sieve that excludes large molecules like lysozyme. The coat contains enzymes that are involved in spore germination. Furthermore, it allows interaction with the surroundings of the spore (Driks, 1999; Henriques and Moran, 2007). Underneath the spore coat lies the cortex, which consists of peptidoglycan. Next follows the inner membrane, which encloses the spore core (Takamatsu and Watabe, 2002). The inner membrane is made up of closely packed lipids that display far less lateral mobility than lipids in the plasma membrane of vegetative cells (Cowan *et al.*, 2003). It is selectively permeable for small and large molecules (Setlow and Setlow, 1993;

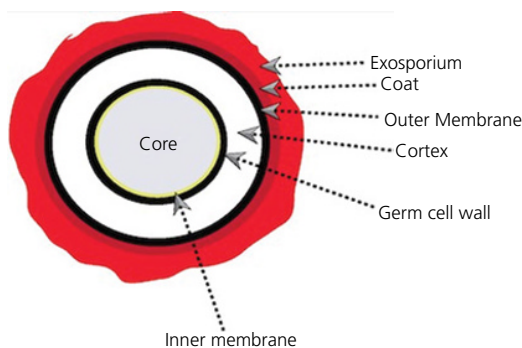


Figure 32.3 Spore anatomy (see text for the details).

Cortezzo *et al.*, 2004). It also contains germination receptor proteins, which are important for the regulation of the start of germination and the outgrowth process of spores.

The core is present beneath the cortex. It contains the chromosomal DNA, which is saturated with α/β -type small acid-soluble spore proteins (SASPs) (5–10%) (Raju *et al.*, 2006). In dormant spores the SASPs provide the spore's resistance to heat and UV light but during germination and outgrowth the SASPs get degraded and generate amino acids, which are subsequently used for protein synthesis (Mason *et al.*, 1986; Setlow, 1981, 1987). The core has a low water content amounting to about 25–50% wet weight (Beaman and Gerhardt, 1986). Under optimal non-stress conditions the pH in the core has been reported to be around 6.0–6.5 and hence to be slightly more acidic than vegetative cells (Magill *et al.*, 1994; Setlow and Setlow, 1993; Van Beilen and Brul, 2013). The acidic nature of the core is due to a large amount of pyridine-2,6-dicarboxylic acid or dipicolinic acid (DPA) present in the core, which is synthesized in the mother cell (Setlow, 2007). This DPA chelates with divalent calcium ions in a 1:1 ratio. The chelation in spores is merely in the solid phase as the DPA concentration is above its level of water solubility (Ablett *et al.*, 1999; Leuschner and Lillford, 2001, 2000). The compound stabilizes the DNA and protects it from heat and UV radiation by enhancing the amount of spore photoproduct (SP) production (Setlow and Setlow, 1993; Setlow, 2006).

32.5 Spore germination and outgrowth

Spore germination is a process by which the dormant and stress-resistant spore wakes up and initiates vegetative cell growth. All of the components necessary to facilitate germination are pre-packaged into the spore during sporulation. Figure 32.4 shows a schematic representation of different layers and elements that comprise the germination receptor system of *Bacillus* spores. Germination starts by interaction of appropriate germinants such as amino acids and sugars with germinant receptor proteins (Corfe *et al.*, 1994; Sammons *et al.*, 1981; Zuberi *et al.*, 1987). The germinant receptor proteins are present at a very low level (24–40 per spore) in the inner membrane of *B. subtilis* spores. The germinant receptors are encoded by tricistronic operons, which are composed of three subunits proteins (A, B, and C, Figure 32.4) (Paidhungat and Setlow, 1999; Moir *et al.*, 2002) and are under the control of a σ^G promoter (Corfe *et al.*, 1994; Kemp *et al.*, 1991). The “A” subunit is composed of two parts: the first part has five or six predicted membrane-spanning domains facing toward the core and the second part has a large hydrophilic N-terminal domain facing the cortex. The “B” and “C” subunits include ten predicted transmembrane domains and predicted lipoprotein signal sequence facing toward the cortex, respectively. Thus the “B” subunit is known as an integral membrane protein and the “C” subunit is known as membrane anchored (Moir *et al.*, 2002; Zuberi *et al.*, 1987). Although the exact roles of each of these proteins are not well comprehended, it is likely that their mutual interaction(s) (Hudson *et al.*, 2001; Igarashi and Setlow, 2005; Paidhungat and Setlow, 2001) is (are) required for proper functioning of the receptor complex as a whole. To start the germination process, germinants must reach receptors in the inner membrane of the spore by crossing the exosporium, coat, and cortex. The process is not well understood. It is known from *B. cereus* and *B. subtilis* studies that the germinant entry may be facilitated by GerP

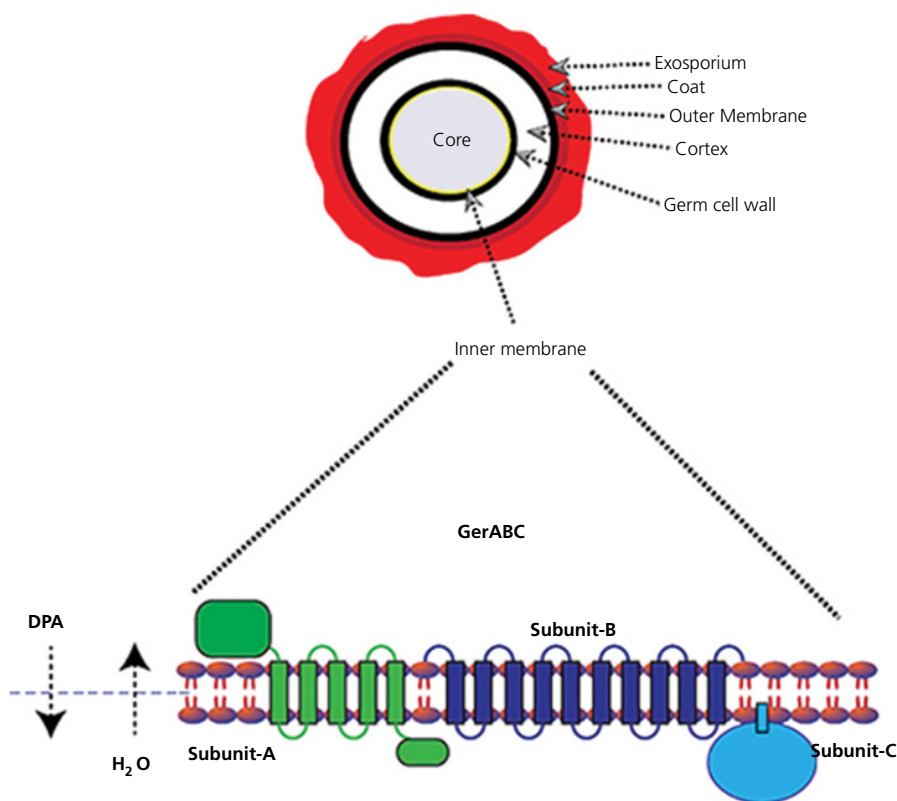


Figure 32.4 Schematic diagram of spore layers and elements that comprise the germination receptor system of *Bacillus* spores (modified from Moir *et al.*, 2002).

proteins (Behravan *et al.*, 2000). The germination process is divided into two different stages: stage I, early germination and stage II, the actual germination stage (Figure 32.5). In the early germination the germinant receptors, located in the inner membrane of the spore, sense the presence of germinant in their environment. This event commits spores to germinate. Once germination is committed, it becomes irreversible and continues even after removal of the germinants (Stewart *et al.*, 1981; Yi and Setlow, 2010b). After this, the release of monovalent cations such as H^+ , Na^+ , and K^+ , as well as divalent Ca^{2+} of dipicolinic acid (DPA) from the spore core occurs. Simultaneously the pH of the core rises from 6.5 to 7.7 (Setlow *et al.*, 2008; Van Beilen and Brul, 2013).

Due to partial rehydration and release of DPA, the heat resistance property of the spores

is reduced. In Stage II, the degradation of cortex peptidoglycan by two lytic enzymes called SleB and CwlJ takes place (Wuytack *et al.*, 1998; Paidhungat and Setlow, 1999, 2001; Paidhungat *et al.*, 2002; Setlow, 2003). This allows further uptake of water, due to which the core and inner membrane expand, dormancy breaks, and hence enzyme activity recommences (Christie, 2012). At this stage, the spores are completely germinated and become phase dark. On the one hand, the germination process is complex but, on the other hand, it is extremely efficient and rapid as 95% of spores germinate within minutes in rich media. The spore can germinate in any of the three ways. Firstly, in nutrient-mediated germination the germinants come in contact with the germination receptors and hence activate them. Secondly, the germination is independent of nutrient or germinant receptors.

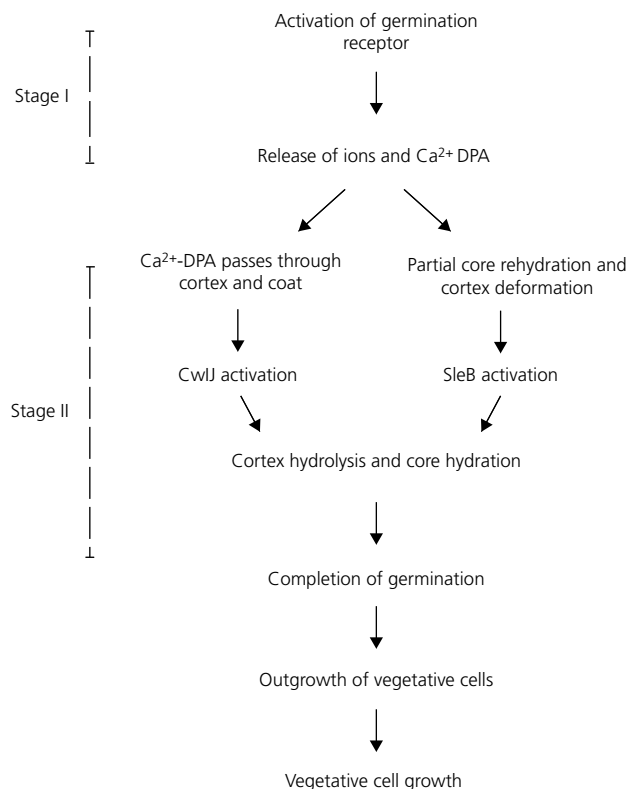


Figure 32.5 Spore germination and outgrowth pathway in *Bacillus* spp.

The dormant spores sense peptidoglycan fragments that are released into the environment by other bacteria in an as yet unknown way and thus stimulate germination (Shah *et al.*, 2008). Thirdly, in the non-nutrient route germination occurs with the help of surfactants, pressure, or exogenous Ca-DPA. The exogenous Ca-DPA allows spores to germinate by activating the lytic enzyme CwlJ, which in turn degrades the peptidoglycan of the cortex. Germination is an irreversible process in which physical and chemical properties of spores change, such as the release of Ca-DPA, loss of heat resistance, and a decrease in absorbance (Stewart *et al.*, 1981; Yi and Setlow, 2010b). Spore germination can be measured indirectly by correlating the mentioned properties of germinating spores (Stewart *et al.*, 1981; Yi and Setlow, 2010b). The time-resolved analysis of the mentioned properties of the spores is frequently used to inspect

germination (Paidhungat *et al.*, 2002). Other methods could be used to analyze the germination mutants where genes involved in germination are mutated, thus allowing us to study different germination components of the spore with respect to spore germination. The germination process is followed by the outgrowth stage. This outgrowth stage is also divided into two stages. In the first stage of outgrowth, the conversion of 3-phosphoglycerate results in the generation of ATP. After ATP production the second stage of outgrowth starts. In this stage the spore starts using extracellular nutrients and producing macromolecules needed to reconstitute biochemical pathways, nutrient uptake, and the replication process. After approximately 30 min chromosomal replication is initiated. Due to the action of germination-specific lytic enzymes (GSLEs) full rehydration of the spore occurs and activation of other

enzymes and more ATP production begin. During this process the degradation of SASPs in the spore core and transcription of core DNA resume (Setlow, 2007). In the outgrowth phase the RNA, protein, and DNA synthesis results in the formation of metabolically active vegetative cells. The SASPs in the spore core responsible for maintaining DNA stability are degraded, hence allowing transcription to resume (Setlow, 2007). The initiation of RNA, protein, and DNA synthesis during outgrowth results in a newly formed, metabolically active vegetative cell.

32.6 Heterogeneity in bacterial (spore) physiology during germination and outgrowth

Conventional microbial culturing methods allow heterogeneity to be measured at the population level, but the analyses average out the single-cell effects. An example of such data is the continuous measurement of the change in optical density (OD) during the germination and outgrowth phase of bacterial spores. Hence different methods have been developed to study the heterogeneity at the single-cell level. These methods include the use of Anopore filters, flow cytometry, laser tweezers Raman microscopy, as well as differential interference contrast

microscopy, etc. (Chen *et al.*, 2006; den Besten *et al.*, 2007, 2010; Smelt *et al.*, 2008; Wang *et al.*, 2011; Zhang *et al.*, 2009). Heterogeneity is not only evident in the germination phase but also in different phases of spore outgrowth as well as the growth of vegetative cells (Pandey *et al.*, 2013; Stringer *et al.*, 2011). Some spores are often called superdormant spores, as they remain dormant when exposed to germinant or, alternatively, they start germinating extremely slowly. Hence, such spores may potentially “come back to life” long after inactivation (preservation) treatments have been applied (Ghosh and Setlow, 2010, 2009; Chen *et al.*, 2014). The generally agreed view is that the cause of germination heterogeneity may be found in the number of receptors and accessory germination proteins present in (super)dormant spores and the number of cations available during sporulation (Ghosh *et al.*, 2012; Chen *et al.*, 2014). It is believed that outgrowth heterogeneity may well be influenced by stresses incurred during spore formation, for example, the temperature at the time of sporulation (Melly *et al.*, 2002). In addition, stress in the spore’s environment, for example, a thermal stress treatment, may influence the heterogeneity observed at this stage of transition of a dormant spore to a vegetative cell (Smelt *et al.*, 2008). Figure 32.6 exemplifies this by schematically indicating the effect of a thermal treatment on a spore population.

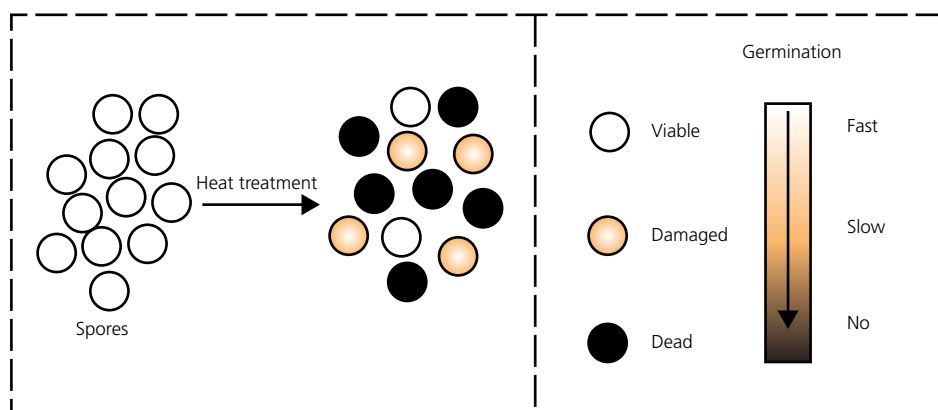


Figure 32.6 Effect of heat treatment on spores, which generates a subpopulation of sublethal damage spores.

When a dormant spore population is treated with heat, the single spores may subsequently be divided into three subpopulations, i.e., viable, damaged, and dead spores. The viable spores can germinate as fast as non-treated spores. The damaged spore tends to germinate slower than the viable spore, as most likely the germination machinery is itself damaged. In addition, outgrowth may be affected, as time may be needed to repair damage in the machinery that regulates DNA duplication and general cellular metabolism (Smelt *et al.*, 2008; Stringer *et al.*, 2011; Pandey *et al.*, 2013). Clearly irreversibly damaged spores often do not even germinate (Coleman *et al.*, 2007). A higher level of stress tends to correlate with an increased heterogeneity in spore behavior (e.g., Smelt *et al.*, 2008). In fact, heat treatment may have various effects on spores. It may, dependent on its intensity, trigger activation, germination, as well as the destruction of dormant spores through a complex multistage process. In order to minimize or eliminate food spoilage caused by the bacterial spore formers, it is very important to detect and estimate the number of viable spores in the food. Effective elimination of spores is only possible once spore detection and inactivation systems are available.

To develop such systems it is necessary to understand the molecular mechanisms behind the processes of germination and outgrowth as well as the stress-resistance properties of spores. Not much is known about the mechanisms that operate during the germination and outgrowth phase. Keijser *et al.* (2007) have shown in a population-based transcriptome study prominent functional modules in spore germination and outgrowth. Interestingly some of the modules contain stress response genes, which are transiently expressed during outgrowth, even in the absence of the cognate stress. In this way, spores may anticipate adverse environmental conditions. The vegetative cell (metabolically active form) is much easier to kill than the dormant spore. Therefore, it would be advantageous to deliberately drive spores in food into their vegetative form in order to facilitate their inactivation with food preservation

treatments. In practice, this should be done by triggering spores with germinants or physical treatments that allow their “rapid return to life” through the process of germination and outgrowth. The timing of this process is difficult to predict accurately as spores are generally seen to germinate at different times and at different rates. There have been few reports on spore germination at the single-spore level. A study on the timing of germination and outgrowth at the level of individual cells has shown that spore outgrowth occurs in up to 150 hr with or without a prior thermal treatment (Smelt *et al.*, 2008). For this study wild-type thermally treated spores were sorted individually in 96 wells of microtiter plates and tested for (out) growth under product-relevant conditions. The method gave a clear insight into the heterogeneity of the timing of outgrowth, but did not allow for the monitoring of the individual successive developmental processes of spore germination, outgrowth to vegetative cells, and subsequent cell divisions. These complex cell processes have been traditionally studied at the population level. The studies have shown that single-cell heterogeneity is a widespread phenomenon in biology and have directed the development of the single cell approaches. In this regard, flow cytometry, fluorescence in situ hybridization, microscopy, etc., was deployed allowing measurement of cell properties at an individual level. Stringer *et al.* used phase-contrast microscopy to determine the relation between different stages in germination and the subsequent outgrowth of spores from an anaerobically growing non-proteolytic *Clostridium botulinum* strain (Stringer *et al.*, 2011; Smelt *et al.*, 2013). Their conclusion was that the distribution of the times to germination, as well as outgrowth and subsequent growth showed considerable variability. All stages contribute to the overall variability in the observed lag time, with the time to germination and outgrowth being most affected by a thermal stress. The time to germination, spore emergence, cell maturation and cell doubling were not correlated. In a relatively recent study Veening *et al.* presented a method to mount *Bacilli* spp. on a

microscope slide and study their sporulation. The authors reported qualitative details on the growth of vegetative cells (De Jong *et al.*, 2011). However, no quantitative validation of the growth performance of vegetative cells growth under the condition for live imaging versus those prevailing in a well-aerated shake flask was performed. Huang *et al.* (2007) showed that microfluidic Raman tweezers can be used on a variety of *Bacillus* species and strains to measure levels of Ca-DPA in individual spores in populations. Phase-contrast microscopy allows us to observe at the single-spore level the process of germination by assessing the transition of spores from phase bright to phase dark, their outgrowth by measuring the time interval between the phase dark formation and the first cell division, as well as vegetative cell divisions for every individual cell of a population (Pandey *et al.*, 2013, 2015). By using such high-resolution single-cell analysis techniques in the combination with time-lapse microscopy, quantitative image analysis and observation of the activity of fluorescent reporter proteins is facilitated. This has enabled the present authors and other researchers to make a time-resolved study of the expression and activity of various proteins in individual cells and even bacterial spores (amongst others, this paper). In fact we can analyze individual spores, study their molecular physiology, and thus the mechanistic basis of the observed heterogeneity in spore behavior during germination and outgrowth. In this way we provide a framework for future contemporary studies aimed at further detailing the mechanistic basis of food preservation and spoilage models targeting bacterial spores.

32.7 Steps towards single-cell physiology and “omics” measurements

An internal pH measurement over time can be a direct method to investigate the physiological state of individual cells or germinating spores when stress is applied. This can be done with fluorescence microscopy using the pH-sensitive

green fluorescent protein pHluorin (Miesenböck *et al.*, 1998). pHluorin expression in the cells allows single-cell live-imaging ratiometric pH_i assessment, thus facilitating a time-resolved measurement of the internal pH during growth of the bacteria. Similar approaches can be used for the measurement of the internal pH behavior in spores during different stages of germination and outgrowth. Preliminary population-based data have been reported in Van Beilen and Brul (2013). Such single-cell live-imaging approaches will give relevant information about the heterogeneity in different phases of germination and an outgrowth of spores, which leads to a better understanding of the behavior of microorganisms in food products and should facilitate the development of more accurate models for bacterial growth under preservative stress as well as allowing appropriate combinations of preservation strategies to be optimized. This contributes to improved microbial food stability and food safety (McMeekin *et al.*, 2010). This knowledge can also be extended to other bacterial groups such as *Clostridium* spp. However, GFP needs oxygen for the maturation of its chromophore, which is composed of threonine, tyrosine, and glycine. Hence GFP cannot be used in anaerobic bacteria but another fluorescent protein called flavin-binding fluorescent protein (FbFPs) could be used instead. It is a recently developed a new class of genetically encoded probes that have a small size and do not require oxygen for maturation of their fluorophores (Cui *et al.*, 2012; Drepper *et al.*, 2007). The fluorescent microscope setup for live imaging that has been developed here has a limitation that due to the fully closed nature of the incubation environment it prevents the long-term monitoring of germination and outgrowth that is needed for analyses that aim at approaching real-life conditions of food preservation. The use of microfluidics (Ducret *et al.*, 2009) might be an avenue that could offer a way to face this challenge. Such tools should allow for the study of the dynamic behavior of cells/spores exposed to changing environmental conditions of interest to the industry. Furthermore, next to studying intracellular pH cells could be interrogation for

key regulators of metabolism and cellular homeostasis using the respective reporter constructs. In this way a functional germination profile can be generated and queried for its behavior under various environmental conditions. In conclusion, the study of the effect of sorbic acid, heat, and the combination of both stresses and tea compounds at single-spore and cell level described in this paper provides a better insight into the population heterogeneity and mode of action of common preservation methods on single spore-forming bacteria. Thereby the result obtained in this paper contributes to the improvement or design of new concepts of microbial food stability and food safety.

References

- Abhyankar, W., Pandey, R., Ter Beek, A., Brul, S., de Koning, L.J., and de Koster, C.G. 2015. Reinforcement of *Bacillus subtilis* spores by cross-linking of outer coat proteins during maturation. *Food Microbiology*, **45** (Part A), 54–62. doi: org/10.1016/j.fm.2014.03.007.
- Ablett, S., Darke, A.H., Lillford, P.J., and Martin, D.R. 1999. Glass formation and dormancy in bacterial spores. *International Journal of Food Science and Technology*, **34**, 59–69. doi: 10.1046/j.1365-2621.1999.00240.x.
- Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH and osmotic stress: a review. *Comprehensive Reviews in Food Science and Food Safety*, **3**, 1–20.
- Beaman, T.C. and Gerhardt, P. 1986. Heat resistance of bacterial spores correlated with protoplast dehydration, mineralization, and thermal adaptation. *Applied and Environmental Microbiology*, **52**, 1242–1246.
- Behravan, J., Chirakkal, H., Masson, A., and Moir, A. 2000. Mutations in the gerP locus of *Bacillus subtilis* and *Bacillus cereus* affect access of germinants to their targets in spores. *Journal of Bacteriology*, **182**, 1987–1994. doi: 10.1128/JB.182.7.1987-1994.2000.
- Brul, S., Bassett, J., Cook, P., Kathariou, S., McClure, P., Jasti, P.R., and Betts, R. 2012. Omics technologies in quantitative microbial risk assessment. *Trends in Food Science and Technology*, **27**, 12–24.
- Chen, D., Huang, S.S., and Li, Y.Q. 2006. Real-time detection of kinetic germination and heterogeneity of single *Bacillus* spores by laser tweezers Raman spectroscopy. *Analytical Chemistry*, **78**, 6936–6941. doi: 10.1021/ac061090e.
- Chen, Y., Ray, W.K., Hekm, R.F., Melville, S.B., and Popham, D.L. 2014. Levels of germination proteins in *Bacillus subtilis* dormant, superdormant, and germinating spores. *PloS One*, **9**. doi: 10.1371/journal.pone.0095781.
- Christie, G. 2012. Initiation of germination in *Bacillus* and *Clostridium* spores. In: *Bacterial Spores: Current Research and Applications* (ed. Erneso-Abel-Santos E.), pp. 89–106.
- Coleman, W.H., Chen, D., Li, Y.-Q., Cowan, A.E., and Setlow, P. 2007. How moist heat kills bacterial spores. *Journal of Bacteriology*, **189**, 8458–8466.
- Corfe, B.M., Sammons, R.L., Smith, D.A., and Mauël, C. 1994. The gerB region of the *Bacillus subtilis* 168 chromosome encodes a homologue of the gerA spore germination operon. *Microbiology*, **140**, 471–478. doi: 10.1099/00221287-140-3-471.
- Cortezzo, D.E., Koziol-Dube, K., Setlow, B., and Setlow, P. 2004. Treatment with oxidizing agents damages the inner membrane of spores of *Bacillus subtilis* and sensitizes spores to subsequent stress. *Journal of Applied Microbiology*, **97**, 838–852. doi: 10.1111/j.1365-2672.2004.02370.x.
- Cowan A.E., Koopel, D.E., Setlow, B., and Setlow, P. 2003. A soluble protein is immobile in dormant spores of *Bacillus subtilis* but is mobile in germinated spores: implications for spore dormancy. *Proceedings of the National Academy of Sciences USA*, **100**, 4209–4214.
- Cui, G., Hong, W., Zhang, J., Li, W., Feng, Liu, Y., and Cui, Q. 2012. Targeted gene engineering in *Clostridium cellulolyticum* H10 without methylation. *Journal of Microbiology Methods*, **89**, 201–208. doi:10.1016/j.mimet.2012.02.015.
- De Jong, I.G., Beilharz, K., Kuipers, O.P., and Veening, J.W. 2011. Live cell imaging of

- Bacillus subtilis* and *Streptococcus pneumoniae* using automated time-lapse microscopy. *Journal of Visualized Experiments (JoVE)*, **3145**. doi: 10.3791/3145.
- Den Besten, H.M.W., Ingham, C.J., van Hylckama Vlieg, J.E.T., Beerthuyzen, M.M., Zwietering, M.H., and Abee, T. 2007. Quantitative analysis of population heterogeneity of the adaptive salt stress response and growth capacity of *Bacillus cereus* ATCC 14579. *Applied and Environmental Microbiology*, **73**, 4797–4804. doi: 10.1128/AEM.00404-07.
- Den Besten, H.M.W., Garcia, D., Moezelaar, R., Zwietering, M.H., and Abee, T. 2010. Direct-imaging-based quantification of *Bacillus cereus* ATCC 14579 –population heterogeneity at a low incubation temperature. *Applied and Environmental Microbiology*, **76**, 927–930. doi: 10.1128/AEM.01372-09.
- Drepper, T., Eggert, T., Circolone, F., Heck, A., et al. 2007. Reporter proteins for *in vivo* fluorescence without oxygen. *Nature Biotechnology*, **25**, 443–445. doi: 10.1038/nbt1293.
- Driks, A. 1999. *Bacillus subtilis* spore coat. *Microbiology and Molecular Biology Reviews*, **63**, 1–20.
- Ducret, A., Maisonneuve, E., Botareschi, P., Grossi, A., Mignot, T., and Dukan, S. 2009. A microscope automated fluidic system to study bacterial processes in real time. *PloS One*, **4**, e7282. doi: 10.1371/journal.pone.0007282.
- Errington, J. 2003. Regulation of endospore formation in *Bacillus subtilis*. *Nature Reviews. Microbiology*, **1**, 117–126. doi: 10.1038/nrmicro750.
- Ghosh, S. and Setlow, P. 2009. Isolation and characterization of superdormant spores of *Bacillus* species. *Journal of Bacteriology*, **191**, 1787–1797. doi: 10.1128/JB.01668-08.
- Ghosh, S. and Setlow, P. 2010. The preparation, germination properties and stability of superdormant spores of *Bacillus cereus*. *Journal of Applied Microbiology*, **108**, 582–590. doi: 10.1111/j.1365-2672.2009.04442.x.
- Ghosh, S., Scotland, M., and Setlow, P. 2012. Levels of germination proteins in dormant and superdormant spores of *Bacillus subtilis*. *Journal of Bacteriology*, **194**, 2221–2227. doi: 10.1128/JB.00151-12.
- Gould, G.W. 2006. History of science – spores. *Journal of Applied Microbiology*, **101**, 507–513. doi: 10.1111/j.1365-2672.2006.02888.x.
- Henriques, A.O. and Moran, C.P. 2007. Structure, assembly, and function of the spore surface layers. *Annual Review of Microbiology*, **61**, 555–588. doi: 10.1146/annurev.micro.61.080706.093224.
- Huang, S.-S., Chen D., Pelczar, P.L., Vepachedu, V.R., Setlow, P., and Li, Y.-Q. 2007. Levels of Ca²⁺–dipicolinic acid in individual *Bacillus* spores determined using microfluidic Raman tweezers. *Journal of Bacteriology*, **189**, 4681–4687. doi: 10.1128/JB.00282-07.
- Hudson, K.D., Corfe, B.M., Kemp, E.H., Feavers, I.M., Coote, P.J. and Moir, A. 2001. Localization of GerAA and GerAC germination proteins in the *Bacillus subtilis* spore. *Journal of Bacteriology*, **183**, 4317–4322. doi: 10.1128/JB.183.14.4317-4322.2001.
- Igarashi, T. and Setlow, P. 2005. Interaction between individual protein components of the GerA and GerB nutrient receptors that trigger germination of *Bacillus subtilis* spores. *Journal of Bacteriology*, **187**, 2513–2518. doi: 10.1128/JB.187.7.2513-2518.2005
- Jensen, G.B., Hansen, B.M., Eilenberg, J., and Mahillon, J. 2003. Minireview, the hidden lifestyles of *Bacillus cereus* and relatives. *Environmental Microbiology*, **5**, 631–640.
- Keijser, B.J.F., Ter Beek, A., Rauwerda, H., Schuren, F., Montijn, R., van der Spek, H., and Brul, S. 2007. Analysis of temporal gene expression during *Bacillus subtilis* spore germination and outgrowth. *Journal of Bacteriology*, **189**, 3624–3634. doi: 10.1128/JB.01736-06.
- Kemp, E.H., Sammons, R.L., Moir, A., Sun, D., and Setlow, P. 1991. Analysis of transcriptional control of the gerD spore germination gene of *Bacillus subtilis* 168. *Journal of Bacteriology*, **173**, 4646–4652.
- Krebs, H.A., Wiggins, D., Stubbs, M., Sols, A., and Bedoya, F. 1983. Studies on the mechanism of the antifungal action of benzoate. *The Biochemical Journal*, **214**, 657–663.

- Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., and Danchin, A. 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature*, **390**, 249–256. doi: 10.1038/36786.
- Leuschner, R.G. and Lillford, P.J. 2000. Effects of hydration on molecular mobility in phase-bright *Bacillus subtilis* spores. *Microbiology*, **146**, 49–55.
- Leuschner, R.G.K. and Lillford, P.J. 2001. Investigation of bacterial spore structure by high-resolution solid-state nuclear magnetic resonance spectroscopy and transmission electron microscopy. *International Journal of Food Microbiology*, **63**, 35–50. doi: 10.1016/S0168-1605(00)00396-2.
- Mason, J.M. and Setlow, P. 1986. Essential role of small, acid soluble spore proteins in resistance of *Bacillus subtilis* spores to UV light. *Journal of Bacteriology*, **167**, 174–178.
- Magill, N.G., Cowan, A.E., Koppel, D.E., and Setlow, P. 1994. The internal pH of the forespore compartment of *Bacillus megaterium* decreases by about 1 pH unit during sporulation. *Journal of Bacteriology*, **176**, 2252–2258.
- McMeekin, T.A., Hill, C., Wagner, M., Dahl, A., and Ross, T. 2010. Eco-physiology of food-borne pathogens: essential knowledge to improve food safety. *International Journal of Food Microbiology*, **139**, S64–S78. doi: 10.1016/j.ijfoodmicro.2010.01.041.
- Melly, E., Genest, P.C., Gilmore, M.E., Little, S., Popham, D.L., Driks, A., and Setlow, P. 2002. Analysis of the properties of spores of *Bacillus subtilis* prepared at different temperatures. *Journal of Applied Microbiology*, **92**, 1105–1115.
- Miesenbock, G., De Angelis, D.A., and Rothman, J.E. 1998. Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent protein. *Nature*, **394**, 192–195. doi: 10.1038/28190.
- Moir, A., Corfe, B.M., and Behravan, J. 2002. Spore germination. *Cellular and Molecular Life Sciences*, **59**, 403–409.
- Moszer, I. 1998. The complete genome of *Bacillus subtilis*: from sequence annotation to data management and analysis. *FEBS Letters*, **430**, 28–36. doi: 10.1016/S0014-5793(98)00620-6.
- Nicholson, W.L. 2002. Roles of *Bacillus* endospores in the environment. *Cellular and Molecular Life Sciences*, **59**, 410–416.
- Paidhungat, M. and Setlow, P. 1999. Isolation and characterization of mutations in *Bacillus subtilis* that allow spore germination in the novel germinant D-alanine. *Journal of Bacteriology*, **181**, 3341–3350.
- Paidhungat, M. and Setlow, P. 2001. Localization of a germinant receptor protein (GerBA) to the inner membrane of *Bacillus subtilis* spores. *Journal of Bacteriology*, **183**, 3982–3990. doi: 10.1128/JB.183.13.3982-3990.2001.
- Paidhungat, M., Setlow, B., Daniels, W.B., Hoover, D., Papafragkou, E., and Setlow, P. 2002. Mechanisms of induction of germination of *Bacillus subtilis* spores by high pressure. *Applied and Environmental Microbiology*. **68**, 3172–3175, doi: 10.1128/AEM.68.6.3172-3175.2002.
- Pandey, R., Ter Beek, A., Vischer, N.O.E., Smelt, J., Brul, S., and Manders, E.M.M. 2013. Live cell imaging of germination and outgrowth of individual *Bacillus subtilis* spores; the effect of heat stress quantitatively analyzed with SporeTracker. *PloS One*, **8**, e58972. doi: 10.1371/journal.pone.0058972.
- Pandey, R., Ter Beek A., Vischer, N.O.E., Smelt, J.P.P.M., Kemperman, R., Manders, E.M.M., and Brul, S. 2015. Quantitative analysis of the effect of specific tea compounds on germination and outgrowth of *Bacillus subtilis* spores at single cell resolution. *Food Microbiology*, (Part A), 63–70. doi: 10.1016/j.fm.2014.03.006.
- Piggot, P.J. and Hilbert, D.W. 2004. Sporulation of *Bacillus subtilis*. *Current Opinion in Microbiology*, **7**, 579–586. doi: 10.1016/j.mib.2004.10.001.
- Piper, P., Calderon, C.O., Hatzixanthis, K., and Mollapour, M. 2001. Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. *Microbiology*, **147**, 2635–2642. doi: 10.1099/00221287-147-10-2635.
- Raju, D., Waters, M., Setlow, P., and Sarker, M.R. 2006. Investigating the role of small, acid-soluble

- spore proteins (SASPs) in the resistance of *Clostridium perfringens* spores to heat. *BMC Microbiology*, **6**, 50. doi:10.1186/1471-2180-6-50.
- Sammons, R.L., Moir, A., and Smith, D.A. 1981. Isolation and properties of spore germination mutants of *Bacillus subtilis* 168 deficient in the initiation of germination. *Journal of General Microbiology*, **124**, 229–241.
- Setlow, P. 1981. *Biochemistry of Bacterial Forespore Development and Spore Germination*, pp.13–28. American Society for Microbiology, Washington, DC, USA.
- Setlow, P. 2003. Spore germination. *Current Opinion in Microbiology*, **6**, 550–556. doi: 10.1016/j.mib.2003.10.001.
- Setlow, P. 2006. Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, **101**, 514–525. doi:10.1111/j.1365-2672.2005.02736.x.
- Setlow, P. 2007. I will survive: DNA protection in bacterial spores. *Trends in Microbiology*, **15**, 172–180. doi: 10.1016/j.tim.2007.02.004.
- Setlow, B. and Setlow, P. 1987. Thymine-containing dimers as well as spore photoproducts are found in ultraviolet-irradiated *Bacillus subtilis* spores that lack small acid-soluble proteins. *Proceedings of the National Academy of Sciences USA*, **84**, 421–424.
- Setlow, B. and Setlow, P. 1993. Dipicolinic acid greatly enhances production of spore photoproduct in bacterial spores upon UV irradiation. *American Society for Microbiology*, **59**, 640–643.
- Setlow, B., Wahome, P.G., and Setlow, P. 2008. Release of small molecules during germination of spores of *Bacillus* species. *Journal of Bacteriology*, **190**, 4759–4763. doi: 10.1128/JB.0039908.
- Shah, I.M., Laaberki, M.H., Popham, D.L., and Dworkin, J. 2008. A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell*, **135**, 486–496. doi: 10.1016/j.cell.2008.08.039.
- Smelt, J.P.P.M., Bos, A.P., Kort, R., and Brul, S. 2008. Modelling the effect of sub (lethal) heat treatment of *Bacillus subtilis* spores on germination rate and outgrowth to exponentially growing vegetative cells. *International Journal of Food Microbiology*, **128**, 34–40. doi: 10.1016/j.ijfoodmicro.2008.08.023.
- Smelt, J.P.P.M., Stringer, S., and Brul, S. 2013. Behaviour of individual spores of non-proteolytic *Clostridium botulinum* as an element in quantitative risks assessment. *Food Control*, **29**, 358–363.
- Stephens, C. 1998. Bacterial sporulation: a question of commitments? *Current Biology*, **8**, 45–48.
- Stewart, G.S., Johnstone, K., Hagelberg, E., and Ellar, D.J. 1981. Commitment of bacterial spores to germinate. A measure of the trigger reaction. *The Biochemical Journal*, **198**, 101–106.
- Stringer, S.C., Webb, M.D., and Peck, M.W. 2011. Lag time variability in individual spores of *Clostridium botulinum*. *Food Microbiology*, **28**, 228–235. doi: 10.1016/j.fm.2010.03.003.
- Takamatsu, H. and Watabe, K. 2002. Assembly and genetics of spore protective structures. *Cellular and Molecular Life Sciences*, **59**, 434–444.
- Tam, N.K.M., Uyen, N.Q., Hong, H.A., Duc, L.H., Hoa, T.T., Serra, C.R., Henriques, A.O., and Cutting, S.M. 2006. The intestinal life cycle of *Bacillus subtilis* and close relatives. *Journal of Bacteriology*, **188**, 2692–2700. doi: 10.1128/JB.188.7.2692-2700.2006.
- Ter Beek, A., Wijman, J.G.E., Zakrzewska, A., Orij, R., Smits, G.J., and Brul, S. 2014. Comparative physiological and transcriptional analysis of weak organic acid stress in *Bacillus subtilis*. *Food Microbiology*, **45** (Part A), 71–82.
- Theron, M.M. and Lues, J.F.R. 2007. Organic acids and meat preservation: a review. *Food Reviews International*, **23**, 141–158. doi: 10.1080/87559120701224964.
- Van Beilen, J.W.A. and Brul, S. 2013. Compartment-specific pH monitoring in *Bacillus subtilis* using fluorescent sensor proteins: a tool to analyze the antibacterial effect of weak organic acids. *Frontiers in Microbiology*, **4**, 157. doi: 10.3389/fmicb.2013.00157.
- Van Beilen, J.W.A., de Mattos, M.J., Hellingwerf, K.J., and Brul, S. 2014. Sorbic acid and acetic

- acid have distinct effects on the electrophysiology and metabolism of *Bacillus subtilis*. *Applied and Environmental Microbiology*, **80**, 5918–5926. doi: 10.1128/AEM.01391-14.
- Vilain, S., Luo, Y., Hildreth, M.B., and Brözel, V.S. 2006. Analysis of the life cycle of the soil saprophyte *Bacillus cereus* in liquid soil extract and in soil. *Applied and Environmental Microbiology*, **72**, 4970–4977. doi: 10.1128/AEM.03076-05.
- Waller, L.N., Fox, N., Fox, K.F., Fox, A., and Price, R.L. 2004. Ruthenium red staining for ultra structural visualization of a glycoprotein layer surrounding the spore of *Bacillus anthracis* and *Bacillus subtilis*. *Journal of Microbiological Methods*, **58**, 23–30. doi: 10.1016/j.mimet.2004.02.012.
- Wang, G., Zhang, P., Paredes-Sabja, D., Green, C., Setlow, P., Sarker, M.R., and Li, Y.-Q. 2011. Analysis of the germination of individual *Clostridium perfringens* spores and its heterogeneity. *Journal of Applied Microbiology*, **111**, 1212–1223. doi: 10.1111/j.1365-2672.2011.05135.x.
- Wuytack, E.Y., Boven, S., and Michiels, C.W. 1998. Comparative study of pressure-induced germination of *Bacillus subtilis* spores at low and high pressures. *Applied Environmental Microbiology*, **64**, 3220–3224.
- Yi, X. and Setlow, P. 2010b. Studies of the commitment step in the germination of spores of *Bacillus* species. *Journal of Bacteriology*, **192**, 3424–3433. doi: 10.1128/JB.00326-10.
- Zhang, P., Setlow, P., Li, Y., and Author, V. 2009. Characterization of single heat-activated *Bacillus* spores using laser tweezers Raman spectroscopy. *Optics Express*, **17**, 16480–16491.
- Zuberi, R., Moir, A., and Feavers, I.M. 1987. The nucleotide sequence and gene organization of the gerA spore germination operon of *Bacillus subtilis* 168. *Gene*, **51**, 1–11.

CHAPTER 33

Role of stress response on microbial ecology of foods and its impact on the fate of food-borne microorganisms

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33.1 Introduction

Foods are complex environments in which present microorganisms may face natural stress conditions such as limited nutrient availability, adverse pH, osmolarity, oxidation, and extreme temperatures among others. In addition, industrial food preservation regimes commonly rely upon imposing extreme physical and chemical stresses with the aim to inactivate or limit the growth of pathogenic bacteria. Thus, a variety of preservation technologies such as thermal processing, high hydrostatic pressure, pulsed electric fields, radiation, drying, etc., impose a challenge to bacterial cells and can determine the fate of food-borne pathogens along the food chain.

Bacteria have evolved a range of adaptive strategies to cope with rapidly changing environmental conditions and ensure their survival in inhospitable niches. Such adaptations sometimes derive from the acquisition of stochastic genomic mutations that are positively selected and fixed in the microbial population due to the beneficial phenotype they confer under selective pressure environments (Rando and Verstrepen, 2007). On other occasions, a transient stress response generally consisting of a characteristic change in the pattern of gene expression occurs (Abee and Wouters, 1999). This results in the induced

synthesis of stress proteins, which repair damaged DNA and proteins, and enzymatic systems with specific stress-counteracting activities. Transient stress responses can also occur through modulation of the envelopes and other cellular structures. These transient stress responses help to protect vital processes and to restore cellular homeostasis, and increase the cellular resistance against subsequent stress challenges.

This chapter reviews some of the strategies used by pathogenic bacteria to respond to stress in a permanent or transient manner and discusses the implications that such adaptive responses pose for food safety.

33.2 Acquisition of permanent stress tolerance through adaptive mutagenesis

The known edges of the biosphere are typically delineated by extremophilic bacteria that manage to resist the severe physical and chemical conditions encountered in these environments (extreme temperature, pH, high pressure, etc.). However, further to the existence of extremophiles, some mesophilic bacteria have shown the capacity to develop resistance against extreme environments within a defined genomic context

and a limited number of generations, in a clear example of bacterial adaptability (Vanlint *et al.*, 2011) (Table 33.1). Such adaptability could be the basis for the existence or persistence of classic mesophilic bacteria in extreme environments that so far have been reported as being inaccessible by current experimental techniques.

High hydrostatic pressure (HHP) processing is becoming a valuable non-thermal food pasteurization technique. While the degree of natural HHP resistance has already been shown to vary greatly among and within bacterial species, a still unresolved question remains as to what extent different food-borne pathogens can actually develop HHP resistance, and how this impacts food safety of HHP processed products. Vanlint *et al.* (2011) used a direct evolution approach to assess the potential of the mesophilic model bacterium *Escherichia coli* to acquire stable resistance to extreme high temperatures or pressures. They observed that while heat resistance could only be marginally increased, piezoresistance (i.e., resistance to high pressures) could readily and reproducibly be extended into the gigapascal range. The currently recognized maximum pressures for growth or survival of any vegetative microorganisms (including *E. coli*) are in the range of 120 and 800–900 MPa, respectively (Zeng *et al.*, 2009; Jofré *et al.*, 2010). Indeed, it was generally assumed that vegetative microorganisms would never be able to survive exposures in the GPa range (Daniel *et al.*, 2006). However, in the study by Vanlint and co-workers the developed piezotolerance was extraordinary, since the upper pressure limit for survival increased from the initial 600 to 2 GPa, thereby greatly exceeding the currently recognized maximum high pressure for growth or survival, and the pressure levels commonly used in food industries for food preservation.

Acquisition of extreme piezotolerance among food-borne pathogens might not be limited to *E. coli*. Vanlint *et al.* (2012) examined the potential for HHP resistance development among strains of *E. coli*, *Shigella flexneri*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Yersinia*

enterocolitica, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *Listeria innocua* and reported that extreme HHP resistance was only observed in some of the *E. coli* strains. These authors concluded that a specific genetic predisposition could be required for HHP resistance development. However, piezotolerant variants have been found by other research groups in other food-borne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus* (Karatzas and Bennik, 2002; Karatzas *et al.*, 2005, 2007; Van Boeijen *et al.*, 2010). In the study by Karatzas and Bennik (2002) a piezotolerant strain of *Listeria monocytogenes* was isolated after long-term exposure to HHP. This isolate showed morphological alterations, including increased cell size and lack of flagella, and also presented increased stress tolerance to heat, acid, and hydrogen peroxide treatments. This resistant phenotype was attributed to a single codon deletion in a glycine-encoding repeat region of the *ctsR* gene, which encodes a class 3 heat shock response regulator. In a subsequent study Karatzas *et al.* (2005) exposed wild-type cultures of *L. monocytogenes* to 350 MPa for 20 minutes and evaluated the piezotolerance of individual surviving isolates, finding 33 isolates with a stable piezotolerant phenotype. Interestingly, 21 of these 33 piezotolerant variants had mutations in the *ctsR* gene. These mutations mainly consisted of deletions or codon insertions in the glycine repeat region of the *ctsR* gene. However, a piezotolerant phenotype could not be linked to mutations in the *ctsR* gene for the rest of variants, which evidenced that other unknown mechanisms may also lead to stable piezotolerance in *L. monocytogenes*. Similarly, Van Boeijen *et al.* (2010) isolated 24 *L. monocytogenes* LO28 stress-resistant variants after HHP treatment. Genetic analysis of these variants revealed two variants with deletions in the upstream region of *ctsR* and seven variants with mutations in the *ctsR* gene itself. Interestingly, all variants were more resistant to heat than the wild-type strain. Piezotolerant variants have been also found for *Staphylococcus aureus* by Karatzas *et al.* (2007) following an HHP treatment of a clonal culture at 400 MPa for

Table 33.1 Studies describing the acquisition of hyper-resistance to food-related stresses.

Reference	Stress	Microorganism	Selection approach	Other comments
Karatzas and Bennik, 2002	High hydrostatic pressure (HHP)	<i>L. monocytogenes</i>	Single pressurization treatment of 400 MPa for 20 min	Variants lacked flagella, were elongated, and showed slightly lower maximum specific growth rates than the wild type and increased resistance to heat, acid, and H ₂ O ₂
Karatzas et al., 2005	High hydrostatic pressure (HHP)	<i>L. monocytogenes</i>	Exposure to 350 MPa for 20 min and isolation of survivors	Two-thirds of the variants had mutations in the <i>ctsR</i> gene
Karatzas et al., 2007	High hydrostatic pressure (HHP)	<i>S. aureus</i>	Exposure to 400 MPa for 30 min and isolation of survivors	The majority of the variants showed increased thermotolerance, impaired growth, and reduced antibiotic resistance compared to the wild type
Karatzas et al., 2008	Acid stress	<i>S. Typhimurium</i> and <i>S. Enteritidis</i>	15 cycles of exposure to pH 2.5 for up to 4 h, followed by growth at pH 7 for 48 h	Variants showed changes in colony morphology, expression of SEF17 fimbriae, growth, increased heat resistance, and reduced virulence
Van Boeijen et al., 2008, 2010	High hydrostatic pressure (HHP)	<i>L. monocytogenes</i>	Exposure to 350 MPa and isolation of survivors from the tail (concave upward survival curve)	All variants were heat resistant; however, differences among variants were observed in acid resistance, growth rate, motility, and biofilm-forming capacity
Van Boeijen et al., 2011	Heat stress	<i>L. monocytogenes</i>	Treatment at specific heating conditions selected through a kinetic modeling based sampling scheme and isolation of survivors	Resistant variants were even isolated after a heat inactivation at 72 °C in milk, and these variants showed high resistance to standard pasteurization conditions
Vanlint et al., 2011, 2012, 2013	High hydrostatic pressure (HHP)	<i>E. coli</i>	Serial cycles of pressurization at increasing pressures	Piezoresistance could readily and reproducibly be extended into the GPa range
Sagarzazu et al., 2013	Pulsed electric fields (PEF)	<i>S. Typhimurium</i>	Repeated rounds of PEF treatment and outgrowth of survivors	PEF-resistant variants showed a higher tolerance to acidic pH, hydrogen peroxide, and ethanol, but not to heat
Metselaar et al., 2013	Acid stress	<i>L. monocytogenes</i>	Exposure of late-exponential phase cells to pH 3.5 for 90 min and isolation of survivors	Increased acid resistance showed to be significantly correlated to reduced growth rate

30 minutes. The majority of these isolated variants showed increased thermotolerance, impaired growth, and reduced antibiotic resistance as compared to the wild type. However, no mutations in the *ctsR* gene were detected for any of the variants, which suggests that genes and regulatory mechanisms involved in acquisition of resistance to HHP by *S. aureus* may differ from those of *L. monocytogenes*.

Selection of resistant variants has been also reported for certain food-borne pathogens after exposure to other stress conditions or food processing technologies different to HHP. Van Boeijen *et al.* (2011) isolated highly heat-resistant variants for two strains of *L. monocytogenes* by using a kinetic modeling-based sampling scheme. Interestingly, they revealed for one of these strains the presence of heat-resistant variants under industrially relevant conditions, i.e., through growth in ultra-high temperature (UHT)-processed whole milk followed by heat treatment cycles at 72°C. Variants thus obtained showed high resistance to standard pasteurization conditions. Several of the highly heat-resistant variants had also mutations in the *ctsR* gene, while the mechanisms leading to increased resistance in the rest of variants were not elucidated. Karatzas *et al.* (2008) showed that exposure of cells of *S. Typhimurium* and *S. Enteritidis* to extreme acid environments (15 cycles of exposure to pH 2.5 for up to 4 h followed by growth at pH 7.0 for 48 h) gave rise to an increase in the population acid resistance by three- to fourfold. In addition, these authors isolated from those acid-exposed populations variants with a stable increased acid resistance, which also showed increased heat resistance, reduced virulence and changes in colony morphology, expression of fimbriae, and growth. Moreover, stable highly acid resistant variants of *L. monocytogenes* have been recently isolated by Metselaar *et al.* (2013) after exposure of late-exponential phase cells to pH 3.5 for 90 minutes. Sixteen of the twenty-three variants isolated by these authors were highly resistant to a treatment at pH 2.5 for 3.5 minutes, showing less than 1 log reduction after acid treatment,

compared to an average of 4 log reduction for the wild-type strain. Nevertheless, the genetic shifts behind the acquisition of stable extreme acid resistance in these bacterial populations have not been unravelled yet.

33.3 Transient adaptive responses to stress: modulation of membrane fluidity as an example

Apart from the above-discussed stable and selection-driven acquisition of high resistance to stress, microorganisms can respond to adverse conditions in a transient manner, i.e., once the stressor disappears the bacterial population recovers its conventional tolerance to the particular stress agent. These transient responses to stress are usually known as stress tolerance responses, and can be defined as the induced resistance to normally lethal stress conditions following growth or short-term exposure to mild sublethal stress conditions (e.g., the acid tolerance response of *Salmonella* spp., reviewed by Alvarez-Ordóñez *et al.*, 2012b). Stress tolerance responses are commonly mediated through activation of particular response regulators that control the expression of specific sets of genes and orchestrate very complex responses involving the induction of cellular enzymes, the synthesis of stress-shock proteins that protect or repair cellular proteins and DNA, and the modulation of the composition and physical properties of the cellular envelopes. The next section will focus on a well-studied cellular process involved in the transient response of bacteria to stress, i.e., the modulation of membrane fatty acid composition and membrane fluidity in changing growth conditions.

The cellular envelopes are the first barrier of defence of microorganisms against the stressful environmental conditions they face. Consequently, modulation of composition and physical properties of the main components of the cellular envelopes, the cytoplasmic membrane and the cell wall, play an important role

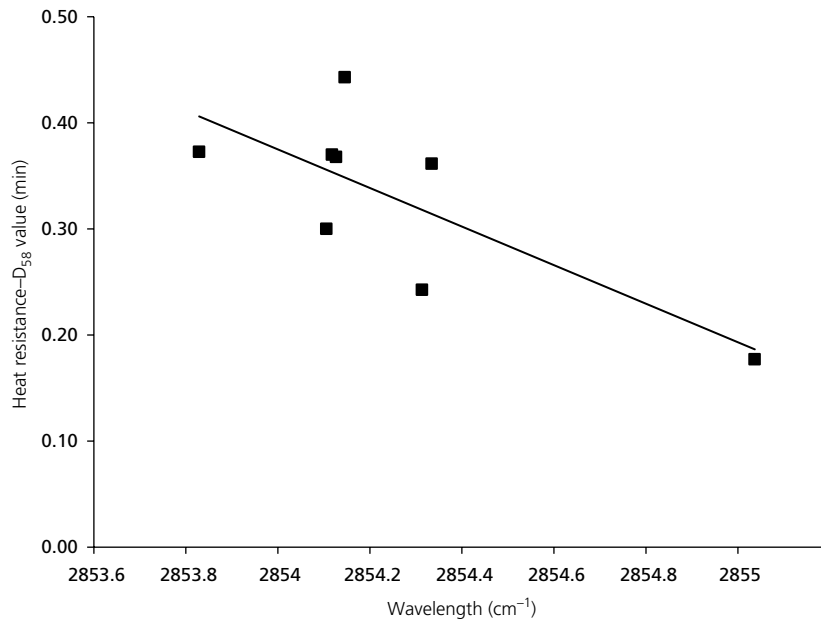


Figure 33.1 Relationship between heat resistance (D_{58} values in min) and membrane fluidity (as determined by FTIR spectroscopy, wavelength in cm^{-1}) of *Salmonella* Typhimurium.

when counteracting the odds of a changing environment. As an adaptive mechanism to stress, bacteria can change their membrane composition in response to the environmental signals found in their surroundings, with the purpose of maintaining a degree of membrane fluidity compatible with life (Beney and Gervais, 2001). It is well known that variations in growth temperature (Suutari and Laakso, 1994; Casadei *et al.*, 2002; Wang *et al.*, 2005; Alvarez-Ordóñez *et al.*, 2008), pH (Russell *et al.*, 1995; Brown *et al.*, 1997; de Jonge *et al.*, 2003; Sampathkumar *et al.*, 2004; Wang *et al.*, 2005), ethanol concentration (Teixeira *et al.*, 2002), and external osmolality (Guillot *et al.*, 2000), as well as transition to the stationary phase (Russell *et al.*, 1995; Casadei *et al.*, 2002), lead to changes in membrane fatty acid composition. It is also well known that changes in membrane fatty acid (FA) composition guarantee the maintenance of optimum membrane fluidity. Interestingly, a number of studies have suggested a relationship between membrane fluidity and stress tolerance (de Jonge

et al., 2003; Álvarez-Ordóñez *et al.*, 2008, 2010a). For instance, the impact that variations in membrane fatty acid composition and membrane fluidity in response to changing growth conditions have on bacterial tolerance to heat treatments has been well studied (Figure 33.1).

Several studies agree with the fact that reduced membrane fluidity is linked to enhancements in heat resistance. For example, Sampathkumar *et al.* (2004) showed an increased thermotolerance in *Salmonella* Enteritidis when it was pre-treated at alkaline pH values, and linked this behavior to an increased saturated to unsaturated fatty acid ratio, and consequently reduced membrane fluidity. Similarly, research work carried out by our team showed that *S. Typhimurium* cells grown at acid pH undergo a membrane adaptation characterized by a decrease in the unsaturated to saturated fatty acid ratio and in the relative concentration of octadecenoic (oleic or vaccenic) acids (C18:1), with a concomitant increase in the content in cyclic fatty acids, mainly methylenoctadecenoic

(dihydrosterculic or lactobacillic) acids (cyc19). These changes in membrane composition resulted in cells that in general showed a higher ability to survive to lethal heat treatments (Alvarez-Ordóñez *et al.*, 2008). The actual effect that such changes in membrane composition occurring in response to acid pH have on the cellular membrane fluidity were subsequently monitored by Fourier transform infrared spectroscopy (Alvarez-Ordóñez *et al.*, 2010a). The vibrational modes of the acyl chain ν_{CH_2} symmetric stretching band, which are indicators of bacterial membrane fluidity, were assessed after growth of *S. Typhimurium* and *S. Enteritidis* in acidic media at different pH values. Results obtained showed evidence that cells grown in acidified media showed a reduction in their membrane fluidity. In addition, it has been reported that cells with enhanced membrane fluidity show a decrease in thermotolerance. Thus, Annous *et al.* (1999) showed that cells of *Pediococcus* spp. grown at low temperatures have an increased concentration of membrane unsaturated fatty acids, and consequently in membrane fluidity, and were able to link this to a reduced thermal resistance phenotype. Growth of *S. Typhimurium* at low temperatures (up to 10°C) or cold shock of *S. Senftenberg* (4°C) were also shown to result in an increase in the unsaturated to saturated fatty acids ratio and in membrane fluidity, and were related to reduced heat resistance parameters (Alvarez-Ordóñez *et al.*, 2009a).

Exposure to different sorts of stress have been shown to cause the conversion of a significant proportion of the membrane unsaturated fatty acids to their cyclic derivatives, known as cyclopropane fatty acids (CFAs). The CFA are formed by a CFA synthase (encoded by the *cfa* gene), predominantly as cultures enter into the stationary phase (Grogan and Cronan, 1997). Kim *et al.* (2005) suggested for the first time that the formation of CFA in the membrane may be a major factor that protects *S. Typhimurium* from lethal stress exposures. Although the contribution of CFAs to bacterial membrane properties is not fully understood, it

has been hypothesized that the presence of a cyclopropane ring within a membrane FA increases the stability of the structural and dynamic properties of biological membranes and restricts the overall mobility and disorder of the acyl chains, thus decreasing the membrane fluidity (Duforc *et al.*, 1984; Grogan and Cronan, 1997). Other authors have postulated that the primary function of CFA formation is to change chemical rather than physical properties of the membrane. As an example, CFAs appear to be considerably less reactive than the corresponding unsaturated FA toward certain forms of oxidation (Grogan and Cronan, 1997), which suggests that CFAs exert their protective effect against lethal stress exposures through reduction of cellular oxidative damage. Finally, the cyclization of membrane FA has been also proposed as a means of controlling the penetration of undesirable molecules from the cellular surroundings (Chang and Cronan, 1999).

33.4 Using food components to survive under harsh conditions

Several authors have described that food-borne pathogenic bacteria are able to survive better under extreme environmental conditions or to food preservation treatments when they are inoculated into certain foods or food-derived media than when laboratory media is used as treatment media (Waterman and Small, 1998; Alvarez-Ordóñez *et al.*, 2009b). This suggests that microorganisms can benefit from the physicochemical properties of foods or can use some food components or molecules to respond to situations of stress (Figure 33.2).

33.4.1 Food components and the response to acid stress

A number of studies have demonstrated a role for particular amino acids in the maintenance of intracellular pH homeostasis in *Enterobacteriaceae* under lethal acidic environments (Kieboom and Abee, 2006; Lee *et al.*, 2007; Alvarez-Ordóñez

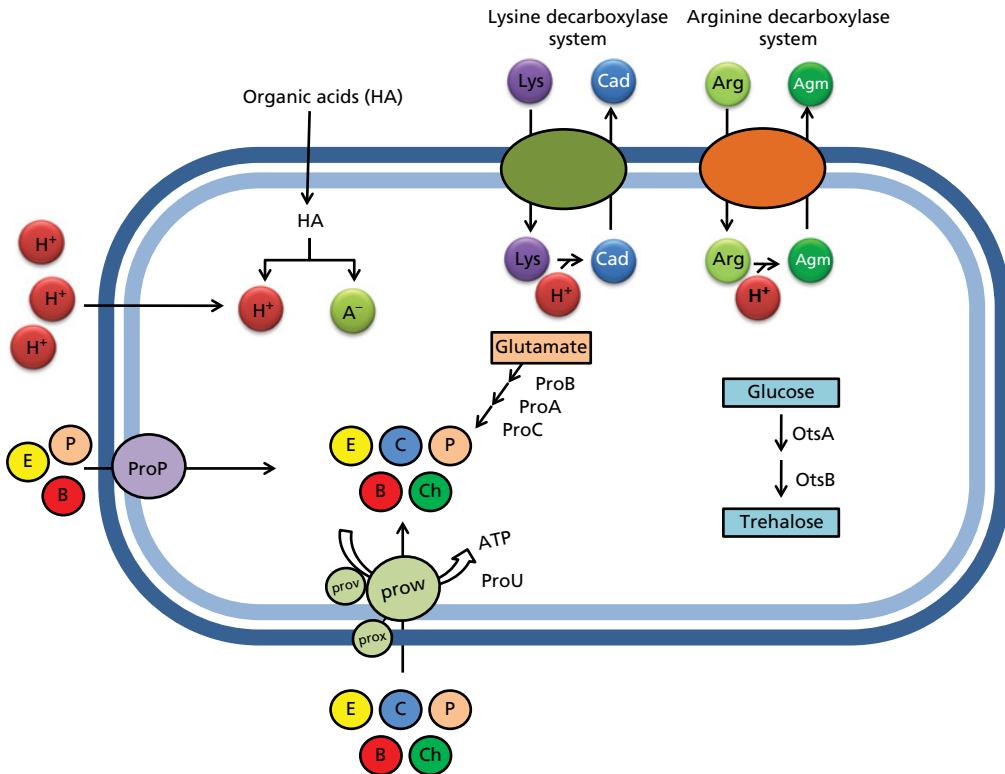


Figure 33.2 Protective role of the amino acids arginine and lysine and the compatible solutes trehalose, ectoine (E), carnitine (C), proline (P), betaine (B), and choline (Ch) against acid stress and osmotic stress, respectively, in *Salmonella* Typhimurium.

et al., 2010b; Feehily and Karatzas, 2012). Arginine, lysine, and glutamate are among those amino acids used by inducible decarboxylase systems responsible for maintenance of intracellular pH. The arginine decarboxylase system is composed of an arginine decarboxylase (AdiA), which converts arginine into agmatine in the cytoplasm with the consumption of a proton, an arginine/agmatine antiporter (AdiC), which expels agmatine from the cell in exchange for external arginine, and a transcriptional activator (AdiY) (Kieboom and Abee, 2006; Alvarez-Ordóñez *et al.*, 2010b). Similarly, the lysine decarboxylase system is composed of a transcriptional regulator of the *cadBA* operon (CadC), a lysine decarboxylase enzyme (CadA), and a lysine-cadaverine antiporter (CadB). Under conditions of low external pH in the

presence of lysine, CadC acts both as a signal sensor and a transcriptional regulator that activates the transcription of the *cadBA* operon. Following induction, the enzyme CadA converts intracellular lysine to cadaverine with the consumption of a proton, thus increasing the intracellular pH. Subsequently, cadaverine is exchanged for extracellular lysine via the CadB antiporter (Park *et al.*, 1996; Lee *et al.*, 2007). The glutamate decarboxylase system catalyses the decarboxylation of glutamate to γ -aminobutyrate (GABA) with the consumption of a proton. This is the first step of the GABA shunt pathway that leads to the production of succinate by the sequential actions of GABA-aminotransferase and succinic semialdehyde dehydrogenase. An antiporter system is usually present to couple the uptake of glutamate to the

efflux of GABA (Feehily and Karatzas, 2012). Whereas the GAD system has been described as the most important mechanism of acid resistance in *E. coli*, it appears to be absent in other members of the *Enterobacteriaceae* such as *Salmonella* Typhimurium, which uses instead the arginine and lysine decarboxylase systems (Audia *et al.*, 2001).

A recent study by Madeo *et al.* (2012) has evidenced the importance of thiamine to withstand lethal acid stress in *L. monocytogenes*. Madeo and co-authors reported that a mutant in the *thiT* gene, which encodes a thiamine uptake system, was highly sensitive to acid exposure and evidenced that thiamine-depleted cultures failed to produce significant concentrations of acetoin, consistent with the known thiamine dependence of acetolactate synthase, an enzyme of the 2,3-butanediol fermentation pathway required for acetoin synthesis from pyruvate. They suggested that as acetoin synthesis is a proton-consuming process, the acid sensitivity observed in thiamine-depleted cultures may be due to the inability to produce acetoin. The 2,3-butanediol fermentation pathway consists of a three-step conversion. Firstly, two molecules of pyruvate from glycolysis are combined to α -acetolactate by α -acetolactate synthase (α -ALS); α -Acetolactate is then decarboxylated to acetoin by α -acetolactate decarboxylase (α -ALD) and, finally, 2,3-butanediol can be formed by reduction of acetoin by 2,3-butanediol dehydrogenase (BDH) (Celińska and Grajek, 2009). The 2,3-butanediol fermentation pathway is often regarded as a strategy to avoid excessive and potentially lethal intracellular acidification (Vivijis *et al.*, 2014). Evidence for this protective role was first reported for the pathogenic bacteria *Vibrio cholerae*, where inactivation of the genes encoding the α -ALS and α -ALD resulted in a growth defect in broth with 1% glucose due to enhanced medium acidification (Yoon and Mekalanos, 2006). Later on, similar findings were reported in other microorganisms, including *Aeromonas hydrophila* (Van Houdt *et al.*, 2007; Moons *et al.*, 2011).

Urea, which is present in various environments in which bacteria can be found including soil, food, and the human host, can also be used by microorganisms to counteract lethal acid pH. Food-borne pathogens encounter urea upon their interaction with the human host: human saliva contains 2.3 to 4.1 mM, and the human stomach contains approximately 4.8 mM. In addition, foods of animal origin generally contain urea; for example, milk contains 4.4 to 6.4 mM urea (Mols and Abee, 2008). Urease activity, which is present in various microorganisms, plays a prominent role in the pathogenesis of the gastric pathogen *Helicobacter pylori* by allowing its survival to the low pH conditions of the stomach. Urea hydrolysis occurs in *H. pylori* inside the cytoplasm, resulting in the production of NH_3 , which is immediately protonated to form NH_4^+ . This ammonium is then metabolized or effluxed because its presence within the cell is counterproductive to the goal of raising pH while maintaining cellular viability (Miller and Maier, 2014). Urease utilization clusters similar to those of *H. pylori* have been identified in the genome of various food-borne pathogenic bacteria, although an equivalent role of ureolytic activity in acid survival has not been convincingly demonstrated for them yet (e.g., Mols and Abee, 2008).

33.4.2 Food components and the response to osmotic stress

Another relevant example of the ability of microorganisms to use food components to withstand stress conditions is the use of carbohydrates, amino acids, and other molecules as compatible solutes in the response to conditions of osmotic stress. Bacteria are known to react to environments of elevated osmolarity by means of a biphasic response, which involves the stimulation of potassium uptake (and its counterion glutamate) followed by a dramatic increase in the cytoplasmic concentration (by synthesis and/or uptake) of several so-called compatible solutes – trehalose, proline, ectoine, betaine, carnitine, and choline, among others (Sleator

and Hill, 2002). These compatible solutes play a dual role in osmoregulating cells, helping to restore cell volume while also stabilizing protein structure and function under adverse environmental conditions (Sleator *et al.*, 2003). In fact, several authors have demonstrated that their presence stimulates microbial growth at elevated osmolarity (mainly by inoculation in media with salt supplementation). Nevertheless, synthesis and/or uptake of compatible solutes can also protect bacterial cells against other forms of osmotic stress. For example, trehalose, a non-reducing disaccharide of glucose, plays a pivotal role in protection of bacteria against drying (Breeuwer *et al.*, 2003). These authors reported that addition of trehalose to the growth medium of *Cronobacter sakazakii*, a particularly desiccation-resistant bacterial species, improved its survival after drying. They also described that intracellular trehalose concentration was increased by more than fivefold in stationary-phase *C. sakazakii* cells exposed to drying. Similarly, other compatible solutes have been shown to improve the survival of microorganisms to high hydrostatic pressure treatments (Smiddy *et al.*, 2004). Smiddy and co-authors observed that addition of both betaine and L-carnitine to the treatment medium improved the barotolerance of *L. monocytogenes* and that betaine was more effective than L-carnitine in protection of the microbial cell from the detrimental effects of high hydrostatic pressure treatments. The uptake or synthesis of compatible solutes has also been shown to allow growth of microorganisms at low temperatures (Hill *et al.*, 2002). Indeed, a study by Wemekamp-Kamphuis *et al.* (2004) described that the addition of betaine or carnitine to the growth medium resulted in a significant increase of *L. monocytogenes* growth at low temperatures, with an associated betaine and carnitine accumulation. They also showed that single and multiple deletions of the known compatible solute transporters BetL, Gbu, and OpuC (involved in the uptake of betaine and carnitine) significantly reduced growth of *L. monocytogenes* at

low temperatures. Therefore, some natural components of foods or solutes added to foods may inadvertently promote the growth of food-borne pathogens under sublethal stress conditions of low temperature. For instance, Sleator *et al.* (2009) evaluated the growth of *L. monocytogenes* at 7°C in two infant formula preparations that differed only by the presence or absence of L-carnitine (1.5 mg/100 ml), and observed that *L. monocytogenes* grew faster in the formula with L-carnitine. They also described that the addition of L-carnitine to the formula that originally contained no L-carnitine resulted in a significant increase in the growth rate and final cell numbers of *L. monocytogenes*, demonstrating a role for L-carnitine in providing *L. monocytogenes* tolerance to cold.

33.5 The balance between self-preservation and nutritional competence (SPANC)

Acquisition of transient stress tolerance by bacteria is subject to a considerable cellular cost since it normally implicates large-scale host protection mechanisms involving several hundred cell components (e.g., proteins and stress-protectant metabolites), which accumulate to counteract the environmental challenges faced (Hengge-Aronis, 2002). Consequently, in return, high stress resistance is commonly accompanied by a reduction in the bacterial capacity to compete for the utilization of nutrients present in the environment at low concentrations. Indeed, an inverse relationship between competitiveness and the resistance of strains to stresses has been described for various food-borne pathogens. This fact can be regarded as the balance between the processes of self-preservation and nutritional competence (SPANC) balance and is responsible for the heterogeneity in stress tolerance among bacterial species (Phan and Ferenci, 2013). One of the best-known examples of a SPANC balance is the one driven by mutations in the general stress response regulator *rpoS* (alternative sigma

factor σ^{38} or σ^S) in members of the *Enterobacteriaceae* family. Sigma factors are dissociable subunits of RNA polymerase that play a key role in the regulation of the production of specific sets of proteins under specific environmental conditions. The core of RNA polymerase consists of five subunits, but this form is unable to initiate transcription from promoters without a sigma factor (the sixth subunit). Sigma factors can bind to RNA polymerase and this complex can recognize promoters and melt DNA, thus initiating transcription (Van Schaik and Abee, 2005). Most transcription events are mediated by the sigma factors σ^{70} (in Gram-negative bacteria) and σ^A (in Gram-positive bacteria). However, under specific conditions, including exposure to stress, other sigma factors (i.e., alternative sigma factors) are activated and control the expression of specialized sets of genes (regulons) (Gruber and Gross, 2003). One of the best-studied alternative sigma factors is the alternative sigma factor σ^S (RpoS) of Gram-negative bacteria. RpoS is the master regulator of the general stress response in Gram-negative bacteria. While in rapidly growing cells levels of RpoS are low, exposure to many different stress conditions results in a rapid and strong RpoS induction, and a consequent transcription of numerous RpoS-dependent genes, many of them encoding gene products with stress-protective functions (Hengge-Aronis, 2002). Despite the critical importance of RpoS for cellular survival and protection under hostile environments, several studies have shown that the *rpoS* gene is located in a highly polymorphic segment of the bacterial chromosome (Ferenci, 2003, 2008). In addition, various authors have described the frequent isolation of *rpoS* defective natural isolates in different members of the *Enterobacteriaceae* family (Table 33.2), including the food-borne pathogens *E. coli* (Waterman and Small, 1996; Bhagwat *et al.*, 2005, 2006; Dong *et al.*, 2009; Chiang *et al.*, 2011; Alvarez-Ordóñez *et al.*, 2013a), *Salmonella enterica* (Robbe-Saule *et al.*, 2003), and *Cronobacter sakazakii* (Alvarez-Ordóñez *et al.*, 2012a). In the first comprehensive study

on *rpoS* polymorphisms, Waterman and Small (1996) found that almost 20% of 58 tested *E. coli* strains had mutations in the *rpoS* gene that were linked to a reduced stress tolerance. Subsequent studies on *E. coli*, *Salmonella* spp., and *C. sakazakii* have described similar percentages of isolates with truncated RpoS among field collections of microorganisms (Robbe-Saule *et al.*, 2003; Chiang *et al.*, 2011; Alvarez-Ordóñez *et al.*, 2012a). The majority of *rpoS* mutations are loss-of-function mutations with little or no residual RpoS protein and include stop codons, deletions, and insertions as well as point mutations, although partial or attenuated *rpoS* mutations have also been described in other research articles. Most of those studies have reported a relationship between *rpoS* status (intact versus truncated RpoS) or expression level and overall intraspecies variability in tolerance to a wide range of stress conditions (e.g., acid, alkaline, oxidative, osmotic, thermal, and HHP exposures). Maintenance of a defective or less active RpoS may appear paradoxical, as it is likely to affect cellular survival under harsh conditions. However, *rpoS* mutations have been shown to confer a selective advantage for the cell under certain conditions. For instance, Wang *et al.* (2010) have observed that *rpoS* mutations provide *E. coli* populations with a strong fitness benefit under phosphate limitation. Similarly, King *et al.* (2004) reported that *E. coli rpoS* mutant strains are able to use more novel and alternative carbon sources for their growth than strains with a functional RpoS and show faster growth under limited-nutrients environments. Indeed, *rpoS* mutants of increased fitness have been shown to occur by natural selection in bacterial cultures maintained under carbon starvation or in the presence of non-preferred carbon sources (Zambrano *et al.*, 1993; Dong *et al.*, 2009; Alvarez-Ordóñez *et al.*, 2013b). For example, growth on weak acids (e.g., succinate and acetate) strongly selects for *rpoS* mutations in laboratory *E. coli* strains (Chen *et al.*, 2004; Spira *et al.*, 2008). Likewise, a study by our research group has shown that growth of

Table 33.2 Prevalence of *rpoS* mutants among natural populations in *Enterobacteriaceae*.

Reference	Microorganism	Prevalence of <i>rpoS</i> mutants (no. mutants/no. strains analysed)	Other comments
Waterman and Small, 1996	<i>E. coli</i>	10/58	Mutants were only confirmed for two of the strains. The other strains showed an acid-sensitive phenotype which was restored upon complementation with a functional <i>rpoS</i> gene
Sutton <i>et al.</i> , 2000	<i>S. Typhimurium</i>	10/27	Strains analyzed were archival cultures stored for 34 to 45 years
Robbe-Saule <i>et al.</i> , 2003	<i>S. enterica</i>	15/116	<i>rpoS</i> mutations in 15/41 <i>S. Typhi</i> strains and 0/75 <i>S. Typhimurium</i> strains
Bhagwat <i>et al.</i> , 2005	<i>E. coli</i>	20/82	Mutant strains showed an acid-sensitive phenotype which was restored upon complementation with a functional <i>rpoS</i> gene
Dong <i>et al.</i> , 2009	<i>E. coli</i>	1/10	Growth in the presence of succinate as the sole carbon source selected for loss of RpoS in seven of the ten <i>E. coli</i> strains
Chiang <i>et al.</i> , 2011	<i>E. coli</i>	6/2040	All 2040 strains were originally isolated from urban beaches and nearby fecal pollution sources on Lake Ontario (Canada)
Alvarez-Ordóñez <i>et al.</i> , 2012a	<i>C. sakazakii</i>	2/15	<i>rpoS</i> mutant strains were hypersensitive to stress and grew faster in the presence of non-preferred nutrients
Alvarez-Ordóñez <i>et al.</i> , 2013a	<i>E. coli</i>	2/12	<i>rpoS</i> mutant strains were hypersensitive to stress
Van Hoek <i>et al.</i> , 2013	<i>E. coli</i>	30/187	<i>rpoS</i> mutations in 0/73, 2/29, and 28/85 strains from bovine, food, and human origin, respectively

C. sakazakii in a minimal medium supplemented with acetate can select for loss of RpoS, with a consequent reduction in stress tolerance (Alvarez-Ordóñez *et al.*, 2013b). In addition, *C. sakazakii rpoS* mutants have been recently isolated in our laboratory by propagation in minimal medium supplemented with other non-preferred carbon sources such as succinate, fumarate, and propionate (unpublished data). Therefore, it appears clear that selection for loss or gain of RpoS function may be an important adaptive mechanism to ensure that bacteria can survive in complex natural environments.

33.6 Conclusions and future prospects

Microorganisms dispose of a range of adaptive mechanisms that contribute to their fitness in changing stressful environments. Many specific stress responses have been described in a wide range of food-borne pathogenic bacteria, which allow them to respond to environmental signals by temporarily changing the patterns of gene expression or modulating the cellular structure or conformation. These adaptive stress responses are normally transient and highly

energy demanding, and, for this reason, are not always beneficial for the cell. Indeed, selection for loss of key regulators of these transient stress responses can occur in complex bacterial populations in order to guarantee an improved fitness under specific environments. On the other hand, microorganisms can also enhance their chances of survival under stress conditions by increasing the generation of heterogeneity at the population level. This can be achieved either by creating genetic diversity by a variety of mechanisms involving, for example, constitutive or transient adaptive mutations, or by revealing phenotypic diversity that remained dormant.

Exposure to stress can induce various degrees of cellular damage or injury depending on the intensity of the stress and the physiological state of individual cells within the population. Injured cells represent a challenge for traditional food microbiology analytical methods. Upon exposure to lethal stresses a fraction of

the bacterial population may enter a non-culturable physiological state in which cells still remain metabolically active, retain their pathogenic properties, and possess the ability to resuscitate under favorable circumstances (Figure 33.3a). This is known as the viable but not culturable (VBNC) state (Ramamurthy *et al.*, 2014). VBNC cells do not grow in classic pre-enrichment media used for detection of pathogenic bacteria in food and environmental samples and may therefore go undetected during routine monitoring programs. However, subsequent cellular repair may allow for injury recovery and bacterial growth in foods. Future research must focus on the development of reliable analytical methods capable of detecting and enumerating VBNC cells in foods. Such methodologies need to be incorporated into standardized detection and enumeration methodologies.

Bacterial adaptive responses to stress can also result in cross-protection against a wide

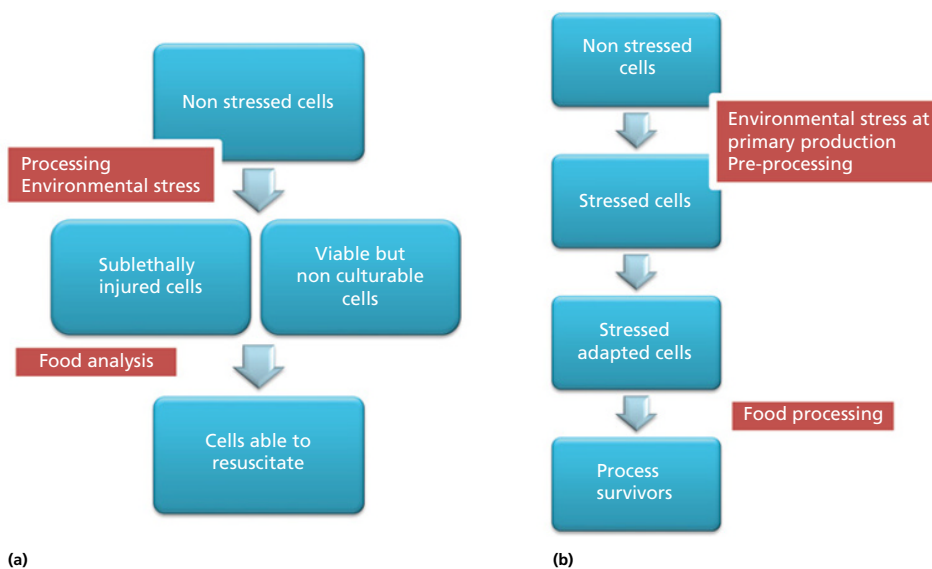


Figure 33.3 Relevance of stress adaptive response for food safety. (a) Bacterial exposure to stress during food processing induces a viable but not culturable state (VBNC). VBNC cells are not detected by conventional food analytical methods but may resuscitate during their transit by the gastrointestinal tract. (b) Bacterial exposure to stress at primary production or at pre-processing may select stress hyper-resistant variants or induce stress tolerance responses, thus improving the survival to food processing or during the transit by the gastrointestinal tract.

variety of lethal exposures different from that inducing the response. Such cross-protection responses are particularly significant when minimal processing of foods is considered, where the imposition of one sublethal stress may lead to the induction of multiple stress responses that may reduce the efficacy of subsequent treatments, and must be considered when implementing new preservation technologies or processing regimes (Figure 33.3b). Regarding this issue, the prediction of the phenotypic behavior using cellular indicators is a key area of research in the field of food-borne pathogens that in the near future will aid in the design and development of more efficient (novel combination) food preservation regimes or control strategies (Abee *et al.*, 2011). Quantitatively correlating microbial responses at molecular and phenotypic levels can assist the identification of cellular indicators for bacterial performance under stress conditions and under particular food preservation regimes. For instance, Den Besten *et al.* (2010) has recently designed a framework for identifying cellular biomarkers for mild stress-induced enhanced microbial robustness towards lethal stress conditions in *Bacillus cereus*. These authors selected several candidate biomarkers (e.g., transcriptional regulators, enzymes, chaperones, and proteases) by comparing the genome-wide transcriptome profiles of *B. cereus* upon exposure to mild stress conditions (heat, acid, salt, and oxidative stress) and quantified all of them at the transcript, protein, and/or activity level alongside the stress-induced robustness acquired towards lethal stress conditions. They were able to identify both short- and long-term biomarkers of bacterial tolerance responses towards lethal heat, acid, and oxidative stresses. In previous sections of this chapter we have identified two additional cellular components that could be used as indicators of bacterial robustness towards stress exposures, i.e., membrane fluidity as a biomarker of *S. Typhimurium* heat resistance (Figure 33.1), and *rpoS* activity as a biomarker of general stress resistance in *C. sakazakii* and other *Enterobacteriaceae*.

Nevertheless, promising candidate biomarkers could be genus, species, or even strain specific, since the functional conservation of stress-related cellular factors differs in nature. Further investigations testing a wide range of microorganisms under variable stress intensities will reveal how reliable those described biomarkers are. Application of systematic approaches such as the one followed by Den Besten and co-workers will facilitate the identification of wide spectrum and narrow spectrum biomarkers, which will aid the prediction of the history-dependent microbial behavior in foods. Incorporation of promising biomarkers into risk assessment schemes would help improve the safety of industrial processes.

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References

- Abee, T. and Wouters, J.A. 1999. Microbial stress response in minimal processing. *International Journal of Food Microbiology*, **50**, 65–91.
- Abee, T., Wels, M., de Been, M., and den Besten, H. 2011. From transcriptional landscapes to the identification of biomarkers for robustness. *Microbial Cell Factories*, **10**, S9.
- Alvarez-Ordóñez, A., Fernández, A., López, M., Arenas, R., and Bernardo, A. 2008. Modifications in membrane fatty acid composition of *Salmonella* Typhimurium in response to growth conditions and their effect on heat resistance. *International Journal of Food Microbiology*, **123**, 212–219.
- Alvarez-Ordóñez, A., Fernández, A., López, M., and Bernardo, A. 2009a. Relationship between membrane fatty acid composition and heat resistance of acid and cold stressed *Salmonella* Senftenberg CECT 4384. *Food Microbiology*, **26**, 347–353.

- Alvarez-Ordóñez, A., Fernández, A., Bernardo, A., and López, M. 2009b. Comparison of acids on the induction of an acid tolerance response in *Salmonella* Typhimurium, consequences for food safety. *Meat Science*, **81**, 65–70.
- Álvarez-Ordóñez, A., Halisch, J., and Prieto, M. 2010a. Changes in Fourier transform infrared spectra of *Salmonella enterica* serovars Typhimurium and Enteritidis after adaptation to stressful growth conditions. *International Journal of Food Microbiology*, **142**, 97–105.
- Álvarez-Ordóñez, A., Fernández, A., Bernardo, A., and López, M. 2010b. Arginine and lysine decarboxylases and the acid tolerance response of *Salmonella* Typhimurium. *International Journal of Food Microbiology*, **136**, 278–282.
- Alvarez-Ordóñez, A., Begley, M., and Hill, C. 2012a. Polymorphisms in *rpoS* and stress tolerance heterogeneity in natural isolates of *Cronobacter sakazakii*. *Applied and Environmental Microbiology*, **78**, 3975–3984.
- Alvarez-Ordóñez, A., Prieto, M., Bernardo, A., Hill, C., and López, M. 2012b. The acid tolerance response of *Salmonella* spp.: an adaptive strategy to survive in stressful environments prevailing in foods and the host. *Food Research International*, **45**, 482–492.
- Alvarez-Ordóñez, A., Alvseike, O., Omer, M.K., Heir, E., Axelsson, L., Holck, A., and Prieto, M. 2013a. Heterogeneity in resistance to food-related stresses and biofilm formation ability among verocytotoxigenic *Escherichia coli* strains. *International Journal of Food Microbiology*, **161**, 220–230.
- Alvarez-Ordóñez, A., Begley, M., and Hill, C. 2013b. Selection for loss of RpoS in *Cronobacter sakazakii* by growth in the presence of acetate as a carbon source. *Applied and Environmental Microbiology*, **79**, 2099–2102.
- Annous, B.A., Kozempel, M.F., and Kurantz, M.J. 1999. Changes in membrane fatty acid composition of *Pediococcus* sp. strain NRRL B-2354 in response to growth conditions and its effect on thermal resistance. *Applied and Environmental Microbiology*, **65**, 2857–2862.
- Audia, J.P., Webb, C.C., and Foster, J.W. 2001. Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. *International Journal of Medical Microbiology*, **291**, 97–106.
- Beney, L. and Gervais, P. 2001. Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Applied Microbiology and Biotechnology*, **57**, 34–42.
- Bhagwat, A.A., Chan, L., Han, R., Tan, J., Kothary, M., Jean-Gilles, J., and Tall, B.D. 2005. Characterization of Enterohemorrhagic *Escherichia coli* strains based on acid resistance phenotypes. *Infection and Immunity*, **73**, 4993–5003.
- Bhagwat, A.A., Tan, J., Sharma, M., Kothary, M., Low, S., Tall, B.D., and Bhagwat, M. 2006. Functional heterogeneity of RpoS in stress tolerance of enterohemorrhagic *Escherichia coli* strains. *Applied and Environmental Microbiology*, **72**, 4978–4986.
- Breeuwer, P., Lardeau, A., Peterz, M., and Joosten, H.M. 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. *Journal of Applied Microbiology*, **95**, 967–973.
- Brown, J.L., Ross, T., McMeekin, T.A., and Nichols, P.D. 1997. Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. *International Journal of Food Microbiology*, **37**, 163–173.
- Casadei, M.A., Mañas, P., Niven, G., Needs, E., and Mackey, B.M. 2002. Role of membrane fluidity in pressure resistance of *Escherichia coli* NCTC 8164. *Applied and Environmental Microbiology*, **68**, 5965–5972.
- Celińska, E. and Grajek, W. 2009. Biotechnological production of 2,3-butanediol – current state and prospects. *Biotechnology Advances*, **27**, 715–725.
- Chang, Y.Y. and Cronan, J.E. 1999. Membrane cyclopropane fatty acid content is a major factor in acid resistance of *Escherichia coli*. *Molecular Microbiology*, **33**, 249–259.
- Chen, G., Patten, C.L., and Schellhorn, H.E. 2004. Positive selection for loss of RpoS function in *Escherichia coli*. *Mutation Research*, **554**, 193–203.
- Chiang, S.M., Dong, T., Edge, T.A., and Schellhorn, H.E. 2011. Phenotypic diversity caused by

- differential RpoS activity among environmental *Escherichia coli* isolates. *Applied and Environmental Microbiology*, **77**, 7915–7923.
- Daniel, I., Oger, P., and Winter, R. 2006. Origins of life and biochemistry under high-pressure conditions. *Chemical Society Reviews*, **35**, 858–875.
- de Jonge, R., Ritmeester, W.S., and van Leusden, F.M. 2003. Adaptive responses of *Salmonella enterica* serovar Typhimurium DT104 and other *S. typhimurium* strains and *Escherichia coli* O157 to low pH environments. *Journal of Applied Microbiology*, **94**, 625–632.
- Den Besten, H.M.W., Arvind, A., Gaballo, H.M.S., Moezelaar, R., Zwietering, M.H., and Abee, T. 2010. Short- and long-term biomarkers for bacterial robustness: a framework for quantifying correlations between cellular indicators and adaptive behavior. *PLoS One*, **5**, e13746.
- Dong, T. and Schellhorn, H.E. 2010. Role of RpoS in virulence of pathogens. *Infection and Immunity*, **78**, 887–897.
- Dong, T., Chiang, S.M., Joyce, C., Yu, R., and Schellhorn, H.E. 2009. Polymorphism and selection of *rpoS* in pathogenic *Escherichia coli*. *BMC Microbiology*, **9**, 118.
- Duforc, E.J., Smith, I.C., and Jarrell, H.C. 1984. Role of cyclopropane moieties in the lipid properties of biological membranes: A2 H NMR structural and dynamical approach. *Biochemistry*, **23**, 2300–2309.
- Feehily, C. and Karatzas, K.A.G. 2012. Role of glutamate metabolism in bacterial responses towards acid and other stresses. *Journal of Applied Microbiology*, **114**, 11–24.
- Ferenci, T. 2003. What is driving the acquisition of *mutS* and *rpoS* polymorphisms in *Escherichia coli*? *Trends in Microbiology*, **11**, 457–461.
- Ferenci, T. 2008. The spread of a beneficial mutation in experimental bacterial populations: the influence of the environment and genotype of the fixation of *rpoS* mutations. *Heredity*, **100**, 446–452.
- Grogan, D.W. and Cronan, J.E. 1997. Cyclopropane ring formation in membrane lipids of bacteria. *Microbiology and Molecular Biology Reviews*, **61**, 429–441.
- Gruber, T.M. and Gross, C.A. 2003. Multiple sigma subunits and the partitioning of bacterial transcription space. *Annual Reviews of Microbiology*, **57**, 441–466.
- Guillot, A., Obis, D., and Mistou, M.Y. 2000. Fatty acid membrane composition and activation of glycine-betaine transport in *Lactococcus lactis* subjected to osmotic stress. *International Journal of Food Microbiology*, **55**, 47–51.
- Hengge-Aronis, R. 2002. Signal transduction and regulatory mechanisms involved in control of the sigma(S) (RpoS) subunit of RNA polymerase. *Microbiology and Molecular Biology Reviews*, **66**, 373–395.
- Hill, C., Cotter, P.D., Sleator, R.D., and Gahan, C.G.M. 2002. Bacterial stress response in *Listeria monocytogenes*: jumping the hurdles imposed by minimal processing. *International Dairy Journal*, **12**, 273–283.
- Jofré, A., Aymerich, T., Bover-Cid, S., and Garriga, M. 2010. Inactivation and recovery of *Listeria monocytogenes*, *Salmonella enterica* and *Staphylococcus aureus* after high hydrostatic pressure treatments up to 900 MPa. *International Microbiology*, **13**, 105–112.
- Karatzas, K.A.G. and Bennik, M.H.J. 2002. Characterization of a *Listeria monocytogenes* Scott A isolate with high tolerance to high hydrostatic pressure. *Applied and Environmental Microbiology*, **68**, 3183–3189.
- Karatzas, K.A.G., Valdramidis, V.P., and Wells-Bennik, M.H.J. 2005. Contingency locus in *ctsR* of *Listeria monocytogenes* Scott A: a strategy for occurrence of abundant piezotolerant isolates within clonal populations. *Applied and Environmental Microbiology*, **71**, 8390–8396.
- Karatzas, K.A.G., Zervos, A., Tassou, C.C., Mallidis, C.G., and Humphrey, T.J. 2007. Piezotolerant small-colony variants with increased thermotolerance, antibiotic susceptibility, and low invasiveness in a clonal *Staphylococcus aureus* population. *Applied and Environmental Microbiology*, **73**, 1873–1881.
- Karatzas, K.A.G., Hocking, P.M., Jørgensen, F., Mattick, K., Leach, S., and Humphrey, T.J. 2008. Effects of repeated cycles of acid challenge and growth on the phenotype and virulence of *Salmonella enterica*. *Journal of Applied Microbiology*, **105**, 1640–1648.

- Kieboom, J. and Abee, T. 2006. Arginine-dependent acid resistance in *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, **188**, 5650–5653.
- Kim, B.H., Kim, S., Kim, H.G., Lee, J., Lee, I.S., and Park, Y.K. 2005. The formation of cyclopropane fatty acids in *Salmonella enterica* serovar Typhimurium. *Microbiology*, **151**, 209–218.
- King, T., Ishihama, A., Kori, A., and Ferenci, T. 2004. A regulatory trade-off as a source of strain variation in the species *Escherichia coli*. *Journal of Bacteriology*, **186**, 5614–5620.
- Lee, Y.H., Kim, B.H., Kim, J.H., Yoon, W.S., Bang, S.H., and Park, Y.K. 2007. CadC has a global translational effect during acid adaptation in *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, **189**, 2417–2425.
- Madeo, M., O’Riordan, N., Fuchs, T.M., Utratna, M., Karatzas, K.A., and O’Byrne, C.P. 2012. Thiamine plays a critical role in the acid tolerance of *Listeria monocytogenes*. *FEMS Microbiology Letters*, **326**, 137–143.
- Metselaar, K.I., den Besten, H.M.W., Abee, T., Moezelaar, R., and Zwietering, M.H. 2013. Isolation and quantification of highly acid resistant variants of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **166**, 508–514.
- Miller, E.F. and Maier, R.J. 2014. Ammonium metabolism enzymes aid *Helicobacter pylori* acid resistance. *Journal of Bacteriology*, **196**, 3074–3081.
- Mols, M. and Abee, T. 2008. Role of ureolytic activity in *Bacillus cereus* nitrogen metabolism and acid survival. *Applied and Environmental Microbiology*, **74**, 2370–2378.
- Moons, P., Van Houdt, R., Vivijis, B., Michiels, C.W., and Aertsen, A. 2011. Integrated regulation of acetoin fermentation by quorum sensing and pH in *Serratia plymuthica* RVH1. *Applied and Environmental Microbiology*, **77**, 3422–3427.
- Park, Y.K., Bearson, B., Bang, S.H., Bang, I.S., and Foster, J.W. 1996. Internal pH crisis, lysine decarboxylase and the acid tolerance response of *Salmonella typhimurium*. *Molecular Microbiology*, **20**, 605–611.
- Phan, K. and Ferenci, T. 2013. A design-constraint trade-off underpins the diversity in ecologically important traits in species *Escherichia coli*. *The ISME Journal*, **7**, 2034–2043.
- Ramamurthy, T., Ghosh, A., Pazhani, G.P., and Shinoda, S. 2014. Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria. *Frontiers in Public Health*, **2**, 103.
- Rando, O.J. and Verstrepen, K.J. 2007. Timescales of genetic and epigenetic inheritance. *Cell*, **128**, 655–668.
- Robbe-Saule, V., Algorta, G., Rouilhac, I., and Norel, F. 2003. Characterization of the RpoS status of clinical isolates of *Salmonella enterica*. *Applied and Environmental Microbiology*, **69**, 4352–4358.
- Russell, N.J., Evans, R.I., ter Steg, P.F., Hellemons, J., Verheul, A., and Abee, T. 1995. Membranes as a target for stress adaptation. *International Journal of Food Microbiology*, **28**, 255–261.
- Sagarzazu, N., Cebrián, G., Pagán, R., Condón, S., and Mañas, P. 2013. Emergence of pulsed electric fields resistance in *Salmonella enterica* serovar Typhimurium SL1344. *International Journal of Food Microbiology*, **166**, 219–225.
- Sampathkumar, B., Khachatourians, G.G., and Korber, D.R. 2004. Treatment of *Salmonella enterica* serovar Enteritidis with a sublethal concentration of trisodium phosphate or alkaline pH induces thermotolerance. *Applied and Environmental Microbiology*, **70**, 4613–4620.
- Sleator, R.D. and Hill, C. 2002. Bacterial osmo-adaptation: the role of osmolytes in bacterial stress and virulence. *FEMS Microbiology Reviews*, **26**, 49–71.
- Sleator, R.D., Gahan, C.G.M., and Hill, C. 2003. A postgenomic appraisal of osmotolerance in *Listeria monocytogenes*. *Applied and Environmental Microbiology*, **69**, 1–9.
- Sleator, R.D., Banville, N., and Hill, C. 2009. Carnitine enhances the growth of *Listeria monocytogenes* in infant formula at 7°C. *Journal of Food Protection*, **72**, 1293–1295.
- Smiddy, M., Sleator, R.D., Patterson, M.F., Hill, C., and Kelly, A.L. 2004. Role for compatible solutes glycine betaine and L-carnitine in listerial barotolerance. *Applied and Environmental Microbiology*, **70**, 7555–7557.

- Spira, B., Hu, X., and Ferenci, T. 2008. Strain variation in ppGpp concentration and RpoS levels in laboratory strains of *Escherichia coli* K-12. *Microbiology*, **154**, 2887–2895.
- Sutton, A., Buencamino, R., and Eisenstark, A. 2000. *rpoS* mutants in archival cultures of *Salmonella enterica* serovar Typhimurium. *Journal of Bacteriology*, **182**, 4375–4379.
- Suutari, M. and Laakso, S. 1994. Microbial fatty acids and thermal adaptation. *Critical Reviews in Microbiology*, **20**, 285–328.
- Teixeira, H., Gonçalves, M.G., Rozès, N., Ramos, A., and San Romao, M.V. 2002. Lactobacillic acid accumulation in the plasma membrane of *Oenococcus oeni*: a response to ethanol stress? *Microbial Ecology*, **43**, 146–153.
- Van Boeijen, I.K., Moezelaar, R., Abee, T., and Zwietering, M.H. 2008. Inactivation kinetics of three *Listeria monocytogenes* strains under high hydrostatic pressure. *Journal of Food Protection*, **71**, 2007–2013.
- Van Boeijen, I.K.H., Chavarroche, A.A.E., Valderrama, W.B., Moezelaar, R., Zwietering, M.H., and Abee, T. 2010. Population diversity of *Listeria monocytogenes* LO28: phenotypic and genotypic characterization of variants resistant to high hydrostatic pressure. *Applied and Environmental Microbiology*, **76**, 2225–2233.
- Van Boeijen, I.K.H., Francke, C., Moezelaar, R., Abee, T., and Zwietering, M.H. 2011. Isolation of highly heat-resistant *Listeria monocytogenes* variants by use of a kinetic modeling-based sampling scheme. *Applied and Environmental Microbiology*, **77**, 2617–2624.
- Van Hoek, A.H., Aarts, H.J., Bouw, E., van Overbeek, W.M., and Franz, E. 2013. The role of *rpoS* in *Escherichia coli* O157 manure-amended soil survival and distribution of allelic variations among bovine, food and clinical isolates. *FEMS Microbiology Letters*, **338**, 18–23.
- Van Houdt, R., Aertsen, A., and Michiels, C.W. 2007. Quorum-sensing-dependent switch to butanediol fermentation prevents lethal medium acidification in *Aeromonas hydrophila* AH-1N. *Research in Microbiology*, **158**, 379–385.
- Vanlint, D., Mitchell, R., Bailey, E., Meersman, F., McMillan, P.F., Michiels, C.W., and Aertsen, A. 2011. Rapid acquisition of gigapascal-high pressure resistance by *Escherichia coli*. *Mbio*, **2**, e00130-10.
- Vanlint, D., Rutten, N., Michiels, C.W., and Aertsen, A. 2012. Emergence and stability of high-pressure resistance in different food-borne pathogens. *Applied and Environmental Microbiology*, **78**, 3234–3241.
- Vanlint, D., Rutten, N., Govers, S.K., Michiels, C.W., and Aertsen, A. 2013. Exposure to high hydrostatic pressure rapidly selects for increased RpoS activity and general stress-resistance in *Escherichia coli* O157:H7. *International Journal of Food Microbiology*, **163**, 28–33.
- Van Schaik, W. and Abee, T. 2005. The role of sigma B in the stress response of Gram-positive bacteria – targets for food preservation and safety. *Current Opinion in Biotechnology*, **16**, 218–224.
- Vivijns, B., Moons, P., Geeraerdb, A.H., Aertsen, A., and Michiels, C.W. 2014. 1,2-Butanediol fermentation promotes growth of *Serratia plymuthica* at low pH but not survival of extreme acid challenge. *International Journal of Food Microbiology*, **175**, 36–44.
- Wang, Y., Corrieu, G., and Béal, C. 2005. Fermentation pH and temperature influence the cryotolerance of *Lactobacillus acidophilus* RD758. *Journal of Dairy Science*, **88**, 21–29.
- Wang, L., Spira, B., Zhou, Z., Feng, L., Maharjan, R.P., Li, X., Li, F., McKenzie, C., Reeves, P.R., and Ferenci, T. 2010. Divergence involving global regulatory gene mutations in an *Escherichia coli* population evolving under phosphate limitation. *Genome Biology and Evolution*, **2**, 478–487.
- Waterman, S.R. and Small, P.L.C. 1996. Characterization of the acid resistance phenotype and *rpoS* alleles of Shiga-like toxin-producing *Escherichia coli*. *Infection and Immunity*, **64**, 2808–2811.
- Waterman, S.R. and Small, P.L.C. 1998. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. *Applied and Environmental Microbiology*, **64**, 3882–3886.
- Wemekamp-Kamphuis, H.H., Sleator, R.D., Wouters, J.A., Hill, C., and Abee, T. 2004. Molecular and

- physiological analysis of the role of osmolyte transporters BetL, Gbu, and OpuC in growth of *Listeria monocytogenes* at low temperatures. *Applied and Environmental Microbiology*, **70**, 2912–2918.
- Yoon, S.S. and Mekalanos, J.J. 2006. 2,3-Butanediol synthesis and the emergence of the *Vibrio cholerae* El Tor biotype. *Infection and Immunity*, **74**, 6547–6556.
- Zambrano, M.M., Siegele, D.A., Almiron, M., Tormo, A., and Kolter, R. 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. *Science*, **259**, 1757–1760.
- Zeng, X., Birrien, J.L., Fouquet, Y., Cherkashov, G., Jebbar, M., Querellou, J., Oger, P., Cambon-Bonavita, M.A., Xiao, X., and Prieur, D. 2009. *Pyrococcus* CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life. *The ISME Journal*, **3**, 873–876.

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