MICROBIOLOGY HANDBOOK

MEAT PRODUCTS

Edited by

Rhea Fernandes

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FOREWORD

The Microbiology Handbook series includes Dairy Products, Fish and Seafood, and Meat Products, published by Leatherhead Food Research and RSC Publishing. These books are designed to be an easy-to-use guide to the microorganisms found in foods. Each book provides a brief overview of the processing factors that determine the nature and extent of microbial growth and survival in product, potential hazards associated with the consumption of a range of products, and the growth characteristics of key pathogens associated with the product. All the handbooks also contain a review of the related legislation in Europe and UK, a guide to HACCP, and a detailed list of contacts for various food authorities. The books are a source of valuable information for microbiologists and food scientists working in the food industry and responsible for food safety, both in the UK and elsewhere.

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Rhea Fernandes
Leatherhead Food International

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INTRODUCTION

The high water and protein content of meat, and other water-soluble constituents, make meat and their products a suitable medium for growth of microorganisms. The animal itself, the environment, and processing conditions all have a bearing on the diversity of microflora of these products; thus, meat and meats products are susceptible to spoilage, and are often involved in the transmission of pathogenic microorganisms.

Being such a highly perishable commodity, preservation plays a key role in extension of shelf life. The various preservation methods used, such as chilling, freezing, cooking, curing, drying, and packaging, have lead to an increased choice of meat products available on the market.

For the purpose of this book, *Microbiology Handbook- Meat Products*, the meat products (including poultry and eggs) have been divided into the following chapters: chilled and frozen raw meat, poultry, and their products; cooked meats, poultry, and their products; dried meats, poultry, and related products; cured meats and poultry, including cooked cured meats; fermented meats; and eggs. The book also contains sections on HACCP; Commission Regulations on general hygiene rules, hygiene rules for food of animal origin and microbiological criteria for foodstuffs; and contact information for various food authorities.

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1. CHILLED AND FROZEN RAW MEAT, POULTRY AND THEIR PRODUCTS

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1.1 Definitions

Carcass refers to the body of any slaughtered animal or bird, often, but not always, after bleeding and dressing.

Chilled meat is meat that has been cooled to, and maintained at or below 7 °C but not below -2 °C during storage, transportation and sale.

Dressing is the progressive separation of a slaughtered animal or bird into a carcass (or sides of a carcass), offals and inedible by-products. Dressed carcasses (or sides) can be skin-on (e.g. chicken, pork) or skin-off (e.g. lamb, beef).

Evisceration is the removal of the viscera from a carcass.

Fresh meat is meat that has not been treated in any way other than refrigeration, with or without preservative packaging, to maintain its fitness for human consumption.

Frozen meat is meat that has been cooled to, and maintained at or below -2 °C (normally below -12 °C) during storage, transportation and sale.

Initial microflora is the association of microorganisms present on an eviscerated carcass after skin removal (if appropriate) but prior to washing, grading, chilling, and further processing.

Meat is the edible part (musculature and edible offal) of an animal or bird slaughtered for human consumption.

Microbial contamination refers to microorganisms directly or indirectly transferred onto a carcass or edible offal; hence, *contaminating microflora* means those microorganisms present as a consequence of such transmission.

Modified-atmosphere packaging (MAP) refers to packaging systems in which the natural gaseous environment around the product is intentionally altered, and then gradually changes as a consequence of the interaction between product, packaging and the external environment.

Controlled-atmosphere packaging (CAP) refers to packaging systems in which the altered internal gaseous environment is maintained at a specified composition regardless of product respiration, temperature or other environmental changes.

Offal is all the edible and inedible parts of a slaughtered animal or bird other than the carcass.

Poultry refers to domesticated birds slaughtered for human consumption; hence, *poultry meat* means all edible parts of any domesticated bird slaughtered for human consumption.

Preservative packaging is a general term describing any packaging system that prevents or restricts the growth of spoilage microorganisms.

Raw meat refers to meat that has not been cooked but excludes meat treated with curing salts and/or subjected to fermentation; hence, *raw meat products* refers to uncured non-fermented whole tissue or comminuted meat products intended for sale in the raw state.

Spoilage describes changes that render meat objectionable to consumers; hence, *spoilage microflora* describes an association of microorganisms that through its development on meat renders that meat objectionable to consumers.

Spoilage potential is a measure of the propensity of microorganisms to render meat objectionable to consumers through the production of offensive metabolic by-products.

Slaughter is the killing of an animal or bird for human consumption generally, but not necessarily performed within a premise (abattoir) that is approved and registered for that purpose.

Viscera mean the organs of the thoracic and abdominal cavities, and include the kidneys.

1.2 Initial Microflora

The initial microflora associated with skin-off meat processing is composed of microorganisms transferred onto the naked carcass during slaughter and dressing. With skin-on meat processing, the initial microflora will also contain microorganisms resident on the skin of the live animal that are not removed during dressing.

Since slaughter and dressing procedures differ not only between meat animal species but also for a given species, the term initial microflora must be precisely defined. The association of microorganisms present on dressed eviscerated carcasses before washing was selected for this chapter as being the initial microflora, to allow the effects of processing variables such as washing and chilling to be considered more fully in the next section, 1.3 Processing and its Effects on the Microflora.

The major sources of the initial microflora found on carcasses are the slaughter animals themselves, the process workers, and the processing environment. The animal sources of contaminating microorganisms include external body surfaces (skin, hide, fleece, feathers, feet and hooves), and the gastrointestinal and respiratory tracts. Contact, either direct or indirect (e.g. via a process worker's hands or implements), between the carcass and external body surface of a slaughtered animal will result in a heterogeneous mixture of microorganisms derived from the animal's pre-slaughter environment, including those of faecal, soil, water and feed origin, being transferred to the carcass. Consequently, the microbiological quality of a dressed carcass is determined by a complex interaction between the microflora carried by the live animal and the hygienic efficiency of the slaughter and dressing process. Despite species and processing differences, the mainly Gram-positive-dominated initial microflora found on dressed meat and poultry carcasses are remarkably similar (1, 2). Unless subsequent processing includes antimicrobial treatments, microbial numbers increase progressively with further processing, at first because of additional contamination associated with handling, and later because of microbial growth.

Pathogenic microorganisms, particularly those of animal origin, can also be expected to be found in the saprophyte-dominated initial microflora; except in unusual and highly undesirable circumstances, their numbers are generally low. With the exception of *Staphylococcus aureus* and *Yersinia enterocolitica*, most of the major food poisoning organisms (*Salmonella* spp., *Clostridium perfringens*, pathogenic *Escherichia coli,* and *Campylobacter jejuni*) are associated with direct or indirect faecal contamination of carcasses. Other pathogens such as *Listeria monocytogenes* may be animal-associated, but can also be found in the processing environment.

The significance of a particular microorganism being present in the initial microflora is questionable because of the adventitious nature of their occurrence, unless that microorganism poses a health hazard, is likely to contribute to product spoilage, or has specific meat plant or process associations. A compilation of the bacterial genera most frequently reported on raw meat and poultry is presented in Table 1.I.

The majority of the bacteria present in the initial microflora are mesophilic and therefore will not generally contribute to spoilage as they are unable to grow at refrigeration temperatures. Consequently, most microorganisms derived from the intestinal tract will not contribute to spoilage although they may represent a safety hazard. A small but variable proportion of the initial microflora will, however, be psychrotrophic and capable of growth that will eventually result in spoilage of the refrigerated product. Those genera that are most frequently represented in aerobic and anaerobic spoilage microflora are identified in Table 1.I. In respect to spoilage, yeasts and moulds (Table 1.II) do not play an important role except under conditions that preclude the growth of spoilage bacteria. In the initial microflora on beef, yeasts and moulds accounted for only 2.6% of those able to grow at 20 °C but the yeasts accounted for 35% of those able to grow at -1 °C (3). In Saudi Arabia, yeasts and moulds accounted for 2% of the microorganisms on broiler chicken carcasses after evisceration and air chilling that were able to grow at between 25 and 35 °C (4).

1.2.1 Skin-off processing

Included in this grouping are cattle, sheep and all other major food animals including emu and ostrich that are usually skinned (in the case of the two avian species after manual plucking), as part of the normal carcass dressing procedure. For some markets, notably Japan, pig carcasses are skinned rather than processed skin-on. With skin-off processing of healthy slaughter stock, the tissues that are destined to become meat are generally sterile in the intact animal, and their contamination with microorganisms after slaughter is an undesirable but unavoidable consequence of the process by which live animals are converted into meat for human consumption.

Typically, Gram-positive cocci dominate the initial microflora of skinned carcasses. These microflora also frequently contain *Acinetobacter, Aeromonas*, coryneforms, Enterobacteriaceae, *Flavobacterium, Moraxella*, and *Pseudomonas* in addition to *Micrococcus* and *Staphylococcus* spp. (1). Irregular occurrences, often in low numbers, of lactic acid bacteria (LAB), *Brochothrix thermosphacta*, *Bacillus* spp., yeasts and moulds are not infrequently reported. The proportion of psychrotrophs present in such an initial microflora shows both latitudinal and seasonal variation, ranging in temperate latitudes from approximately 1% in summer to 10% in winter (5). Psychrotrophic strains are often virtually absent from the initial microflora of stock slaughtered in tropical regions. The presence

of psychrotrophs is of concern because not only can they directly potentiate product spoilage but also they may pose a secondary contamination hazard should they become established in a chilled processing environment through inadequate or ineffective sanitation.

1.2.2 Skin-on processing

This group includes most pig and poultry processing. With skin-on processing the initial microflora is made up of skin microorganisms surviving the dressing process and those introduced during slaughter and dressing. With both pig and poultry processing, carcasses are subjected to a scalding treatment to loosen hair or feathers prior to their mechanical removal.

Scalding and dehairing of pig carcasses removes approximately 95% of the microorganisms initially present on the skin. The remaining population is comparable to that typically found on skinned carcasses. Subsequent singeing operations reduce that residual skin population to levels as low as 100 bacteria/cm^2. From this minimum population the microflora increases through contamination during further processing to reach levels between 10^3 and 10^4 bacteria/cm^2 at chiller entry (6). This initial microflora contains a small proportion of thermoduric cocci and spore formers that survive scalding and singeing. The major part of the microflora is, however, made up of a heterogeneous mixture of microorganisms introduced from the processing environment after singeing and also from the animal during evisceration.

Scalding of poultry results in the removal of many microorganisms from the feathers. The subsequent defeathering process, however, causes considerable spread of mainly skin microorganisms between carcasses and into the processing environment. Skin-associated microorganisms include *Acinetobacter, Corynebacterium, Flavobacterium, Micrococcus, Moraxella, Pseudomonas, Staphylococcus* and yeasts. Despite the violent action of the plucking machines, extrusion of faeces and subsequent dispersion of enteric bacteria are not reported to be a major problem (7). However, *Salmonella* spp. and *C. jejuni* present on the feathers and feet of birds are spread during plucking. Further contamination with these and other enteric microorganisms during high speed automatic evisceration also contributes to the not infrequent presence of these microorganisms on dressed poultry carcasses. Contamination of carcasses with psychrotrophic microorganisms, particularly *Pseudomonas* spp. that grow on the wet product contact surfaces within the processing plant, remains an important process hygiene problem that impinges significantly on the composition of the initial microflora (4). With the addition of enteric bacteria and Gram-negative psychrotrophs to the skin-derived microorganisms, the qualitative if not quantitative similarity of the initial microfloras on plucked poultry and skinned carcasses is unmistakable. Unfortunately for the poultry processor, that population tends to be significantly larger than that typically found on beef or lamb carcasses

and usually shows a predominance of psychrotrophs. The consequences of this quantitative difference are profound with respect to spoilage and not insignificant with respect to product safety.

Table 1.I
Genera of bacteria found on raw meat and poultry and their association with spoilage development (major spoilage organisms in bold)

Genus	Association with		Spoilage development		
	Meat	Poultry	Frequently implicated	Oxygen requirement	Spoilage potential
Gram-positive					
Bacillus	+	+	No	Aerobe	
Brochothrix	+		Yes	Facultative	High
Clostridium	+	+	Yes	Anaerobe	High
Corynebacteria	+	+	No		
(1) Corynebacterium	+	+	No	Aerobe*	
(2) Kurthia	+		No		
(3) Microbacterium	+	+	No		
Lactic acid bacteria (LAB)	+		Yes		
(1) Carnobacterium	+		Yes		
(2) Enterococcus	+	+	Yes		
(3) Lactobacillus	+		Yes		
(4) Lactococcus	+		Yes		
(5) Leuconostoc	+		Yes	Aerotolerant	Low
(6) Pediococcus	+		Yes		
(7) Vagococcus		+	Yes		
Listeria	+	+	No	Facultative	
Micrococcus	+	+	No	Aerobe	
Staphylococcus	+	+	No	Facultative	
Gram-negative					
Acinetobacter group	+	+	Yes		
(1) Acinetobacter	+	+	Yes	Aerobe	Low
(2) *Moraxella*	+	+	Yes		
(3) Psychrobacter	+	+	Yes		
Aeromonas	+	+	Yes	Facultative	Low
Alcaligenes	+	+	Yes	Aerobe	Low
Campylobacter	+	+	No	Microaerophile	
Enterobacteriaceae	+	+	Yes		
(1) *Citrobacter*	+	+	No		
(2) Enterobacter	+	+	Yes	Facultative	High
(3) *Escherichia*	+	+	No		
(4) Hafnia	+		Yes		
(5) Pantoea	+	+	Yes		
(6) Serratia	+	+	Yes		
Flavobacterium	+	+	No	Aerobe	
Proteus	+	+	No	Facultative	
Pseudomonas	+	+	Yes	Aerobe	High
Salmonella	+	+	No	Facultative	
Shewanella	+		Yes	Facultative	High
Yersinia	+		No	Facultative	

+ known to occur; + frequently present; * some *Corynebacterium* spp. can be facultative

Table 1.II
Genera of mould and yeast found on raw meat and poultry and their association with spoilage development (major spoilage organisms in bold)

Genus	Association with		Spoilage development		
	Meat	Poultry	Frequently implicated	Oxygen requirement	Spoilage potential
Mould					
Hyaline					
Aspergillus	+	+	No	Aerobe	
Chrysosporium	+		Yes	Aerobe	Low†
Fusarium	+		No	Aerobe	
Geotrichum	+	+	No	Facultative*	
Monascus	+		No	Aerobe	
Monilia	+		No	Aerobe	
Neurospora	+		No	Aerobe	
Penicillium	+	+	Yes	Aerobe	Low†
Sporotrichum[(a)]	+		Yes	Aerobe	Low†
Dematiaceous					
Acremonium[(b)]	+		No	Aerobe	
Aureobasidium	+		No	Aerobe	
Alternaria	+	+	No	Aerobe	
Cladosporium	+	+	Yes	Aerobe	Low†
Mucoraceous					
Mucor	+	+	Yes	Aerobe	Low†
Rhizopus	+	+	Yes	Aerobe	Low†
Thamnidium	+		Yes	Aerobe	Low†
Yeast					
Candida	+	+	Yes	Facultative*	Low
Cryptococcus	+	+	Yes	Aerobe	Low
Debaromyces	+		No	Facultative*	
Rhodotorula	+	+	Yes	Aerobe	Low
Saccharomyces		+	No	Facultative	
Trichosporon	+	+	No	Aerobe	

+ known to occur; + frequently present; * oxygen requirement varies between species; † affects product appearance; [(a)] *Sporotrichum carnis* is known as *Chrysosporium pannorum*, other *Sporotrichum* spp. are currently include in the genus *Sporothrix*; [(b)] may produce white mycelium, spores generally dark

1.3 Processing and its Effects on the Microflora

Process flow diagrams for generic meat animal and poultry processing are presented in Figures 1.1 and 1.2 respectively. It must be appreciated that individual slaughter and dressing processes will differ in the sequence of some operations and that further processing of raw meats into raw meat products has been represented as a simplified series of generalised operations. Notwithstanding obvious differences in abattoir technology, slaughter, dressing and further processing are, irrespective of meat species, sufficiently similar functionally to reasonably allow generic consideration of their microbiological implications.

In the conversion of live slaughter stock into either raw meat or raw meat products, there are no operations that kill or otherwise eliminate microorganisms (i.e. no Hazard Analysis and Critical Control Point (HACCP), critical control points (CCP's), see chapter 8 on HACCP), However, there are many steps in which the introduction, spread or proliferation of microbial contaminants can be minimised (i.e. see CCP's in the chapter on HACCP). Current emphasis on pathogen reduction may see antimicrobial interventions such as decontamination sprays or irradiation treatments mandated into meat processing systems. Despite the introduction of such innovations, microbial status of the animal at the time of slaughter, care and standards of hygiene, and sanitation used during slaughtering and carcass dressing will remain as it is, on the removal of the skin (skinned carcasses) and alimentary tract with minimal transfer of microbial contamination to the carcass surface, and, in the case of skin-on carcasses, on a minimal persistence of the normal skin microflora.

1.3.1 Stock presentation

Approximately 30% of the microflora present on a given area of cattle hide is transferred to a corresponding area of carcass surface following direct hide/carcass contact (3). Consequently, the number of microflora present on hide, hooves, feet, hair and feathers is a major factor determining the microbiological status of slaughter stock. However, the microflora of the intestinal tract cannot be ignored. The latter can contribute significantly to the former when animals and birds become soiled with their own faeces or that of others as a result of poor animal husbandry, transportation, or meat plant holding practices. Therefore, the microbiological status of stock can be expected to show a positive correlation with their visual cleanliness.

Cage rearing of chickens, for example, a husbandry practice that separates the birds from their droppings, results in lower microbial loads and the complete absence of faecal coliforms on live birds compared with floor-reared birds (8). A general trend that woolly and dirty lambs produce carcasses with increased levels of contamination after pelt removal has been observed (9). Similarly, one of the explanations proposed by Empey and Scott in 1939 (3) to explain the more than 100-fold difference between maximum (3.1×10^7 bacteria/cm^2) and minimum (1.0×10^5 bacteria/cm^2) counts on beef hides was the extent to which the hides were contaminated by soil. In the light of these observations, common logic would suggest that even with animals that are dressed skin-off, pre-slaughter cleaning of stock should significantly reduce carcass contamination, since for those animals the major source of carcass contamination is direct or indirect hide/carcass contact.

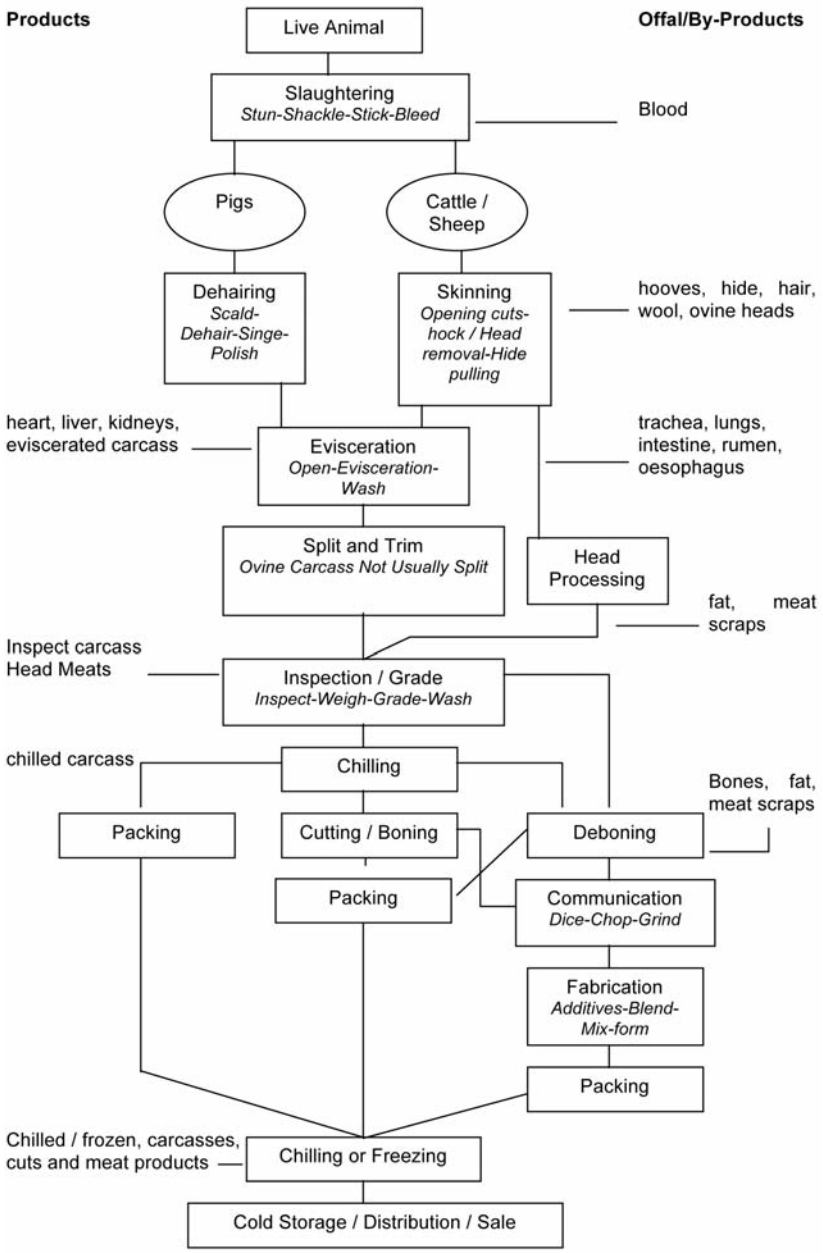

Fig. 1.1. Generic process flow diagram for chilled and frozen raw whole meat carcasses, cuts and meat products

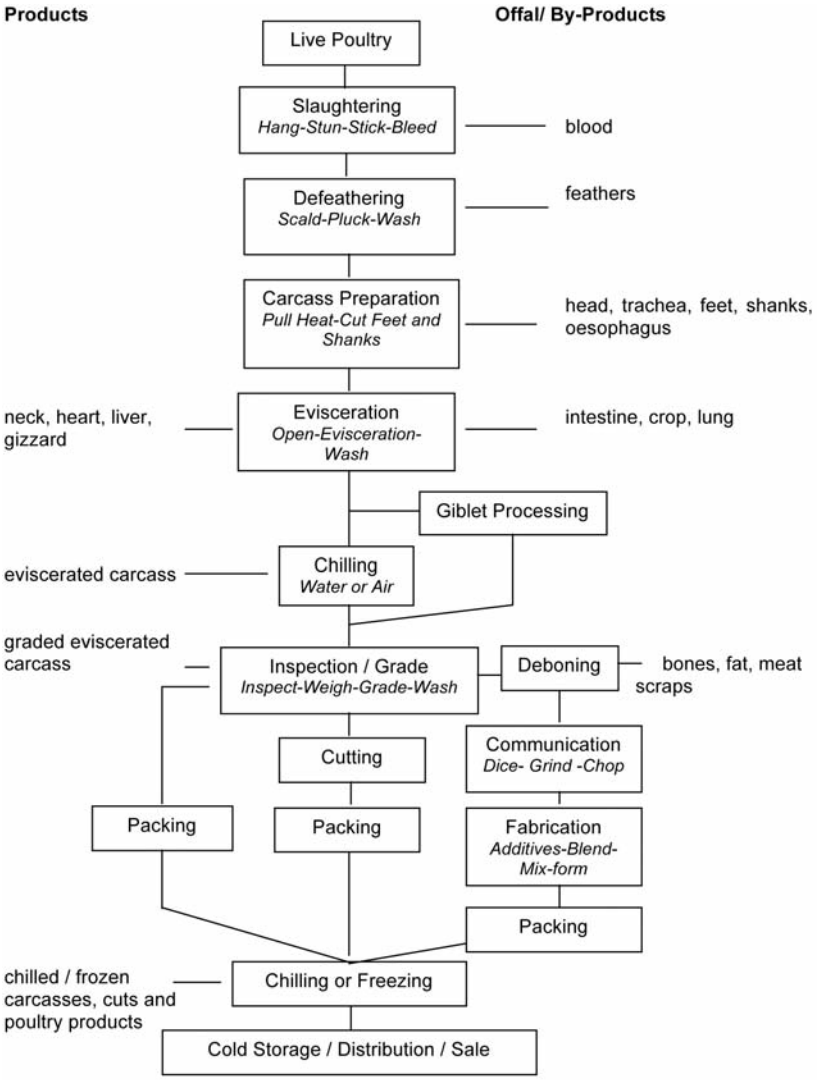

Fig. 1.2. Generic process flow diagram for chilled and frozen raw whole poultry carcasses, cuts and poultry products

Transportation of slaughter stock over long distances not only provides the opportunity for faecal contamination but also, through stress and starvation, potentiates the excretion of pathogenic organisms such as *Salmonella* spp. by symptomless carriers (10). The resulting cross contamination via the faecal oral route can quickly spread the pathogen through entire truck loads of some classes of susceptible stock such as young calves, pigs, old ewes, and dairy cows. Some

approaches being used to control this problem are the pre-transportation use of competitive exclusion strategy, probiotics, or prebiotics (11, 12) The overall goal of any of the strategies used is to promote the growth of groups of beneficial bacteria that are competitive with, or antagonistic to, pathogenic species. In the competitive exclusion strategy, a non-pathogenic bacterial culture (composed of one or more strains or species of bacteria, but derived from the animal of interest) is added to the intestinal tract; probiotic preparations consist of individual species or mixtures of LAB or yeast without being species specific or even being of animal origin; prebiotics are organic compounds that are unavailable to, or indigestible by the host animal, but are available to a specific proportion of the microbial population (11, 12). The so-called "Nurmi effect", which has proven efficacious in reducing salmonellosis in young poultry and pigs, employs the oral administration of viable beneficial gut microorganisms including *Lactobacillus, Bifidobacterium* and *Propionibacterium* strains. These organisms, through mechanisms including competitive exclusion, maintain the balance of the gut microflora during times of stress, thereby preventing gastric disturbances and colonisation by pathogens.

1.3.2 Cleaning the live animals

Pre-slaughter cleaning of stock to remove soil and faecal material can be achieved by either shearing or washing.

1.3.2.1 Shearing

With sheep, especially when the fleece is long, large accumulations of faecal material can form around the anus and down the hind legs. To reduce the chance of transferring this faecal material to the carcass, such animals are generally “crutched", i.e. sheared in the crutch region, prior to slaughter. However, quite contrary to conventional hygienic expectation, such pre-slaughter "crutching" increases microbial contamination of the carcass in the inside leg region of both wet and dry stock (13). Similarly, increased microbial contamination has been observed when woolly lambs were crutched after slaughter before the start of hind leg dressing (14).

1.3.2.2 Washing

It has been shown that spray washing of cattle with approximately 100 litres of cold water per animal can remove up to 50% of the microflora present on hides (3). However, in terms of bacterial numbers, such a change represents only a small improvement (13). More recently, experiments have shown that power washing for 3 minutes significantly reduces *E. coli* O157:H7 counts on contaminated hides (15). Washing is done immediately before slaughter because the addition of moisture has been shown to favour the proliferation of the microflora present on

the hide (3). In addition, the exclusion of visibly soiled animals from slaughter facilities has been thought to be an important preventative measure. The implementation of a Hygienic Assessment Scheme (HAS) in the UK has resulted in bacterial counts of <2 $\log_{10}$ cfu/cm^2 (15). With sheep, although either spray washing of the crotch area or whole animal spray washing followed by a swim wash, produced visibly cleaner animals, microbial contamination of the carcasses was in both cases increased rather than decreased (9, 13). When considering the enigma surrounding these paradoxical results, it may be pertinent to note that swim-washed sheep are often left overnight to reach a "drip-dry" state prior to slaughter the next morning. Consequently, possible explanations of enhanced carcass contamination after sheep washing include microbial growth during holding, aerosol production during pelt removal, facilitated transfer of microorganisms from wet wool, and direct contamination by dirty water draining from saturated fleeces. Not only is the hygienic justification of sheep washing flawed, but the practice must be denigrated for its adverse effect on meat quality: stress-related high ultimate pH meat with inherently poor storage properties.

1.3.3 Slaughter

Legislation in the major developed countries requires that slaughter methods be humane and that blood be rapidly and effectively removed from the carcass. Slaughter stock either must be killed instantly by means of a mechanically operated instrument before bleeding or must be stunned by an approved method to render them insensible to pain until death results from exsanguination. Exception from the requirement for stunning prior to bleeding is made in the case of Jewish (Shechita) and Muslim (Halal) slaughter. Both involve the use of a sharp knife to sever the oesophagus, trachea, carotid arteries and jugular veins. Regurgitation of stomach contents through the severed oesophagus can contaminate the head and neck stump, requiring trimming of the tissue so affected. This problem has been exacerbated in modern meat plants where carcasses are bled hanging from a bleed rail rather than in the recumbent position of traditional slaughter procedures. In the case of Muslim slaughter, where the animal must be alive but not necessarily conscious when the Halal cut is made, regurgitation can be prevented by the application of a stunning electric current that causes a simultaneous closure of the oesophagus that lasts for up to 20 seconds (16). In New Zealand, the contamination of neck meat with ingesta and the consequent need for trimming have been dramatically reduced by the use of electrical stunning and clipping of the oesophagus within seconds of making the Halal cut.

1.3.3.1 Slaughter instruments

Unless properly cleaned, saws, steel-mesh gloves, knives, scabbards and other equipment can be heavy contaminated with microorganisms; there is experimental

evidence that bacteria can be introduced into deep tissues through the use of contaminated slaughter instruments (15).

The practice of pithing to prevent reflex muscular activity at sticking, in which the brain stem and upper part of the spinal column are destroyed by insertion of a long thin rod (pithing cane) through the hole made by a penetrating bolt, is considered particularly unhygienic. The destruction of the brain stem induces reflex vomiting, thereby increasing the risk of ingesta contamination of the carcass. Carcass immobilisation can, however, be more effectively and hygienically induced electrically. In most meat plants electrical immobilisation has replaced pithing.

The practice of pithing has been banned in the EU (Regulation (EC) No. 999/2001, and in England, Transmissible Spongiform (Encephalopathies) (No. 2) Regulations 2006 (as amended)), for animals slaughtered for human or animal consumption, to prevent the possible spread of bovine spongiform encephalopathy (BSE) following the discovery of traces of brain material in the circulation of pithed cattle.

Any microorganisms introduced into the deep tissues during slaughter are largely eliminated by the bactericidal activities of the blood and tissues (17). Consequently, the slaughter process, when compliant with modern slaughter hygiene requirements, should not contribute significantly to carcass contamination.

1.3.3.2 Bleeding

Although the bleeding process should be completed as quickly as possible, it is generally thought to have little effect on the microbial growth on meat (15, 18) on the following grounds: blood of healthy slaughter stock is sterile; agonal invasion, in which body tissues are invaded by bacteria originating from the gastrointestinal tract and rapidly entering the blood stream at the time of death, is not supported by scientific evidence; blood content of muscle tissue is largely independent of blood drainage from the carcass; and a buffering effect of excess blood that maintains high tissue pH and potentiates early spoilage is improbable because of the relatively low proportion of blood to muscle tissue. Furthermore, the early microbial spoilage of high-pH (also called dark, firm, dry (DFD)) meat results from its low glucose content and not from its high pH (19).

Imperfect bleeding at slaughter can, however, indicate a diseased condition in the slaughter animal. Congestion of tissues due to an active hyperaemia associated with pyrexia must therefore be differentiated from that resulting from imperfect bleeding of mechanical aetiology when carcasses are assessed in respect to their fitness for human consumption. Flesh from a poorly bled carcass is more nutritious than that from a well bled one, but is darker and is more prone to exude

blood from cut surfaces. Consequently such meat, particularly in its raw state, is likely to be aesthetically unacceptable to the majority of consumers.

1.3.4 Carcass dressing

Provided reasonable hygienic precautions are taken, the edible tissue, with the exception of pig and poultry skin, of healthy animals will be virtually sterile immediately after slaughter. Microbial contamination of these tissues, however, is inevitable and occurs during carcass dressing and butchering.

1.3.4.1 Skinning

Direct contact between the carcass and the outside surface of a slaughtered animal's skin results in the transfer of a significant proportion of the microflora present on hair, fleece, fur or feathers onto the carcass (see section on 1.2 Initial Microflora). Indirect transfer may also occur, particularly in procedures where operatives must handle both hide and carcass, such as in the manual skinning of bovine hind hocks or manual flank clearing in ovine dressing. With modern multi-operative line systems of slaughter and dressing, the opportunity for cross contamination between carcasses is significant. Consequently, the introduction of mechanisation that reduces the alternate handling of skin and carcass by many pairs of hands should significantly reduce carcass contamination. The use of mechanical hide pullers appears to be particularly effective in this regard, as is the introduction of the inverted dressing system for sheep.

The distribution of microbial contamination on skinned carcasses and direct observation of the skinning process both point to hide "rollback" in the vicinity of opening cuts as being the major cause of hide/carcass contact and hence carcass contamination. Therefore, if a reduction in microbial contamination, rather than its subsequent removal, is required to meet hygiene criteria, attention must be directed towards improving slaughter animal presentation and developing pelt removal practices that reduce opportunities for hide/carcass contact, such as the use of legging papers in ovine dressing.

1.3.4.2 Scalding

The scalding of pig carcasses with water at 58 - 62 °C for about 5 - 6 minutes to loosen the hair significantly reduces the number of contaminants carried on the skin. The high probability that contaminated scald water will be sucked into the lungs as the thoracic muscles relax after their initial reflex contraction induced by immersion in the scald tank accounts for the exclusion of pig lungs from the edible porcine offal in many countries. Steam or spray scalding offers distinct hygienic advantages over tank scalding, although there is little evidence of significant

muscle or organ contamination with the thermoduric microorganisms that progressively accumulate in scald water with successive carcass immersions.

With poultry, inspiration of scald water may contaminate both the lungs and air sacks. Unlike the lungs, the ten paired and one single (clavicular) air sacks are not removed during evisceration. Instead, they remain attached to the inside of the thoracic cavity in close association with the pneumatic bones and if contaminated are not an infrequent focus of early microbial spoilage. Consequently, spray scalding offers a hygienic advantage over tank scalding, where microbial contamination levels can be high because of the use of water at 52 °C for about 3 minutes (soft scald) rather than water at 58 - 62 °C usually used for pigs. A hard scald (>58 °C for about 2.5 minutes) can be counter-productive in respect to chilled storage life because the removal of the cuticle makes the carcasses more susceptible to the growth of spoilage bacteria (20).

1.3.4.3 Dehairing/defeathering (singeing, scraping and polishing)

Immediately after scalding, pigs and poultry are denuded by the mechanical action of scraper blades and rubber-fingered flails, respectively.

Faecal matter escaping from the anus can contaminate the carcass and machinery, resulting in a 100-fold increase in contamination. This recontamination can be reduced if hot water (60 - 62 °C) is used at this stage. Further reduction in bacterial counts, often below 100 cells/cm^2, follows during treatment in the singeing oven (6). Singeing, used to remove any residual hair, also destroys organisms on the skin exposed to heat. Heat treatment may be uneven; bacteria that may have been massaged into the sub-epithelial tissue will remain protected. The carcass is then scraped manually or mechanically and washed to remove burnt material and remaining hair and scurf. However, if the scraping and polishing equipment is inadequately cleaned and sanitised it could result in as high as a 1000-fold increase in bacterial counts as a result of spread of contamination from one site to another. When equipment is properly cleaned and sanitised, the combined effect of scalding and scraping reduces skin counts to 5 to 10% of those found on carcasses at the bleed rail.

With poultry, skin microorganisms that survive scalding cross contaminate carcasses during mechanical defeathering. Faecal microorganisms including pathogenic *Salmonella* spp. and *C. jejuni* are not infrequently present on the surfaces of freshly plucked carcasses, but appear not to be derived directly from the birds' intestinal tracts (cloacal contamination), during plucking (7). Defeathering procedures also result in extensive aerial dispersion of microorganisms - hence the recommendation that these operations be isolated from subsequent activities in the poultry processing plant. The rubber flail fingers are difficult to clean and, because of microbial colonisation, have been implicated in the spread of *Staph. aureus* within poultry processing plants (21).

An alternative method of hair removal for pigs is the coating of the whole body in a hot adhesive resin whose heat turns subcutaneous moisture into steam, which loosens the hair at its roots. After cooling, the hardened resin compound, in which the hairs are firmly embedded, is stripped off, leaving a dehaired carcass ready for further processing. With ducks, after scalding at 60 °C and mechanical plucking, a similar process employing immersion in 87 °C wax, spray-cooling to harden the wax, then hand stripping removes the fine pinfeathers or filoplumes. Both the resin and wax methods appear to offer a hygienic advantage over more conventional dehairing and defeathering processes.

1.3.4.4 Evisceration

The gastrointestinal tract, because of its high microbial load, is a potentially very serious source of carcass contamination and poses a particular hazard in respect to contamination with enteric pathogens. It is, therefore, hygienically imperative that it be removed intact and without puncture. Leakage of stomach contents is prevented by the tying-off or clipping of the oesophagus before, during or after bleeding, as dictated by the method of stunning and/or slaughter. The operation of securing the rectal end of the intestinal tract varies with meat species and, if performed, occurs before evisceration at a position in the dressing sequence convenient to the individual process. With cattle, the skin around the anus is cut and the rectum is raised, placed in a plastic bag, tied-off or clipped and then dropped back into the cavity it originally occupied. With smaller animals, particularly calves, which are prone to scouring, the rectum may be secured by insertion of a cardboard plugging device.

With modern processing, both the respiratory and gastrointestinal tracts, preferably with the latter secured at both its oesophageal and rectal ends, can be removed from the larger meat species with minimal carcass contamination. In cases where the rectum is not secured, e.g. in ovines, faecal contamination is prevented by hygienically effective gutting practices. These same procedures also minimise contamination with urine, which may be expelled if the bladder is squeezed during evisceration. With either manual or automated evisceration of poultry, the processing speed and small carcass size make some gut breakage inevitable. Pre-evisceration head removal by pulling rather than cutting facilitates subsequent automatic evisceration by loosening the crop and lungs while removing both the oesophagus and trachea.

Hygiene regulations require that carcasses be eviscerated soon after slaughter to prevent agonal invasion of the tissues. As discussed previously (see section *1.3.3.2 Bleeding*), there is no scientific justification for believing that bacteria rapidly leave the gut at the time of death. Bacteria in fact remain confined within the intestines until they are released by autolytic action, which requires days, not minutes, at temperate ambient temperatures (22). Aesthetics aside, there are, however, some very practical reasons why evisceration should not be excessively

delayed. Within a few hours of slaughter, distention of the stomach and intestines makes their removal without rupture difficult, and bile may stain the liver and surrounding tissue if rumen distention squeezes bile from the gall bladder. In uneviscerated poultry (New York Dressed), hydrogen sulphide (H_2S) produced by the microflora within the gut can diffuse into surrounding muscle tissue causing a green discolouration that most often first appears around the vent. A similar tissue hue is often evident in game birds after "hanging", and its appearance and distribution will almost certainly be accelerated and extended by shot perforation of the intestines. In such cases it is interesting to speculate whether Epicurean satisfaction and meat hygiene are mutually exclusive.

1.3.4.5 Carcass washing and carcass decontamination

Spray washing of carcasses at the completion of dressing or at several points during the dressing sequence is widely practised. The efficacy of cold potable water carcass washes in respect to the removal of visible contamination including blood, bone dust, hair, and to a lesser extent ingesta and faeces remains beyond dispute. This cosmetic enhancement of carcass appearance is not generally accompanied by an improvement of its microbiological status, however (23). At best, carcass washing with potable water at ambient temperatures redistributes microbial contamination in the direction of the water flow, which is usually posterior to anterior. Similarly, the use of spray washing to remove faecal contamination from the body cavity of poultry, although approved, must be questioned on hygienic grounds because such practices can lead to contamination of the air sacs. If microbial contamination is reduced with such treatments, the reduction is not sufficiently large to significantly extend the storage life of chilled carcasses (24).

In poultry processing, carcass washing at various stages during evisceration has been introduced following the realisation that microbial attachment to skin is a two-stage time-dependent process. Once the process is completed, the attached bacteria can no longer be removed by cold water washing. Multipoint washing during evisceration has proven efficacious in reducing coliform and *Salmonella* numbers on eviscerated poultry carcasses. More recent work with beef (25) suggests that pre-evisceration washing acts prophylactically in respect to microbial attachment to tissue surfaces. Pre-evisceration washing reduces the surface free energy, a measure of the stickiness of the carcass surface, thereby reducing the attraction between microbe and carcass surface. However, the surface free energy increases with time, returning to its pre-wash level within about 60 minutes, which may in part explain the variable and often contradictory claims made for different carcass washing systems. The efficiency of low pressure (85 to 498.5 kPa, 12 to 72 psi) cold water washes for removing bacteria increases with the pressure of application (26), an observation that provides further evidence that the initial physical attachment is not permanent. As the surface free energy increases with time, the ease of removal of microorganisms would be expected to

fall progressively as the time interval between contact and wash treatment increases. Therefore the relevance of some laboratory studies must be questioned in light of the time constraints between contamination and washing imposed by commercial practice.

Carcass decontamination is a remedial measure and should not be regarded as being an acceptable alternative to hygienic slaughter, dressing and handling practices. The separation of carcass washing from carcass decontamination may be academic, but if the former is regarded as relating to removal of visual contamination and the latter to removal of microbial contamination then the differentiation is valid in respect to the major effect, if not the intent, of the two activities. Decontamination may extend the product storage life through a general reduction in product bioload, or may resolve microbiological safety problems by targeting the pathogens concerned.

1.3.4.5.1 Carcass decontamination methods

Physical methods

Trimming is the most widely practised physical method of decontaminating carcasses. Indeed, it is the only approved method in many regulatory codes for removing faecal and ingesta contamination. The practice is, however, of questionable efficacy (27), is labour-intensive, and results in significant economic losses in respect to both discarded tissue and disfigurement of carcasses.

The extent to which the effectiveness of cold water washing, as a means of removing microbial contamination can be increased by elevating the pressure of water application is limited. Those limits relate both to the physical damage incurred by the meat surface and the wash-off protection afforded to microorganisms should they be forced, by water pressure, into the meat tissue. Dye particles have been shown to be forced up to 1.44 mm into meat tissue by water at a pressure of 6200 kPa as compared to a mean penetration depth of 0.56 mm at 690 kPa, which suggests that many bacteria not removed during high pressure washing could be driven into meat tissue (28). If swab sampling rather than excision sampling is used, this effect would erroneously be interpreted as cell removal. The recommended maximum water pressure for the removal of faecal contaminants from beef carcasses is in the 690 - 2070 kPa range (27, 28).

The counts of contaminating bacteria on meat surfaces can be reduced by applying bactericidal treatments such as hot water sprays. From a hygiene point of view, the higher the temperature of the applied wash water the greater the potential for microbial reduction. However, the colour of the meat surface changes permanently if its temperature is raised above 80 °C or is maintained at 80 °C for more than 30 seconds (29). Consequently, for spray treatment of carcasses, a water temperature between 70 and 80 °C at the carcass surface is recommended.

Although colour changes do occur at these temperatures, meat surface colour returns to normal within 24 hours during subsequent carcass chilling. The reductions in bacterial load effected by hot water spray washing range from 1.7 to 3.8 log cycles on beef carcasses (29). The United States Department of Agriculture (USDA) approved the use of a proprietary steam pasteurisation system, the so-called Steam Vacuum Sterilisation Process, for decontamination of beef carcasses. The patented process involves the application of hot water (88 °C), vacuum and steam at 45 psi through means of a nozzle similar to a vacuum carpet cleaner. The hot water, it is claimed, kills more than 90% of the surface bacteria and detaches both faecal and ingesta contamination, which is then removed by the vacuum applied through the nozzle. Another method approved by the USDA is the system of Steam Sterilisation, in which, after removal of surface water, the split carcases are passed thorough a sealed chamber where they are exposed to low pressure steam at over 85 °C for 8 seconds and then cooled with a chilled water spray (30).

Ionising radiation has for many years been proposed as the panacea for eliminating pathogens, particularly *Salmonella*, from poultry and other raw meats. Many experimental studies have shown the efficacy of low dose radiation (2 - 3 kGy) for the elimination of *E. coli* O157:H7, *C. jejuni* and *Salmonella* spp. from red meats and poultry. *Clostridium botulinum* spores may, however, survive such treatments and could subsequently develop in temperature abused anaerobic packs. Despite the increasing availability of irradiation technology, its widespread use to decontaminate meat and poultry within the near future appears unlikely. The reason for this is a deep-rooted and perhaps emotionally rather than factually based consumer reaction against the irradiation of food (31).

Chemical methods

A very diverse range of chemicals, including organic acids, inorganic acids, chlorine, hydrogen peroxide (H_2O_2), triphosphates, and mixtures of nisin with 50 mM ethylene diamine tetraacetic acid (EDTA) and acidified sodium chlorite solutions ($NaClO_2$) have been assessed with respect to their efficacy and practical suitability for use as decontaminating sprays or dips. This activity has been stimulated by the emergence of *E. coli* O157:H7 as an often fatal, low infective dose meatborne pathogen. Spraying trisodium phosphate (TSP, Na_3P0_4), at 55 °C resulted in a reduction of *E. coli* O157:H7; a mixture of nisin and lactate or nisin and EDTA also obtained statistically significant but practically insignificant reductions of *E. coli* O157:H7. A combination of sub lethal concentration of TSP with nisin has been shown to inhibit Gram-negative cells (15).

Among the organic acids, acetic and lactic are the most widely used. Their effectiveness, quite unsurprisingly, is influenced by concentration and temperature of application; they were most effective when sprayed at 55 °C (15). Sensory considerations severely limit the concentration of organic acids that can be applied, as these acids impart distinct odours and flavours as well as causing

surface discolouration of meat. These changes can make the meat unacceptable to consumers, e.g. vinegar flavoured lamb in the Middle East. The antimicrobial effect of organic acids is most marked when contamination levels are high. Consequently, their efficacy against low numbers of pathogens remains uncertain. The sensitivity of *E. coli* O157:H7 and *Salmonella typhimurium* to organic acids is generally similar; however, both are markedly more resistant to organic acid treatment than are the spoilage organisms (32). Such selective antimicrobial activity raises, once again, the safety concern that suppression of the spoilage microflora will, by default, provide an opportunity for unrestricted growth of any pathogens present. Notwithstanding this reservation, the use of antimicrobial sprays and dips are being introduced within the meat and poultry industries as a cost effective method of meeting ever-more stringent microbiological standards or commercial requirements.

1.3.5 Carcass cooling/chilling

Carcass cooling is the stage in meat and poultry processing at which the emphasis of microbiological concern changes from minimising contamination to controlling the growth of the contaminating microflora. The warm and usually moist surface of a freshly dressed carcass provides near ideal conditions for microbial growth. Therefore, if consumption or further processing is to be delayed for more than a few hours, that growth must be slowed if wholesomeness and safety are to be maintained. In this regard, the combined effects of cooling and surface drying are used. These processes, both natural and mechanically assisted, not only determine whether the contaminating microflora proliferates, remains unchanged or declines, but also influence its composition. Low temperature favours the growth of psychrotrophs while drying selects for xerotolerant organisms, particularly micrococci, yeasts and moulds.

Carcasses are cooled, or allowed to cool, under a variety of conditions determined by the facilities available and the storage life required. Before the advent of mechanical refrigeration, storage life was determined by, rather than being a determinant of, the cooling process. At one end of the carcass cooling and storage life spectrum is the local market hot meat trade, in which carcasses are held at ambient temperature. Control of microbial growth depends as much on surface drying as it does on temperature reduction. In hot climates, such meat is consumed before its safety and wholesomeness are compromised, usually within 12 to 18 hours of slaughter. At the other end of the spectrum is blast freezing, in which air at -40 °C and at a velocity near 5 m/s can reduce the deep tissue temperature of a lamb carcass to -12 °C within 6 hours, a temperature at which those bacteria surviving cold shock and freezing damage are unable to grow.

It is generally accepted that mesophilic pathogens do not grow at temperatures below 7 °C. Consequently, cooling of carcasses to below this temperature within a specified time has become a feature of many meat hygiene regulations.

While the requirements of meat hygiene are served by the use of low temperatures and high air velocities, those of meat quality are not. If red meat carcasses are cooled below 10 °C too soon after slaughter, before rigor development, irreversible toughening will result through cold shortening (33). The onset of rigor can be accelerated by electrical stimulation (34), thereby allowing more rapid chilling without prejudicing meat tenderness. Other practical commercial considerations associated with cooling practice include carcass appearance and weight loss. Solutions for, or compromises minimising the hygiene-quality dichotomy have resulted in a range of chilling processes and regimes that include air chilling, spray chilling, immersion chilling and hot deboning systems.

1.3.5.1 Air chilling

Air chilling consists of blowing dry or moist air into and over carcase; various time, temperature and humidity combinations can be used. Methods commonly used for dry chilling involve dry air operated at 0 to -2 °C, or -17 °C/1 hour followed by overnight holding at 4 °C. Moist air chilling involves spraying carcasses with water while blowing air over them to cause evaporative chilling. Moist air chilling can be followed by dry air chilling.

In the 1930s, Scott and Vickery showed the relationship between microbial growth/viability and the water activity (a_w) of the meat surface as affected by air velocity, relative humidity (RH) and temperature. During cooling, evaporation from the warm carcass surface reduces the surface a_w below that required for bacterial growth. Provided the air velocity over the carcass is sufficient to ensure adequate drying (0.7 m/s), the rate of cooling has only a minor effect on bacterial numbers. Even at high or moderate ambient temperatures, as experienced in hot meat trading, surface drying will prevent bacterial growth for several hours, provided evaporation is not restricted. When the carcass has cooled to air temperature, the surface muscle tissue will rehydrate as water diffuses from deeper tissue, but the surface fat tissue tends to remain dry unless the humidity is very high, as moisture migration is less from sub-surface tissues. With the exception of micrococci, staphylococci, enterococci, pediococci, yeasts and moulds, the bacteria (dominating the microflora of carcasses) are not markedly xerotolerant; a modest reduction in a_w from 0.99 (the approximate a_w of muscle tissue) to 0.93 will prevent their growth (35); mesophiles are held in check by the lowered a_w until the surface temperature falls to <7 – 8 °C when temperature prevents growth; psychrotrophs are controlled by the lowered a_w for some time but they will eventually grow (15). Although surface drying and hence control of microbial growth can be maintained by suitable adjustments to air velocity and RH, weight losses become commercially unacceptable.

Changes occurring in the microbial population on carcasses during air chilling are determined by the interaction between the cooling conditions and the contaminating microflora. Consequently, changes occurring at different sites can be expected to differ because of the variation in surface drying effects. It can be argued that there are sites where drying is not a factor in determining microbial growth, and consequently that growth can be predicted by consideration of temperature alone. With the use of an appropriate growth model, e.g. *E. coli*, the temperature function integration technique allows the hygienic adequacy of a cooling process to be calculated from the temperature history obtained at the slowest cooling point (36).

Compared with beef carcasses, chilling of the smaller lamb carcass has much the same microbiological effect, except that the influence of surface drying is less marked because of the more rapid cooling. Despite the difference in drying characteristics between pig skin and subcutaneous tissues of beef and lamb, the effect of chilling on the surface microflora of pig carcasses is similar to that on beef and lamb.

1.3.5.2 Spray chilling

With spray chilling, fine droplets of potable water are intermittently sprayed onto carcass surfaces for periods of 40 to 90 seconds at 30 to 60 minutes intervals during the first 8 to 12 hours of a chilling cycle. This process was developed as a means of preventing the evaporative weight losses associated with conventional air chilling of carcasses. This process also provides a means of decontaminating carcasses. The use of a mild chlorine solution for this purpose has been patented as the Clor-Chil System (37).

Carcass surface drying in conventional air chilling is considered essential for the effective control of microbial growth. Therefore, the deliberate and frequent application of water to the surface of a cooling carcass should theoretically compromise the hygienic efficiency of the cooling process. However, in most studies this has not been found to be the case, and there is no significant difference in microbiological status between spray and conventionally chilled carcasses (38). The Clor-Chil process, on the other hand, typically effects a greater than 95% reduction in the microflora present on the carcass. The higher moisture content of surface adipose and muscle tissue associated with spray chilling does not appear to potentiate enhanced microbial growth during subsequent chilled storage or retail display (39).

1.3.5.3 Immersion chilling

This method involves moving of carcasses through a tank or series of tanks containing chilled water; the flow of water against the direction of carcasses. This

is an efficient and relatively effective method; hence immersion chilling is the most widely used method of cooling poultry. It does, however, pose hygiene problems of cross contamination and quality problems associated with the uptake of water. These concerns have prompted investigation of alternative cooling methods including spray chilling and air blast chilling.

Hygienic operation of both continuous on-line chillers and slush-ice tanks, used for cooling turkeys and other large poultry carcasses, depends on preventing microbial build-up in the cooling water. Microbial numbers are managed by effective temperature control, pre-immersion carcass washing and adequate water flow through immersion chillers. With chiller water usage of between 1 and 2 litres per bird, depending on carcass size, microbial levels can be maintained below 10^5 per ml (40). Immersion chilling not only effects rapid carcass cooling but also has a decontaminating effect, removing between 50 and 90% of the bacteria present on carcasses (40). However, the presence of eluted bacteria in the cooling water, particularly pathogens such as *Salmonella* and *Campylobacter* spp. poses a serious cross contamination problem. The addition of hypochlorite or chlorine dioxide to the cooling water has proven to be an effective remedial measure. It is pertinent to note that the chlorine is effective against the planktonic bacteria but not against those remaining attached to the carcass surfaces. Similarly, in static slush-ice cooling tanks, the use of chlorinated water has been found to be effective in controlling the growth of psychrotrophic microorganisms in the cooling medium.

1.3.5.4 Hot deboning

Hot deboning was developed to address the economic commercial problems of lowering energy usage and chiller space requirements. However, the hygienic consequence of a process that first increases the area of warm moist meat exposed to microbial contamination and then eliminates the effects of surface drying on microbial growth, is nothing short of alarming. The microbiological requirements for safe hot deboning are therefore very simple: minimisation of microbial contamination and very rapid chilling to limit the growth of both spoilage microorganisms and mesophilic pathogens such as *Salmonella* spp. and *E. coli* O157:H7. The technological problems of how to cool a standard 27 kg carton of hot meat rapidly without causing cold shortening (33) lies outside the scope of the present discussion. Temperatures will remain conducive for bacterial proliferation over long periods of time, as meat in the centre of cartons cools slowly. Bacterial growth under these conditions is not as rapid as would be expected because there is a lag period before cell division takes place (41). A further lag will be induced by the shift from aerobic to anaerobic conditions occurring in cartons. Notwithstanding any safety benefit afforded by these lags, product temperature remains a critical factor, as both lag resolution and growth rate are directly related to temperature.

To prevent multiplication of mesophilic bacteria, hot deboned meat must be cooled to less than 7 °C within 3 hours, an impossible requirement for normal commercial refrigeration systems. A commercially attainable objective of limiting growth to a single generation was proposed in Australia. This requires cartoned boneless beef at initial packaging temperatures of 40, 30 and 20 °C to be cooled to less than 8 °C within 4, 6 and 9.5 hours, respectively (42). Such cooling rates necessitate the use of plate freezing techniques and/or the use of half-size cartons. There is, however, no evidence that compliance with New Zealand's regulations that allow a slower cooling rate (to 7 °C or less within 24 hours of leaving the slaughter floor) creates a health hazard. The hygienic adequacy of hot deboning processes can be monitored using the *E. coli* based temperature function integration technique (36).

1.3.6 Further processing

This group of activities includes packaging, cutting, deboning, comminution, mixing and forming. With fresh meat products, all these processes tend to result in the introduction of microbial contamination from the processing environment, and from non-meat ingredients. The microbiological loadings on meat and meat products may increase by as much as 1 log unit with each processing operation, e.g. a carcass with an aerobic plate count of 10^3 cells/cm^2 when broken down produces meat cuts that at packaging carry 10^4 cells/cm^2. Similar increases occur when vacuum packaged (VP) primal cuts are portioned and repackaged for retail sale. Thus, microbiological quality of further processed meat and meat products reflects both the bioload present on the raw material and the hygienic efficiency of processing.

1.4 Spoilage

The microorganisms most frequently associated with the spoilage of raw meats are shown in Tables 1.I and 1.II. Which of these develop to cause spoilage is determined by a complex interaction between the initial microflora; the substrate (meat) parameters, particularly pH, a_w and glucose content; and the environmental variables of temperature and gaseous environment. The spoilage microflora tends to become dominated by those microorganisms that grow most rapidly, i.e. have a growth rate advantage, under a particular set of prevailing conditions. However, spoilage may actually be caused by a minor but high spoilage potential component of the spoilage microflora. In the following discussion of microbial spoilage, temperature, arguably the single most important factor affecting the rate of spoilage onset, will be used as the primary means of classifying spoilage, with gaseous environment (packaging) as a secondary means.

1.4.1 Warm-temperature spoilage

Holding meat at warm temperatures (above 15 °C), because of the potential it affords for pathogen growth, is not generally advocated except in the special case of the hot meat, or wet market meat distribution system. It is, however, pertinent to consider spoilage occurring in the 20 - 35 °C range in respect not only to hot meat distribution, but also to gross temperature abuse within a chilled meat marketing system. Holding meat at warm temperatures should be avoided except where microbial, including pathogen, growth is controlled by surface drying. Under aerobic conditions, growth of saprophytes will usually bring about overt spoilage before the pathogens reach dangerous numbers. However, under anaerobic conditions, spoilage may not be evident before some pathogens have reached high numbers. Consequently, pathogen growth in temperature abused VP meat poses a potentially serious safety problem because there may be no organoleptic warning that high microbial numbers have been reached.

1.4.1.1 Aerobic

Under aerobic conditions at temperatures above 20 °C the pseudomonads do not have a growth rate advantage over competing psychrotrophs or mesophiles present on meat. However, it is not until temperatures reach about 30 °C, the maximum for the growth of many psychrotrophic pseudomonads, that mesophilic strains of *Acinetobacter* and Enterobacteriaceae assume dominance of the spoilage microflora (43).

1.4.1.2 Anaerobic

Under anaerobic conditions, such that prevail within a vacuum pack, the spoilage microflora developing at room temperature is dominated by a mixture of psychrotrophic and mesophilic, including pathogenic, strains of Enterobacteriaceae. This dominance reflects the large numbers of Enterobacteriaceae in the initial microflora rather than any significant growth rate advantage they hold over the lactobacilli. Raising the temperature to 30 °C will allow *C. perfringens*, if present in the initial microflora, to bring about spoilage (43). Similarly, the slow cooling of carcasses can potentiate the growth of clostridia in deep tissue, if present, resulting in the spoilage condition known as bone taint.

In the temperature range considered to be abusive in respect to prolonged chilled storage, 5 to 15 °C, pack distension ("blown pack" spoilage) resulting from the production of large volumes of gas within the pack is associated with the growth of psychrotrophic Enterobacteriaceae.

1.4.2 Chill-temperature spoilage

The optimum temperature for chilled storage of meat is the lowest temperature that can be maintained without freezing the product. With unwrapped products, because of the added effect of evaporative cooling, temperatures below 0 °C should be avoided; however, packaged product can be held at -2 °C, i.e. slightly super cooled, for long periods without freezing.

1.4.2.1 Aerobic

Under aerobic chilled storage, *Pseudomonas* spp. will predominate in the developing spoilage microflora (44), which will usually also contain significant numbers of *Acinetobacter* and *Moraxella* strains. For today's meat trader, psychrotrophic aerobic spoilage is a problem associated either with the storage of carcasses and sides, or with product on retail display.

Onset of spoilage can be retarded if carcass surfaces can be maintained in a dry condition. However, maintenance of a dry meat surface inevitably results in weight loss and surface desiccation. Under these conditions, yeasts and moulds may make their appearance as the falling surface a_w inhibits the growth of the faster growing but xerosensitive aerobic spoilage bacteria. However, where desiccation is prevented by the use of plastic wraps or where air circulation is inadequate to maintain a dry meat surface, rapid spoilage onset results from the unrestricted growth of the *Pseudomonas* spoilage consortium. Off-odours become evident when numbers reach 10^8 cells/cm^2 and surface slime is visible as numbers approach 10^9 cells/cm^2. The spoilage of consumer cuts in the home refrigerator follows a similar course.

With retail display packs, the onset of aerobic spoilage can be delayed by the use of high oxygen (O_2) (60 - 80%) carbon dioxide (CO_2) (40 - 20%) modified atmospheres. The high concentration of O_2 enhances the bright red colour of oxygenated meat while the CO_2 inhibits the growth of the *Pseudomonas*-dominated spoilage microflora to give a chilled display life of 5 to 10 days compared with the 2 to 3 days obtained with an air atmosphere in overwrapped packs.

1.4.2.2 Anaerobic

1.4.2.2.1 Vacuum packaging

Today, VP, in which an oxygen-deficient environment is created around the product by evacuation, is the most widely used preservative packaging system. By changing the gaseous environment from aerobic to anoxic, an ecological shift is affected that sees the strictly aerobic high spoilage potential *Pseudomonas*

microflora replaced by low spoilage potential LAB. Not only does the spoilage potential change significantly, but also the spoilage microflora develops more slowly because fermentative metabolism is considerably less energy-efficient than oxidative metabolism. The overall result is an extension of chilled storage life from 2 - 3 weeks obtainable under aerobic conditions to 8 - 10 weeks or more, depending on the storage temperature and meat species.

With a spoilage microflora dominated by LAB, spoilage does not become evident until several weeks after maximum numbers of 10^7 - 10^8 cells/cm^2 have been reached. Meat odour and flavour then progressively deteriorate, becoming distinctly “cheesy" as volatile fatty acids produced by the metabolism of leucine and valine accumulate. LAB are, however, usually present as an undetectably small fraction of the initial contaminating microflora, so it is possible for early spoilage to be caused by slower growing, higher spoilage-potential species present in the initial microflora in relatively large numbers. Facultative microorganisms that are particularly troublesome in this regard are *Shewanella putrefaciens, B. thermosphacta* and the psychrotrophic Enterobacteriaceae. All these organisms have a propensity to become established in processing environments where sanitation procedures are ineffective. Such pre-packaging contamination can produce serious, if intermittent, early spoilage problems.

Sh. putrefaciens, which produces copious quantities of H_2S under anaerobic conditions, is unable to grow at pH values below 6.0. Consequently, *Sh. putrefaciens* spoilage, characterised by offensive odours and green discolouration resulting from sulphmyoglobin formation (H_2S reacting with oxymyoglobin), is limited to high-pH meat. In the case of poultry, *Sh. putrefaciens* will grow on high-pH chicken leg muscle but not on the lower pH breast muscle (45). Similarly, with *B. thermosphacta* and psychrotrophic Enterobacteriaceae, including the pathogen *Y. enterocolitica*, anaerobic but not aerobic growth is precluded at pH values of 5.8 or below (46, 47). Although high pH allows the growth of these high spoilage potential microorganisms, the poor storage characteristics of high ultimate pH meat (DFD meat) is due to the low level or complete absence of glucose in such meat (19). Under such conditions, the developing microflora is forced to utilise amino acids as an energy source. Early spoilage results when the offensive by-products of amino acid catabolism become detectable to the consumer, often at cell densities as low as 10^6 cells/cm^2.

Of increasing concern with VP meat produced during the winter months in temperate regions is a type of "blown pack" spoilage resulting from the production of hydrogen and CO_2 due to the growth of psychrotrophic clostridia (48). Several causative organisms within this apparently ubiquitous group have been named: *Clostridium laramie, Clostridium estertheticum, Clostridium frigidicarnis* and *Clostridium algidicarnis*, which produces offensive odours without pack distension. Failure to recognise this type of spoilage due to the use of inappropriate methods to isolate obligate anaerobic psychrotrophs, and the

samples experiencing poor temperature control during transportation and storage, allow Enterobacteriaceae, the most probable cause of "blown pack" spoilage in that situation, to be blamed as the causative agent. Although microscopic examination of the purge in non-temperature abused spoiled packs usually shows the presence of Gram-positive sporing bacilli, clostridia, in large numbers, their culture and isolation remain problematical. A clostridial aetiology of "blown pack" spoilage can reasonably be inferred from the detection of butyric acid, butanol and various butyl esters in the pack atmosphere.

1.4.2.2.2 Carbon dioxide packaging

Chilled storage life can be extended by 50 to 100% over that achieved by conventional VP by using elevated concentrations of CO_2 in combination with anaerobiosis. Saturated CO_2 packaging (49) has proven particularly successful for the prolonged chilled storage of raw meats and poultry. Storage life is prolonged by extension of the lag phase before growth of spoilage microorganisms begins, then by differential suppression of the growth of high spoilage potential psychrotrophic Enterobacteriaceae and *B. thermosphacta*. The differential inhibitory effect and lag phase extension also retard the growth of the psychrotrophic pathogens *Aeromonas hydrophila, L. monocytogenes* and *Y. enterocolitica*.

1.4.3 Freezing temperature spoilage

At the temperature at which frozen meat and poultry are normally held, below -12 °C, microbial growth is effectively prevented. However, at temperatures only marginally higher, fungal growth has been reported. As meat freezes, ice crystals begin to form and the dissolved solutes become concentrated in the remaining liquid. The a_w of the free liquid in frozen meat also decreases, because of the concentration of solutes dissolved in it (50). Consequently, microorganisms attempting to grow on frozen meat face two hurdles: firstly, the low temperature and secondly, the reduced a_w. As discussed previously with respect to surface drying, meat spoilage bacteria are not xerotolerant and consequently cannot grow at a_w values associated with frozen meat cooled to below about -2 °C.

With bacterial growth effectively eliminated at temperatures below -2 °C, the more xerotolerant moulds and yeasts are frequently observed growing on frozen meat stored at marginal temperatures. Mould spoilage of meat has received more attention than yeast spoilage because mould growth causes dramatic changes to the meat appearance. Despite literature claims of mould growth occurring at temperatures as low as -18 °C, most of the common meat spoilage moulds, causing conditions descriptively referred to as "black spot", "white spot", "blue green mould" and "whiskers", have a minimum growth temperature near -5 °C. At this temperature several months are required for visible colonies to develop (51).

The mould spoilage of greatest concern is "black spot", caused by the growth of *Cladosporium herbarum, Cladosporium cladosporoides* or *Penicillium hirsutum*. Black spot, commonly associated with meat transported long distances by sea, is recognised by black colonies up to 1 cm in diameter with distinct hyphaI penetration into the surface layer of the meat. Although such growth does not pose a health hazard, considerable financial loss may be incurred when carcasses are downgraded following the trimming necessary to remove the affected areas. White spot, most frequently caused by *Chrysosporium pannorum* (formerly known as *S. carnis*), and the other forms of mould spoilage are superficial and can be wiped off without leaving visible evidence of growth. Like black spot, they do not pose a health hazard; indeed, one of the causes of "whiskers", *Thamnidium elegans*, is the subject of several meat ageing patents and is claimed not only to tenderise the meat, but also to impart a pleasant nutty, aged flavour (52).

Mould growth on frozen meat is indicative of marginal conditions during frozen storage and transportation. The appearance of yeasts on frozen meat, another sign of marginal storage, may not result in adverse sensory changes, but visual acceptability can be compromised by the appearance of colonies, particularly on fat surfaces. Unsightly brown spots, resulting from the deposition of haem pigments, have been reported in the vicinity of yeast colonies growing on the fat of VP beef stored at 0 °C (53). It is not known whether this phenomenon occurs in frozen products.

At chill temperatures, the reduction in a_w associated with surface drying will also provide yeasts and moulds an opportunity to develop. Many of the species associated with frozen spoilage also occur on unwrapped carcasses stored for several weeks at chill temperatures.

1.4.4 Spoilage ecology

Product spoilage occurs as a direct consequence of the development of a microbiological community. Competition between species under the selective conditions found on meat surfaces tends to produce a climax population, the spoilage microflora, lacking in species diversity. From the investigating microbiologist's perspective, this selective process makes examination of spoiled products relatively simple, as the number of organisms concerned is small and these can be differentiated on the basis of a small number of tests (5).

The surprise appearance of an organism under inappropriate conditions, such as finding *Pseudomonas* in the microflora of VP products, indicates that the conditions experienced were not those expected, e.g. the pack leaked, or the wrong packaging material was used. A more complex example is provided by an incident of yeast-mediated brown spot fat spoilage of VP beef (51), which should not have occurred because of the vast growth rate advantage afforded to the LAB by the packaging. The probable explanation is an unusual combination of process

contamination and micro-habitats. Yeasts were present in high numbers, as the meat had become heavily contaminated with yeasts present in chiller condensate that had dripped onto carcasses during chilling. The growth of these yeasts was then enhanced by O_2 availability, as growth was restricted to areas of poor contact between the packaging film and meat surfaces.

In another example, clostridial "blown pack" spoilage, conventional cultural examination revealed an absence of Enterobacteriaceae in physiologically significant numbers in a LAB microflora typical of normal VP products. As this microflora is not consistent with the spoilage symptoms, the culprit had obviously eluded detection. In such cases, routine examination of the spoiled product becomes a research exercise, firstly, in microbiological deduction and subsequently in microbiological methodology.

1.5 Pathogens: Growth and Survival

With few exceptions, raw fresh meats receive a bactericidal but not sporicidal treatment; cooking, before consumption. Therefore, meat poses a direct food poisoning hazard only through its consumption in a raw or undercooked form. However, raw meat poses an indirect food poisoning hazard through cross contamination of cooked meats and of other foods that are not cooked before consumption. Worldwide, meat and meat products have unenviably high placings in the league tables of foods associated with food poisoning.

The microbiological characteristics of meatborne pathogens causing food poisoning are briefly outlined in Table 1.III. Further comment on individual microorganisms is presented in alphabetical order and consequently the order does not indicate priority with respect to either their prevalence or their severity. The discussion relates primarily to the microorganisms and their association with meat. Readers requiring clinical information about the food poisoning events these pathogens cause are referred to major texts on foodborne infections (e.g. 54, 55) from which the following microbiological cameos have been drawn.

1.5.1 Aeromonas hydrophila

Aeromonads are widely distributed in freshwater and marine environments and attain high numbers in sewage and in traps and floor drains associated with domestic and food processing wastewater systems. In addition to transient human carriage, aeromonads may also be found in the faeces of healthy animals. Prevalence in livestock is variable, 4 to 12%, with a high level of carriage often found in poultry.

Aeromonas spp. are not infrequently present in the initial microflora on meat (Table 1.I) and because of their psychrotrophic nature develop to form part of the

spoilage microflora in vacuum packs, and to a lesser extent in CO_2 packs. With the exception of poultry, intestinal carriage cannot explain the high incidence of *Aeromonas* spp. on meat (56). Water used for washing of carcasses and plant is believed to be the most likely source of aeromonad introduction into the meat processing environment.

Table 1.III
Food-poisoning pathogens associated with chilled and frozen raw meats, poultry and their products

Organism	Gram reaction Cell morphology	Oxygen Requirement	Temperature Requirement	Food Poisoning
A. hydrophila	G-ve rod	Facultative	Psychrotrophic	Infection
Bacillus cereus	G+ve sporing rod	Facultative	Mesophilic	Intoxication
Campylobacter spp.	G-ve spiral rod	Microaerophilic	Mesophilic	Infection
C. botulinum	G+ve sporing rod	Anaerobe	Mesophilic*	Intoxication
C. perfringens	G+ve sporing rod	Anaerobe	Mesophilic	Intoxication†
E. coli	G-ve rod	Facultative	Mesophilic	Infection
L. monocytogenes	G+ve rod	Facultative	Psychrotrophic	Infection
Salmonella spp.	G-ve rod	Facultative	Mesophilic	Infection
Staph. aureus	G+ve cocci	Facultative	Mesophilic	Intoxication
Y. enterocolitica	G-ve rod	Facultative	Psychrotrophic	Infection

* non-proteolytic strains grow to 3.3 °C
† toxin is usually associated with sporulation in the intestine rather than preformed in food

1.5.2 Bacillus cereus

The presence on carcasses of *B. cereus* and other *Bacillus* spp. of soil origin, including *Bacillus subtilis* and *Bacillus licheniformis*, is not unusual although their numbers are generally low. In raw meat products such as sausage, these organisms are both more numerous and more frequently present because of their introduction in cereal fillers and spices (57). The endospores of contaminating *Bacillus* spp. survive cooking, but pose a health hazard only when post-cooking conditions, particularly temperature, allow them to germinate and develop to high numbers.

1.5.3 Campylobacter spp.

Campylobacter infection was the most commonly reported cause of bacterial food poisoning in humans in 2007, which is why the reduction of *Campylobacter* in chicken is a priority for the Food Standards Agency (FSA) (58). Most cases are caused by the "thermophilic" species *C. jejuni, Campylobacter coli* and *Campylobacter laridis*. These microorganisms are frequently isolated from food

animals, particularly poultry, with *C. jejuni* the most common, although *C. coli* has a high incidence in pigs.

Campylobacter infections have been traced directly to the consumption of undercooked poultry, particularly chicken. However, cross contamination of other foods that are not cooked before consumption probably accounts for the largest proportion of cases of campylobacteriosis. Despite the relatively rapid die-off of *Campylobacter* spp. at refrigeration temperatures, *Campylobacter* infection remains a problem associated with chilled poultry at retail.

With some localised exceptions relating to porkborne *C. coli* infections, campylobacteriosis is not commonly acquired as a consequence of beef, lamb or pork consumption. This may in part reflect the low meat pH of these species, compounded by the adverse conditions for *Campylobacter* survival prevailing at the carcass surface during air chilling. As with poultry, direct infections have been linked to undercooking. As *Campylobacter* spp. are effectively eliminated by adequate cooking, post-cooking contamination, and hence kitchen hygiene, remain matters of some concern.

1.5.4 Clostridium botulinum

Botulism is rare, but the disease is severe, with a high mortality rate. Endospores of the obligate anaerobe *C. botulinum* are widely distributed in the environment, including soil and bottom deposits in lakes, rivers and coastal marine waters. The presence of *C. botulinum* endospores in meat and meat products as a consequence of chance contamination cannot be excluded, although the incidence of their occurrence must be expected to be low and variable. The mere presence of spores does not, however, pose a hazard, as botulism results only from the consumption of foods in which toxin has accumulated as a consequence of *C. botulinum* growth. With the exception of non-proteolytic types B, E and F, strains of *C. botulinum* are generally mesophilic and so require temperatures above 15 °C for significant growth and toxin production. Botulism remains a possible health hazard in anaerobically packaged meats and meat products, especially those that have been subjected to heat treatment and are then stored at ambient rather than refrigeration temperatures.

1.5.5 Clostridium perfringens

C. perfringens is commonly found in the intestines of man and food animals as well as in agricultural soils. The gastrointestinal association means that *C. perfringens* is found in sewage and in receiving waters contaminated with either agricultural run-off or domestic sewage. Both spores and vegetative cells of *C. perfringens* are frequently present in small numbers on raw meats, probably as a result of direct or indirect faecal contamination, with the level of that

contamination reflecting the hygienic adequacy of the abattoir. As with *B. cereus*, food poisoning caused by *C. perfringens* requires the extensive growth of the organism in inadequately cooled cooked meats and meat products, or during warm holding of such products. Unlike food poisoning caused by *B. cereus* or *C. botulinum*, which are true intoxications, that caused by *C. perfringens*, although due to an enterotoxin, classically results from the ingestion of high numbers of vegetative cells. Those that survive passage through the stomach produce toxin on reaching the intestine, usually in association with sporulation. Toxin production unrelated to sporulation has been reported and toxin accumulation in food demonstrated. The importance of such non-intestinal production of toxin in respect to *C. perfringens* food poisoning has yet to be established.

In Papua New Guinea, *C. perfringens* type C causes a necrotic enteritis known locally as Pigbel. The condition is associated with the consumption of pork, often transported in carcass form over long distances for distribution to friends and relatives unable to attend major tribal or family celebrations featuring often severely undercooked roast whole pig.

1.5.6 Escherichia coli

E. coli is the most abundant aerobic microorganism found in the gastrointestinal tract of man and many other animals. In recent years, interest in *E. coli* has expanded from its traditional role in food and water microbiology as a harmless indicator of faecal contamination, first to being associated with "travellers and infantile diarrhoea", and then to being recognised as a specific and often highly virulent pathogen. Five types of diarrhoea-producing strains of *E. coli* are now recognised: enteropathogenic *E. coli* (EPEC) associated with infantile diarrhoea; enterotoxigenic *E. coli* (ETEC) associated with "traveller's diarrhoea"; enteroinvasive *E. coli* (EIEC); enterohaemorrhagic *E. coli* (EHEC), which includes as a subgroup the verocytotoxigenic *E. coli* (VTEC) or colohaemorrhagic strains; and enteroadherent-aggregative *E. coli* (EA-AgEC). Of these, EHEC, in particular serotype O157:H7, has a strong epidemiological link with beef. Evidence from outbreaks has established beyond doubt the association between the consumption of undercooked hamburgers and infection with *E. coli* O157:H7, haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Proper cooking will, however, eliminate the health hazard posed by this and other *E. coli* serovars.

As with any other organism associated with the gastrointestinal tract of slaughter stock, meat contamination with pathogenic *E. coli* results primarily from direct or indirect faecal contamination. The frequent presence of other, generally harmless serotypes of *E. coli* on carcass meat suggests that the incidence of EHECs, including O157:H7, will reflect their prevalence in the slaughter stock. Complete prevention of faecal contamination appears impossible, so the typically extremely low infective dose of EHECs and the severity of the diseases they cause

are prompting the development and introduction of effective decontamination measures.

1.5.7 Listeria monocytogenes

Until the early 1980s *L. monocytogenes* was of concern as a causative agent of disease in animals, particularly ruminants, with infection resulting in abortion in sheep, encephalitis in cattle, and of an occupational disease of those working with animals. Its recognition as a foodborne pathogen of man was alarming, not because of the mild self-terminating enteric phase in healthy individuals, but because of the sequelae occurring in "at-risk" groups: pregnant women, neonates, and the immuno-compromised. Abortions and stillbirths caused by *L. monocytogenes* gained media attention, leading to the aptly named "*Listeria* hysteria". The very practical outcome of this has been a list of foods, which includes some types of meat products, that pregnant women are advised to avoid.

L. monocytogenes is ubiquitous and can be isolated from vegetation, soil, water, and faeces. Infection of livestock has been linked to the feeding of silage, and is especially prevalent when fermentation has not been completed properly. *L. monocytogenes* can grow at chill temperatures and therefore has a propensity to colonise food processing environments, particularly floor drains and moist surfaces. The tendency to form sanitiser-resistant biofilms on the latter, including stainless steel, is a serious problem with respect to plant sanitation and hygiene measures. Consequently, contamination of meat can occur not only during dressing but also, and probably more frequently, during further processing.

L. monocytogenes is able to grow in vacuum packs at chill temperatures. The use of CO_2 packaging confers a considerable safety advantage over VP in that growth is delayed or numbers decline during chilled storage. Which of these scenarios occurs is determined by an interaction of substrate pH and storage temperature (59). Growth is favoured by high temperature and elevated pH. The prolonged storage of refrigerated fresh meats under CO_2 appears unlikely to increase the associated *Listeria* hazard, and then only if that meat is eaten raw, e.g. as steak tartare. The major risk of contracting listeriosis is associated with the consumption of chill-stored ready-to-eat (RTE) meat products that are either lightly cooked, e.g. paté, allowing *L. monocytogenes* survival, or adequately cooked products that become contaminated prior to packaging.

1.5.8 Salmonella spp.

Salmonella gastroenteritis can be caused by a large number of *Salmonella* strains that are not highly host-adapted, i.e. strains that can infect both man and animals. The typhoid fevers caused by the human-adapted *Salmonella typhi, Salmonella paratyphi* and *Salmonella sendai* are either waterborne or foodborne. With

foodborne infection, contamination occurs at a point close to consumption and is often traced to asymptomatic carriers involved in food preparation.

The salmonellae inhabit the intestinal tract of a wide range of animals, birds and reptiles, including all the common meat animal species. Consequently, raw meat, particularly poultry, is not infrequently contaminated with salmonellae. Incidence varies with species, age, husbandry practices, slaughter hygiene, and subsequent product handling. The latter two considerations concern cross contamination while the first three relate to prevalence in the slaughter stock. In extreme cases with intensively reared poultry, incidence on carcasses can approach 100%.

Salmonellosis is rarely acquired directly from fresh meats and then only when these are consumed raw, e.g. steak tartare and raw sausage. As with *Campylobacter* infection, most outbreaks of salmonellosis are associated with the consumption of undercooked meat, particularly poultry, mishandling, or meat that has been contaminated after cooking. Drip released during the thawing of frozen poultry is particularly hazardous in respect to cross contamination of cooked meats and other foods. The presence of *Salmonella* spp. on raw meat and especially poultry must always be assumed and reasonable precautions taken to assure its elimination and/or containment.

1.5.9 Staphylococcus aureus

The growth of enterotoxigenic *Staph. aureus* in foods can lead to staphylococcal intoxication on their ingestion. *Staph. aureus* strains of animal origin are frequently present on raw meat and poultry. Strains derived from human sources can be introduced during processing. Numbers on poultry carcasses can be high as a consequence of *Staph. aureus* colonisation of processing equipment (21).

The presence of *Staph. aureus* in raw meat has little direct food safety significance if the organism is unable to grow; however, further processing of raw product containing high numbers of *Staph. aureus* is likely to be reflected in elevated levels in end-products, including cooked products. Nevertheless, post-cooking contamination, most often by food handlers or by cross contamination with raw product, is probably a more important route by which *Staph. aureus* is introduced onto cooked meats. The scene is then set for a food poisoning outbreak, requiring only warm or prolonged unrefrigerated holding, to allow *Staph. aureus* to reach the high numbers (10^6 cells per gram or cm) required for toxin production to render the food unsafe. Unfortunately for the consumer, there is no organoleptic indication that *Staph. aureus* numbers have reached dangerous levels.

1.5.10 Yersinia enterocolitica

Y. enterocolitica is able to grow at chill temperatures and, as with other yersinias, many of its biochemical reactions are temperature-dependent, being expressed at 28 - 30 °C but not at 37 °C. Although *Y. enterocolitica* serovars are widely distributed in the animal kingdom, the only meat animal that harbours human pathogenic serovars is the pig. Carriage in pigs is either intestinal or more frequently pharyngeal, with isolations readily made from the tonsils.

Despite the frequent association of *Y. enterocolitica* with pigs, the only retail pork cut from which the human pathogenic serovar 03 can be consistently isolated is fresh pork tongue. Consequently, undercooked pork products derived from head meats may pose a direct hazard in respect to yersiniosis. Post-processing contamination, perhaps through contaminated water on work surfaces, is considered to be the major route of *Y. enterocolitica* introduction into food products. The wide range of foods, including meat and poultry products, from which *Y. enterocolitica* has been isolated is consistent with post processing contamination. The significance of such isolations, which have been predominantly of environmental serovars, remains uncertain.

1.5.11 Protozoa

From the above discussion it is obvious that elevated meatborne bacterial disease morbidity and the consumption of raw or undercooked meat and poultry are positively correlated. The same eating habits would also carry increased risk of contracting diseases of protozoal aetiology such as enteritis caused by the ingestion of *Cryptosporidium parvum* oocysts present on the meat as a result of faecal contamination.

Ingestion of raw or undercooked muscle tissue in which cysts containing viable bradyzoites of *Toxoplasma gondii* are present is one of the routes by which humans contract toxoplasmosis (60). To avoid transmission, infected meat should be cooked to an internal temperature of at least 60 °C or frozen (-15 °C for 3 days, -20 °C for 2 days) (15).

Humans can also be infected with *Sarcocystis hominis* and *Sarcocystis suihominis* by eating cysts present in raw beef and pork respectively. Cooking or freezing meat destroys the cysts (15).

Another protozoon commonly responsible for outbreaks is *Trichinosis spiralis*. Trichinellosis is acquired due to the ingestion of encysted larvae in the muscle of raw pork and wild animals. The risk of trichinellosis to consumers can be reduced by cooking meat to at least 58 °C or freezing (-15 °C for 30 days) (15).

Humans can be infected with tapeworm following the ingestion beef muscle and pork offal contaminated with viable, or encysted cysticeri of *Taenia saginata*, and *Taenia solium* respectively. Freezing (-10 °C for at least 10 days) and cooking inactivates the cysticerci (15).

1.5.12 Prion

BSE, also called mad cow disease, is caused by prions, a specific type of misfolded protein. The disease can be transmitted to humans by food. To protect human health, the use of certain bovine organs, called specific risk material, is prohibited for use in food, gelatine, tallow, drugs or cosmetics. The materials include brain, eyes, spinal cord, spleen, thymus, bovine intestine of calves >6 months old, visible lymph and nerve tissues and lymph nodes (15).

An effective disinfectant measure is steam sterilisation at 133 °C and 3 bar pressure for 20 minutes (15).

1.5.13 Avian influenza

Avian influenza has been recorded in birds in a number of countries worldwide. The disease primarily affects birds, but there have been cases of human infection in people that have been closely associated with infected birds. Although it often causes little or no disease in wild birds, it can sometimes cause large outbreaks associated with high mortality in poultry. Under these instances the term 'highly pathogenic avian influenza' is used (58).

The virus was detected on two poultry premises in the UK. It resulted in the culling of birds on the infected premises; the birds were then disposed of and preliminary disinfection had to be carried out. Biosecurity practices on farms are now in place to prevent recurrence of the disease (58).

1.6 Published Microbiological Criteria

The purpose of microbiological criteria is protection of the health of the consumer by assurance that products are both safe and wholesome (61). The rationale for the establishment of microbiological standards is that a relationship exists between the presence of microorganisms and the safety of food. A microbiological criterion should be established and applied only where there is a definite need for it, the method of testing is practical, and its enforcement can be shown to significantly reduce the risk to consumers. The need for a microbiological criterion is demonstrated by epidemiological evidence that the food is a public health hazard or where assurance is required that provisions of hygienic significance in a process have been adhered to. Finally, a criterion should not set a standard that is unattainable by currently accepted Good Manufacturing Practice (GMP).

The microbiological status of meat and poultry is determined by the interaction between the slaughter animal, and the process by which it is turned into an edible product. Microorganisms of public health significance are often present in the microflora carried by the slaughter animal. Today, even the best processing practices will not eliminate these pathogens from raw meats. Therefore, until effective antimicrobial interventions are introduced, limits in microbiological criteria for pathogenic microorganisms in raw meats may be inappropriate. Similarly, limits for indicator bacteria are of dubious value, as there is no direct relationship between the presence of these microorganisms and the presence or absence of pathogens (62, 63). However, in order to contribute to the protection of public health and to prevent differing interpretations, it was felt appropriate to establish harmonised safety criteria on the acceptability of food.

Microbiological criteria (Food Safety Criteria and Process Hygiene Criteria) relating to raw meat, poultry and their products are specifically prescribed in national and international food legislation. The legal aspects of the application of microbiological criteria to meat and meat products are considered more fully in the Legislation chapter. This section covers other published guidance on microbiological criteria for this category of product.

Other widely accepted microbiological criteria for raw meat and poultry are those relating to aerobic plate counts (APC) proposed by the International Commission for the Microbiological Specifications of Foods (ICMSF) (63) (see Table 1.IV). The values for "m" reflect current commercial attainment based on examination of meats from different sources, at the point of production, and are supported by experience that meat with plate counts lower than the value “m” have not been subjected to excessive contamination or undue faulty handling; this meat would have a normal shelf life. Those for "M" are based on experience and are indicative of gross contamination or conditions that have permitted microbial growth to a level approaching incipient spoilage. Consequently, the values of "m" and "M" are independent of each other and so have no constant relationship. The microbiological quality of comminuted meat reflects both the hygienic status of the whole tissue from which it was derived, and the conditions under which it was prepared. Counts in ground (minced) meat can generally be expected to be one or two orders of magnitude higher than those on the carcasses from which that meat originated.

The values contained in Table 1.IV provide a useful guide to assess the hygienic adequacy of the conditions under which the meat was produced. In the case of chilled products, they should be applied only at the abattoir or processing facility. Generally, in-plant quality control criteria are significantly more stringent than the limits shown in Table 1.IV.

APCs are of limited usefulness for port of entry assessment because of the perishable nature of chilled meat and the modifying influence of VP and MAP on

the microflora. However, commercial expediency in response to "due diligence" legislation is promoting the increased use of microbiological standards in product specifications. It is important for all concerned to appreciate that acceptance or rejection using APC as a criterion is based on experience relating to the probability of a particular outcome being attained, e.g. adequate retail display life, and not on public health concerns relating to pathogens.

Table 1.IV
Recommended APC (30 °C) limits for three class sampling plans for raw meat* and poultry (63)

Product	n	c	Limit per cm^2 or gram m	M
Carcass meat before chilling	5	3	10^5	10^6
Carcass meat, chilled	5	3	10^6	10^7
Edible offal, chilled	5	3	10^6	10^7
Carcass meat, frozen	5	3	5×10^5	10^7
Boneless meat, frozen (beef, veal, pork, mutton)	5	3	5×10^5	10^7
Comminuted meat, frozen	5	3	10^6	10^7
Edible offal, frozen	5	3	$5 \times 1\ 0^5$	10^7
Raw chicken (fresh / frozen) during processing	5	3	5×10^5	10^{7*}

n Number of units making up a sample
c Number of units in sample allowed to have values between m and M
m Threshold below which a result is considered satisfactory
M Acceptability threshold above which a result is considered unsatisfactory
* Not for port-of-entry sampling; for in-plant quality control

Another widely accepted microbiological criterion is that published by the Institute of Food Science and Technology UK (IFST). It provides microbiological guidelines for a number of food product categories, including raw meats and poultry. It provides recommendations on bacterial counts under GMP values (values expected immediately following production of the food under good manufacturing conditions employing a HACCP-based system of controls) and Maximum values (those regarded as the maximum acceptable at any point in the shelf life of the product) (64).

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2. COOKED MEATS, POULTRY, AND THEIR PRODUCTS

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2.1 Introduction

Despite all the measures that have been taken by the food industry to ensure a high level of safety and quality of products, the number of reported cases of food poisoning continues to increase all over the world. The Centres for Disease Control and Prevention (CDC) estimates 76 million cases of foodborne illness every year in the United States; 325,000 people are hospitalised and nearly 5,000 die every year due to consumption of contaminated food. With the scientific advances over the decades, one might think that the biological, chemical, and physical hazards that contaminate our food and compromise our health would long since have been conquered. Most foodborne outbreaks associated with meat and poultry can be traced to under-cooked or cross contaminated cooked food products.

2.2 Definitions

Contamination is the unintended presence of a harmful substance or microorganism in food. The source of contamination in meat, poultry and their products may be primary - coming directly from an infected food animal and its discharges, or secondary - resulting from mishandling of raw meat and finished products.

The types of cooked meat, poultry and products available can be divided into the following groups:

Cooked cured meat - e.g. hams, hot dogs

Cooked uncured meat - e.g. ground beef, ground turkey

Fermented cooked/partially cooked meat - e.g. sausages

Partially cooked meat - e.g. charcoal marked meat

This chapter refers to cooked products; more detailed information on cured and fermented meats can be found in later chapters.

2.3 Initial Microflora

A freshly slaughtered meat animal harbours very few microorganisms inside the muscle tissues, and most of these are isolated from the lymph nodes. However, the surface of the meat is exposed to varying degrees of contamination during slaughter, evisceration, and other operations after slaughter. Wholesale and retail cuts of fresh meat held under proper refrigeration will develop a predominant microflora of psychrophilic microorganisms, either eurypsychrotroph (eurys, "broad") or stenopsychrotroph (stenos, "narrow"). The most commonly encountered are *Pseudomonas, Acinetobacter* and *Flavobacterium*. These spoilage bacteria are aerobic and require a high water activity (a_w) for optimum growth, and do not grow at the same rate between 0 and 7 °C. The microbial picture of ground beef or hamburger is similar to that of other fresh meat, but because of grinding much more surface is exposed to a considerably greater number of microorganisms.

Greater detail on the initial microflora of raw meat is described in Chapter 1. Chilled and Frozen Raw Meat, Poultry and their Products.

2.4 Processing and its Effects on the Microflora

Microbiological spoilage and pathogen growth associated with cooked meat, poultry and their products are common and usually attributed to several factors. These factors include the use of microbiologically spoiled raw meats and other ingredients, abuse of products during preparation, spoilage of intermediate/final products prior to heat processing, manipulation of product conditions (e.g. reduction of a_w or pH), post-process contamination, and the substantial changes in the gaseous environment of the product due to the wrapping or packaging materials.

2.4.1 Ingredients

Additional sources of microorganisms that can be introduced into the cooked meat products are the seasoning and formulation ingredients that are used in the recipes for products such as sausages and frankfurters. Processed meats such as frankfurters, bologna, sausage, salami, and luncheon meat are expected to reflect the sum of their ingredient make-up with regard to microbial counts and types.

High microbial counts are usually found in many spices, and lactic acid bacteria (LAB) and yeasts can be introduced in the added milk solids. Also, natural pork casings have been shown to contain high counts of bacteria (log 5.26 - 7.77) consisting mainly of *Bacillus* spp., followed by clostridia and pseudomonads (1).

Some additional ingredients are included to reduce the microbial load of cooked meats; these are preservatives such as nitrite and salt. In general, the levels of nitrite and salt used are enough to inhibit the outgrowth of endospores, including those of *Clostridium botulinum*, but have little effect on the growth of the vegetative spoilage organisms, or on the growth rate of non-halotolerant pathogens, including some *Salmonella* serovars.

However, some cooked meat products (e.g. emulsion sausages and patés) contain high enough concentrations of nitrite and sometimes salt, to inhibit both the outgrowth of endospores and the growth of vegetative microorganisms. However, it should be noted that in some cases nitrite is only added at a level sufficient to obtain the desirable pink pigmentation (2).

Some cooked products do not contain nitrite, and also have low salt levels, e.g. roast beef and some types of sausage, and for these products sodium lactate has been used as a preservative. Sodium lactate (2 - 3%) can prevent toxin production by *C. botulinum*, and also inhibit Gram-positive bacteria such as *Listeria monocytogenes*. In general, lactate is effective against bacteria that are able to grow at pH 6.5, and a_w level of 0.95 or below. However, yeasts are resistant even at levels of 10% or above (2).

In addition to the preservatives, other ingredients have demonstrated antimicrobial properties such as phosphates, various antioxidants, and herbs and spices, although these properties, whilst often demonstrated in laboratory experiments, appear limited in actual cooked meat products (2).

2.4.2 Cooking

The most significant process stage in the production of cooked meats is the cooking step. A wide variety of cooked meat products exists, but many of these share a similar basic technology (Figure 2.1). However, the type of cooker/cooking used and the heat treatment applied varies according to the specific product and its expected shelf life.

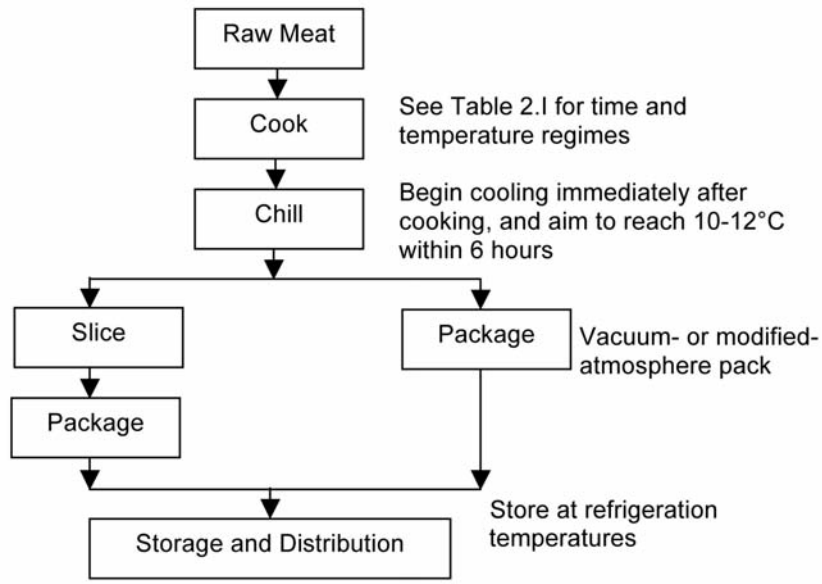

Fig.2.1. Production of cooked meats

2.4.3 Cooker/cooking methods

There is a variety of cooking methods in use commercially. The most common are detailed below.

2.4.3.1 Hot air

This is a traditional means of cooking, and so is widely used. Ovens may be used on a batch basis, or continuously with conveyors moving the product through. The air is heated to about 200 °C (392 °F) either by gas burners or by electricity (2).

2.4.3.2 Steam

Saturated air is heated to 100 °C (212 °F) to generate the steam. This type of cooking does not allow browning of meats, for example, because the temperature does not rise above 100 °C (212 °F), and so it is often used in conjunction with hot air or radiant heating to impart the characteristic appearance of the meats. Steam cooking can be efficiently used with meat stews/soups where direct steam injection can occur (2).

2.4.3.3 Hot water

Hot water at temperatures of about 100 °C is used either to cook stews, soups or pie fillings, where the water is consumed as part of the product, or to cook sausages or joints that have been pre-packed in plastic bags, or patés that are contained in metal moulds (2).

2.4.3.4 Radiant heating

This is essentially a grilling process using the infra-red part of the spectrum (2).

2.4.3.5 Microwave

Microwaves are a form of radiation (frequency 500 - 5,000 MHz), and cause cooking by making water molecules in the product oscillate (move), and the resultant friction generates the heat. Commercially, it has been used to cook products such as sausages, paté and bacon. Also, it could be used post-cooking to further pasteurise in-pack ready meals (this would eliminate any post-heat process contamination) (2).

2.4.4 Heat treatment

Heat treatment varies according to the expected shelf life of the product. Some meats are pasteurised; other canned uncured meats are subject to a botulinum cook (i.e. heated to a core temperature of 115 - 120 °C (239 - 248 °F) to inactivate spores of *C. botulinum*), and some canned products (e.g. soups - where meat is a minor component) are heated to higher temperatures to inactivate thermophilic spores (3).

Typical examples of pasteurised meats are cooked sausages, patés, and similar products, as well as a range of ready-to-eat (RTE) meals. Some require cooking before serving; others can be eaten without further heat treatment (3).

Traditionally, the cooking process has been based upon attaining a 10^7-fold reduction in *Salmonella* numbers (4). Examples of internal time/temperature combinations that meet these criteria have been determined.

The United States Department of Agriculture (USDA) has time/temperature cooking requirements to ensure destruction of the microorganisms in the coldest area of re-cooked roast beef. These requirements do not include additional thermal destruction associated with the long heating and cooling periods during the cooking process. Thus, the critical limits for time/temperature are conservative and ensure that *Salmonella* do not survive if the limits are met. Furthermore, the minimal internal temperatures for cooking uncured meat and poultry products

(Food Safety and Inspection Services (FSIS) Policy Book or Notice), established by USDA-FSIS (Table 2.I) often exceed what is needed for food safety.

TABLE 2.I
USDA minimum internal temperature for cooking uncured meats and poultry products

Products	Internal Temperature °C (°F)	Time
Partially cooked products (comminuted)	62.22 (144) 62.78 (145) 63.33 (146) 64.44 (148) 66.11 (151)	5minutes 4 minutes 3 minutes 2 minutes 1 minute
Pork, ham, sausage and bacon in microwave	76.67 (170)	
Cooked beef and roast beef	54.44 (130) 62.78 (145)	121 minutes <1 second
Baked meat loaf	71.11 (160)	
Pork (suspected trichinae)	48.89 (120) 60 (140)	21 hours <1 second
Baked pork cut	76.67 (170)	
Cooked poultry products	71.11 (160)	
Jellied chicken loaf	71.11 (160)	
All foods previously served and cooled within 2hours that are reheated	73.89 (165)	

Source: FSIS Policy Book

In the United Kingdom (UK), the Government has issued advice on the safe handling of cooked meats, and these guidelines recommend that meat is cooked sufficiently to ensure the destruction of *Escherichia coli* O157, *Salmonella* and *Listeria*. It is recommended that the centre of the meat reaches a core temperature of at least 70 °C (158 °F) for 2 minutes or equivalent (see Table 2.II) (5).

TABLE 2.II
Equivalent heat treatments (5)

Temperature (°C)	Time
60	45 minutes
65	10 minutes
70	2 minutes
75	30 seconds
80	6 seconds

Some ready meals are produced using the "sous-vide" process. This is where products are part cooked, vacuum-packed, then given an extensive in-pack pasteurisation. The meals are then stored under refrigeration until use, at which stage they are either warmed or fully reheated to complete the cooking. There is some concern over these products in relation to the potential growth of *C. botulinum*, and so guidelines concerning the heat treatment and storage of such products have been issued (see Table 2.III) (2).

TABLE 2.III
Guidelines for the heat treatment and storage of sous-vide products (6, 7)

Sous-vide Advisory Committee	
Heat treatment [(a)]	80 °C/26 minutes 85 °C/11 minutes 90 °C/4.5 minutes
Storage	Up to 8 days at 4 °C (or less)
Advisory Committee for Microbiological Safety of Foods	
Heat treatment [(b)]	80 °C/129 minutes 85 °C/36 minutes 90 °C/10 minutes
Storage	10 days max.

(a) based on 6D reduction of *C. botulinum* type E
(b) based on 6D reduction of *C. botulinum* type B

Canned uncured meats and meat products (such as stews, meats in gravy, meat pastes and meat soups) are given a botulinum cook, which is to give a 12D reduction of *C. botulinum*. This is necessary because although good-quality meat normally contains less than 1 *Clostridium* spore/g, and fewer than 10 *Bacillus* spores/g, head meat, diaphragm, and pig skin, which may be used in certain low-quality meat products, frequently carry higher spore counts (3).

2.4.5 Chilling

The USDA-FSIS has established time/temperature requirements for cooling and storing refrigerated products. Cooling procedures require that the product's internal temperature does not remain between 26.6 and 54.4 °C (80 and 130 °F) for more than 1.5 hours, or between 26.6 and 60 °C (80 and 140 °F) for more than 5 hours. Cooling procedures for meat and poultry products with intact muscle (roast beef) require that product chilling should be initiated within 90 minutes of the cooking process. Products shall be chilled from 48.9 to 12.8 °C (120 to 55 °F) in not more than 6 hours. Products should not be packed for shipment until it reaches 4.4 °C (40 °F). The recommended refrigerated storage temperature for

products is 4.4 °C (40 °F) (internal temperature for a storage period exceeding one week it is 1.7 °C (35 °F)).

Cooked meats can be chilled by chilled water or air. If chilled water is used it should be chlorinated and of potable quality to avoid recontaminating the cooked product (2).

2.4.6 Slicing

It is important to prevent recontamination of the meat after cooking, and so the processing facility should be designed so that cooked meat is not handled in the area or with the equipment used for preparing the raw meat. This should be the rule in a commercial processing facility. To control the risk of recontamination of the cooked meat, considerable attention must be given to training personnel and to cleaning and disinfecting the equipment.

Pasteurised meats may be sliced after heat processing (e.g. roast pork, roast beef) and the microflora on the slices will consist of the flora on the equipment used for slicing. This process normally occurs in a room at approximately 10 °C; thus the flora will be psychrotrophic and, even if the sliced cooked product is stored at low temperatures, rapid growth can occur (3).

2.4.7 Packaging

The final stage in the production of cooked meats that can affect the microflora is that of packaging.

Vacuum-packaged (VP) and modified-atmosphere-packaged (MAP) meats are of great interest, owing to their microbiological quality and longer shelf life. Lactobacilli or *Brochothrix thermosphacta* or both are the predominant organisms found in VP meats that undergo long-term refrigeration spoilage (8, 9, 10). However, other microorganisms can be found and may predominate, based on several factors. These factors include product pH, percentage nitrites, type of VP film, initial load of psychrotrophic bacteria, and type of product (raw, cooked, and partially cooked).

The microorganisms that dominate VP fully or partially cooked meats are different from those that dominate VP raw meats. Gill & Newton (11) found that *Yersinia enterocolitica, Serratia liquefaciens, Shewanella putrefaciens*, and *Lactobacillus* spp. were the predominant flora in VP dark, firm, dry meat held at 2 °C (35.6 °F) for 6 weeks. In VP products, when high concentrations of nitrites are present LAB were predominant, while the growth of psychrotrophic Enterobacteriaceae and *B. thermosphacta* is inhibited (12, 13). On the other hand, with low concentrations of nitrites, the growth of *B. thermosphacta* is not

inhibited. LAB seem to be less significant in the spoilage of VP luncheon meat than *B. thermosphacta* (14). Both lactobacilli and *B. thermosphacta* have been found to be responsible for off-odours and off-flavours produced in VP cooked meat products. In a comparison study of *B. thermosphacta* and lactobacilli as spoilage organisms of VP sliced luncheon meats, *B. thermosphacta* grew with no lag phase, reached 10^8 cells/g after 9 days and developed off-flavours 2 - 3 days later. On the other hand, lactobacilli reached 10 cells/g after 12 - 20 days and developed off-flavours 11 - 21 days later.

Volatile compounds such as acetoin and diacetyl produced by *B. thermosphacta* have been found to be responsible for a sharp off-flavour in VP corned beef (15). Several studies have shown that acetoin is the major volatile compound produced by *B. thermosphacta* in raw and cooked meats in oxygen-containing atmospheres (16, 17).

MAP frankfurters, stored at -4 to 7 °C (24.8 to 44.6 °F) for 49 days showed a decrease in the levels of *B. thermosphacta* (from 48 to 5%), and an increase in the levels of lactobacilli (from 6 to 94 - 96%) (18).

In addition, meat sandwiches, stored in 50% $C0_2$/50% air at 4 °C (39.2 °F) had an increased acceptable shelf life of up to 35 days (19). Therefore, both VP and MAP affect the make-up of the microflora, and also its growth.

2.5 Spoilage

It has been previously mentioned that there are various types of cooked meat and poultry products available, and that they may be uncured, cured or fermented. The following section will primarily describe the spoilage organisms associated with uncured cooked products. However, some information on cured and fermented meats is described but greater detail is provided in later chapters.

Some of the spoilage microorganisms associated with processed and cooked meat products are summarised in Table 2.IV. These spoilage microorganisms are usually found in and on processed meat products.

2.5.1 Cooked, uncured products

The spoilage flora associated with the cooked product will largely depend on the heat treatment received.

2.5.1.1 Pasteurised meats

Insufficient heat treatment of cooked meats could result in survival of *Enterococcus* and heat resistant strains of *Lactobacillus*. These bacteria may grow

at temperatures below 5 °C (41 °F), although most growth is achieved at temperatures above 7 °C (44.6 °F). This spoilage is rare and is typified by weak acid production and slime formation. Occasionally, gas is produced by hetero-fermentative species of *Lactobacillus* (2).

TABLE 2.IV
Type of spoilage in various cooked meat products

Meat product	Microflora	Type of spoilage
Processed meats (cured, ham)	*Pseudomonas* *Achromobacter* *Bacillus* *Lactobacillus*	Underneath the casing of the meat Souring
	Streptococcus Clostridia	Gasiness: gas pocket in muscle, "gassy or puffers", greenish discolouration
	Microbacterium *Micrococcus* Yeasts	Slimy spoilage
Bacon	*Streptococcus* Moulds	Slime formation, white to coloured spots or discolourations
	Lactobacillus *Micrococcus* *Streptococcus*	"Cheesy" sour-scented, and putrefied off-odour in VP bacon
Cooked, cured frankfurter sausages	*Micrococcus* Yeasts Microbacterium	Surface slime
	Lactobacillus	Gasiness in VP frankfurters
	Leuconostoc *Micrococcus*	Chill rings (fading of cured colour at outer surfaces)
	Lactobacillus	Greenish discolouration
Canned meats (sterile)	*Bacillus* spores *Clostridium* spores	Thermophilic growth due to inadequate cooling; survival and growth due to excessive initial number
Canned meats (semi-preserved)	*Streptococcus*	Souring and discolouration
	Bacillus *Clostridium*	Liquefaction of gelatin and proteolysis at <10 °C

Cooked meat products provide excellent conditions for the growth of bacteria, moulds and yeasts; hence the problem of recontamination. Handling after the heat treatment can contaminate the surface of the meat with mesophilic Gram-negative rods (e.g. Enterobacteriaceae), Gram-positive cocci and rods, yeasts or moulds. Contamination with bacteria is from hands and surfaces in contact with the cooked meats, and with moulds from the air (3).

Serratia may be present and red-pigmented strains can cause colourful spoilage of cooked meat stored under warm conditions (15 - 18 °C (59 - 64.4 °F)). Also,

formation of a purple slime on roast beef is indicative of *Chromobacterium violaceum* (probably *Janthinobacterium lividum*) spoilage, but this condition is rare (2).

The surface of roast poultry and meat joints cooked in hot air ovens can exhibit extensive drying, and this can lead to mould growth during extended storage at low temperatures. A number of genera may be involved, but the most common are *Penicillium, Mucor, Aspergillus, Cladosporium,* and *Thamnidium*. Yeasts, especially *Candida, Monilia,* and *Torula*, may also be present in significant numbers on both meat and fat surfaces (2).

The meat filling of most pies, puddings and sausages is protected from post-process contamination by the pastry and sausage casing, and the only microorganisms present are those that survive the cooking process. This may be the heat resistant *Enterococcus* or *Lactobacillus* strains, but a greater problem is the potential for germination and outgrowth of spores of *Bacillus* and *Clostridium* spp. If the meat is stored under warm conditions (>20 °C (68 °F)), where spores, especially *Clostridium* spores, may germinate and grow, the spoilage will be putrid, and gas may develop (3).

Meat pies, puddings and sausages are all susceptible to mould growth on their outer surfaces. Spoilage of sausages by yeasts can occur, and a wide range of yeasts may be involved, including *Candida, Cryptococcus, Debaryomyces, Galactomyces, Pichia, Rhodotorula,* and *Torulaspora* (2).

In preparations composed of several ingredients (e.g. in RTE meals), or where ingredients are added after cooking (e.g. paté decorations), the spoilage microflora can come from the other ingredients, e.g. from vegetables, spices, and condiments.

Ready meals may contain significant numbers of *Bacillus* spp. derived from non-meat ingredients such as flour, starches and spices, and herbs and fruit decorations of paté may cause mould and yeast spoilage, respectively (2).

2.5.1.2 "Botulinum cooked" meats

Spoilage of canned, uncured meats results either from survival of heat resistant spores or post process leakage of the cans. As previously mentioned, cans are treated to give a 12D reduction in *C. botulinum*; however, some strains of *Clostridium sporogenes* and putrefactive anaerobes have a greater heat resistance than *C. botulinum*. Therefore, if present, these heat resistant spores may survive, and storage at high temperatures could result in germination and outgrowth. For example, *C. sporogenes*, a proteolytic gas former, can cause cans to swell or even burst (3).

In order to combat this problem, many heat processes are more severe to ensure the destruction of these heat resistant spoilage organisms.

Canned meats can be spoiled by microorganisms entering the can through pinholes (either from a can defect or from external corrosion), through faulty seams (from improper manufacture or closure), or through the mastic that seals the ends to the body (3).

2.5.2 Cooked, cured products

Spoilage in cooked and processed meats such as frankfurters, sausage, bologna, and luncheon meat is generally of three types:

1. *Slimy spoilage*: Microflora isolated from the slime is yeast and LAB such as *B. thermosphacta, Weissella viridescens, Lactobacillus,* and *Enterococcus*. Slimy spoilage starts as discrete colonies on a moist surface, which later form a uniform layer of greenish slime, and usually occur on the surface of the casing.

2. *Souring*: Souring results from the growth of lactobacilli (*B. thermosphacta*) and enterococci underneath the casing of meats. These organisms utilise the lactose and other sugars present and produce acids. Mould and bacterial spoilage usually occur when the products are moist and stored in high humidity.

3. *Discoloration*:

Green: This occurs due to exposure of anaerobically stored processed red meat products (frankfurters, cured and VP meats) to air. Greening is caused by formation of hydrogen peroxide (H_2O_2), which reacts with nitrosohaemochrome and produces a greenish oxidised porphyrin (20). Also, greening can be caused by the growth of H_2O_2 producers such as *Lactobacillus fructovorans* and *Lactobacillus jensenii. W. viridescens, Leuconostoc, Enterococcus faecium,* and *Enterococcus faecalis* can also produce greening in the interior core of products such as smoked pork loins and frankfurter sausage by lowering the oxido-reduction potential (ORP), which allows the accumulation of H_2O_2 (21). The green product is not known to be harmful to humans.

Yellow: Yellow discoloration of cooked cured meat products is caused by the growth of *Enterococcus casseliflavus*. It grows between 4.4 - 10 °C (40 - 50 °F) and can survive a cooking temperature of 71.1 °C (160 °F) for 20 minutes. It requires between 3 and 4 weeks to produce small spots on products, which are fluorescent under long-wave ultra-violet (uv) light, (22) and are usually found in VP luncheon-style meat.

Smoking and brining procedures used in preparation of bacon and cured hams protect these products from most microbial spoilage. In modern commercial methods of curing, bacteria play little or no role in the physical, chemical and organoleptic changes that occur. Cured hams undergo a type of spoilage different from that of fresh or smoked hams. Fermentation of sugars pumped into the hams with the curing solution by the natural flora and spoilage microorganisms (lactobacilli) produces sours of various types in cured hams (22). Bacterial counts during curing are generally held to moderate numbers, and the subsequent heating in the smokehouse kills most of the bacteria present except for the small number of spores that may be present. The smoke also contains bactericidal chemicals, which are deposited on the surface of the product, and the heat causes development of a surface layer of coagulated protein. LAB (lactobacilli) is the main flora of commercial curing brines. Lactobacilli are mainly located below the surface of the product, while yeast and micrococci are mainly present on the surface (Table 2.IV). Salt, nitrite and mild heat usually used in cured ham have an inhibitory effect on the growth of Gram-negative bacteria, and make it more favourable to the growth of Gram-positive bacteria, yeast and moulds. Curing salts tend to reduce the amount of heat necessary to produce stable canned pork luncheon meat.

2.5.3 Fermented products

Specific types of sausages such as salami, Cervelat, Thuringer and Lebanon bologna are fermented by adding a starter culture into the sausage emulsions to produce the desired flavour. The starter culture should be a homofermentative lactic acid bacterium (*Lactobacillus* and *Pediococcus cerevisiae*) that is relatively tolerant to nitrite and salt and capable of producing a low pH. Fermented sausages produced without the use of starter cultures have been found to contain high counts of *Lactobacillus plantarum* (24). In commercially produced fermented sausage, the absence of starter cultures led to high LAB counts, ranging from 10^7 - 10^8 /g and a low range of pH 4.0 - 4.5, whereas the use of starter cultures such as *P. cerevisiae* produced a highly desirable fermented summer sausage (25, 26) with a final pH in the 4.6 to 5.0 range. The use of manufactured pure starter cultures led to a decrease in production times and produced a uniform and safer product (27). *Micrococcus auranticavus*, in addition to lactic acid culture, has also been used in the production of fermented sausages. *M. auranticavus* reduces nitrates to nitrites and produces catalase that benefits the lactic culture (28).

2.6 Pathogens: Growth and Survival

An estimated range of 12.6 to 81 million cases per year of foodborne illness occurs in the USA, causing a great economic loss of $1.9 to 8.4 billion (29). Foods of muscle origin such as meat and poultry are sensitive to contamination and support growth of microorganisms involved in spoilage and foodborne illness. In fresh, unprocessed products organisms multiply rapidly; thus various methods of

processing and preservation are being applied to extend product shelf life. Consumers have begun to prefer meat and poultry products or entrées subjected to minimal processing and preservation treatments. Also, chicken and turkey are being marketed in a variety of forms including turkey bologna, salami, turkey roasts, turkey ham, breaded chicken patties, chicken frankfurters, and breaded precooked pieces. While these products still offer palatability and convenience, their safety can be compromised by increased potential for growth of pathogenic microorganisms because of further processing.

Microbiological hazards can be divided into three types: bacterial, viral and parasitic (protozoa and worms). Table 2.V lists hazardous microorganisms that are of most concern, as classified by the International Commission on Microbiological Specifications for Foods (ICMSF) 1986 (30), according to severity of risk. As shown in Table 2.V, the limited-spread pathogens cause common foodborne outbreaks; however, their subsequent spread is limited. The severe pathogens are a significant hazard; also, moderate-spread pathogens could cause severe illnesses in certain susceptible populations.

TABLE 2.V
Microbiological pathogens of concern in HACCP (30)

Severity	Aetiologic agent
Limited spread (moderate)	*Bacillus cereus* *Campylobacter jejuni* *Clostridium perfringens* *Staphylococcus aureus* *Vibrio cholerae*, non-01 *Vibrio parahaemolyticus* *Y. enterocolitica* *Giardia lamblia* *Taenia saginata*
Extensive spread (moderate)	*L. monocytogenes* *Salmonella* spp. *Shigella* spp. enterovirulent *E. coli* *Streptococcus pyogenes* Rotavirus Norwalk virus *Cryptosporidium parvum* *Ascaris lumbricoides*
Severe	*Salmonella typhi* *Salmonella paratyphi* A, B *Shigella dysenteriae* *C. botulinum* type A, B, E, F Hepatitis A and E *Brucella abortis* *Brucella suis* *V. cholerae* 01 *Trichinella spiralis*

Bacterial hazards in cooked meat and poultry products can result in either foodborne infections or foodborne intoxications. A foodborne infection is caused by ingesting a cross contaminated or recontaminated cooked meat or poultry product containing a sufficient number of pathogenic microorganisms; whereas a foodborne intoxication results from ingestion of pre-formed toxins produced and excreted by bacteria (31, 32). The following summary discusses the notable characteristics of the various foodborne bacterial pathogens of concern to cooked meat, poultry and products, and their mechanism of control. Table 2.VI summarises various foodborne pathogens, the severity of disease caused by these bacteria, and the general conditions required for their control.

2.6.1 Bacillus cereus

Outbreaks of *B. cereus* food poisoning have been implicated in meat products such as meat loaf. Strains of diarrhoeal syndrome *B. cereus, Bacillus licheniformis* and *Bacillus subtilis* have also been responsible for food poisoning following consumption of paté, liver sausage, and hot-eating pies that had been held for long periods at high temperatures. Emetic syndrome *B. cereus* food poisoning may have resulted from consumption of a shepherd's pie, but growth probably occurred in the potato-based topping rather than in the meat filling (2). Proper holding and cooling of cooked meat and poultry products prevent the growth of *B. cereus*. Hot cooked meat and poultry should be held at temperatures greater than 63 °C until served. Cooked foods should be cooled within 2 - 3 hours of cooking to a temperature below 15 °C. Retorting, thermal processes and irradiation destroy spores of *B. cereus*.

2.6.2 Campylobacter jejuni

The most common foods implicated are uncooked or insufficiently cooked meat and poultry products, and foods that have been cross contaminated. Since *C. jejuni* is sensitive to freezing, drying, and acidic conditions, these can be used as barriers to growth. Proper sanitation practices, including good personal hygiene, conscientious temperature control, and the avoidance of cross contamination, are also effective control measures.

2.6.3 Clostridium botulinum

Foods implicated in botulism outbreaks are improperly processed, usually home-canned, low-acid foods. Also, MAP and sous-vide products offer a potential risk because of reduced oxygen. Commercially canned meats have rarely been involved in outbreaks of botulism, although canned liver paste has caused cases of botulism in Canada and New York, where cans contained both type A and type B spores (33). Severe under-processing, although the heat processing was originally designed to give a botulinum cook, was the reason for the outbreak.

Processing conditions had been changed and resulted in a process that was inadequate to destroy *C. botulinum*. In addition, beef stew containing type A toxin resulted in two cases of botulism with one death (34).

TABLE 2.VI
Major foodborne pathogens of concern to cooked meat and poultry products

Microorganism	Source in nature	Foods implicated	Prevention
Salmonella spp.	Water, domestic and wild animals; also, humans, especially as carriers	Poultry and poultry salads, meat and meat products	Avoid cross contamination, refrigerate food, cool cooked meats and meat products properly; avoid faecal contamination by practising good personal hygiene
L. monocytogenes	Humans, domestic animals, fowl, soil, and water	Raw meat sausages; raw and cooked poultry, chicken and other meats and meat products	Cook foods to proper temperatures; avoid cross contamination
Staphylococcus intoxication	Hands, throats and nasal passages of humans; also animals	Warmed-over foods, ham and other meats	Avoid cross contamination from bare hands; practise good personal hygiene; ensure proper heating and refrigeration of foods
C. perfringens toxin-mediated infection	Humans (intestinal tract), animals and soil	Meat that has been boiled, steamed, braised, stewed, or roasted at low temperature for a long period of time, or cooled slowly before serving	Use careful time and temperature control in cooling and re-heating cooked meat, poultry and products
B. cereus intoxication	Soil and dust	Meat loaf	Use careful time and temperature control and quick chilling methods to cool food; hold hot food above 63 °C, reheat leftovers to 74 °C
C. botulinum intoxication	Soil, water	Improperly processed canned goods of low acid foods, beef stew, meat/poultry loaves	as above
C. jejuni	Domestic and wild animals	Poultry, pork, beef, and lamb	Avoid cross contamination; cook poultry, pork, beef and lamb thoroughly
E. coli O157:H7 infection/ intoxication	Animals, particularly cattle; humans (intestinal tract)	Raw and undercooked beef and other red meat	Cook beef and red meats thoroughly; avoid cross contamination; use safe food and water supplies; avoid faecal contamination by practising good personal hygiene

Addition of nitrite to cured meat products has been found to inhibit the growth of *C. botulinum* and prevent growth of germinating spores (35). The addition of sorbic acid (0.2%) in combination with nitrite (156 ppm) in chicken meat frankfurters resulted in significant extension of the time needed for botulinum toxin to develop during elevated temperature abuse (27 °C) (36). Furthermore, the effect of sorbic acid (0.2%) in combination with nitrite (156 ppm) increased at pH values <6.20 (36). Sodium lactate delays toxin production by *C. botulinum* in cooked-in-bag turkey products (37). Preventive and control measures include heat treatment and addition of salt and nitrites to cured processed meats. Foods that have been heated should not remain in the temperature danger zone (7.2 - 60 °C), and VP products should be properly refrigerated below 7.2 °C.

2.6.4 Clostridium perfringens

C. perfringens spores on raw meat and poultry can survive normal heat and cooking processes and can continue vegetative growth when food reaches a suitable temperature. The pathogen grows well in cooked meat and poultry dishes, stews, or gravies that are kept warm, as well as battered poultry products (fillet sandwiches, nuggets). Holding these cooked foods at warm temperatures provides an anaerobic nutrient-rich environment, which favours the growth of *C. perfringens*. Slow cooling of large cooked joints of meat and whole large chickens or turkeys is a common cause of *C. perfringens* food poisoning, particularly in catering operations. The usual cause is inadequate or insufficient refrigeration and it has been known for small catering operations to cool and store cooked meat at ambient temperature (2).

Microbiological safety of these products is important, particularly in regard to pre-cooking and final preparation at fast-food restaurants or at home. Thus, after cooking, rapid uniform cooling to 10 °C within 2 - 3 hours is critical in controlling the growth of these pathogens. During food service, hot meat should always be held at temperatures of 63 °C and above, and cold meat at 4 °C and below. Reheated foods should reach an internal temperature of 75 °C or more before service. However, researchers found that heating pre-cooked chicken by microwave to temperatures of 84 °C was not adequate to eliminate the possibility of *C. perfringens* food poisoning (38).

2.6.5 Escherichia coli O157:H7

Raw or undercooked ground beef and red meats (lamb and pork) are the main vehicles of transmission. A major outbreak in the UK was linked to the consumption of cooked meats; over 400 people showed symptoms and there were a number of fatalities. The organism has no unusual heat resistance, with D-values at 57.2, 60, 62.8 and 64.3 °C of 270, 45, 24, and 9.6 seconds, respectively (39). The organisms can survive in ground beef during frozen storage and grow rapidly

between 30 and 42 °C. *E. coli* O157:H7 has the ability to survive the fermentation, drying and storage steps during the production of fermented dry sausages, and an acidified food product (40). Control of *E. coli* O157:H7 through Good Manufacturing Practices (GMP) in processing of meat requires adequate cooking and avoidance of product recontamination by unsanitary equipment or infected food handlers. Cooking temperatures should be monitored and proper sanitation and hygiene procedures followed. Ground beef should be cooked to a minimum internal temperature of 71.1 °C (160 °F). Cooking certain cuts of beef to at least 54.4 °C and holding it there for 2 hours, or cooking thoroughly are the best control measures to use. Leftover red meat should be reheated to 73.9 °C to kill *E. coli* O157:H7.

2.6.6 Listeria monocytogenes

Although raw meat and poultry products are the main foods implicated in listeriosis outbreaks, cross contamination of cooked foods is a major concern. Schoeni *et al*. (1991) studied the rates of thermal inactivation of *L. monocytogenes* in beef and fermented beaker sausage (41). Results indicated that *L. monocytogenes* was about four times more heat resistant than *Salmonella* in ground beef. The use of bacteriocinogenic (*Lactobacillus sake* ctc494) or pediocin-producing starter cultures in dry fermented sausages are found to inhibit the growth of *L. monocytogenes* (42, 43). During slaughtering and processing, product flow must guard against potential cross contamination between the raw and finished product. A major outbreak of foodborne listeriosis occurred in France in 1992 (44). The major causes of contamination identified were contact of cooked products with solid surfaces, cross contamination between raw and cooked channels, and the inadequacy of cleaning and disinfection procedures. *L. monocytogenes* grew on several processed meat products (ham, bologna, wiener, fermented dry sausages and cooked roast beef) within 4 weeks of refrigerated storage, and grew exceptionally well on sliced chicken and turkey (45). These results indicate the importance of preventing post-processing contamination of *L. monocytogenes* in a variety of RTE meat and poultry products. Any aspect of cross contamination including humans, equipment, water and air should be carefully analysed to eliminate potential cross contamination. A rigid environmental sanitation programme encompassing employee hygiene, separation of raw and RTE processing areas, and sanitation should be developed.

2.6.7 Salmonella spp.

Salmonella is an important public health problem both in the United States and throughout the world. There are over 1,600 *Salmonella* serotypes; however, in the US, *Salmonella typhimurium* is the most common serotype associated with human salmonellosis, followed by *Salmonella heidelberg* and *Salmonella enteritidis* (46).

There are several factors contributing to outbreaks of salmonellosis in cooked meat and poultry; these include inadequate cooling of cooked meats, inadequate time and/or temperature during cooking or heat processing of meats, cross contamination from raw chicken, turkey, and meats to cooked meat or poultry products, and cross contamination from unsanitised equipment and kitchen surfaces to cooked foods. As an example, cooked roast beef has been implicated as a major vehicle associated with outbreaks of salmonellosis due to a combination of the above factors (47). RTE and pre-cooked products such as turkey roasts and turkey rolls, which may be consumed without reheating, could be a source of salmonellosis. However, Wilkinson *et al*. observed that *Salmonella* spp. were not recovered from defrosted turkey roll cooked at an internal temperature of 71 °C in an oven set at 107 °C (48). Commercial water cooking procedures were capable of eliminating the pathogen from encased turkey rolls cooked at 73.9 °C for 5½ hours, or 85 °C for 4½ hours (49). Five *Salmonella* cultures were isolated from baked chicken pot pies following the manufacturer's instructions. In this case, baking time and temperature recommended by the manufacturer were not sufficient to kill all pathogens (50).

The type of cooking procedures applied to the meat and poultry products has a great effect on destroying the pathogens. Drying and semi-drying processes in fermented sausages and pre-cooked roast beef minimised the destroying effect of cooking. Thus, in sausage manufacturing, fermentation should be rapid and must decrease pH and a_w, and cause a build-up of a microbial flora to compete with or inhibit the growth of *Salmonella*.

2.6.8 Staphylococcus aureus

Staph. aureus was recovered from prepared chicken burgers, chicken loaves, and roasted chicken inoculated with known concentrations of *S. typhimurium* and *Staph. aureus* cooked in a microwave oven, or by conventional methods (51). Contamination with *Staph. aureus* is most often caused by human contact with hands after the product is cooked. Sliced cooked meat products are especially prone to contamination with *Staph. aureus* from human sources. Often outbreaks involve large numbers of cases and are associated with special events such as weddings, dances and christening parties. This is because of the advance preparation of meals, with sliced cooked meats held at ambient temperatures for extended periods (2). Staphylococcal food poisoning occurs when cooked food is held at high temperatures, which allows growth to a million cells/g of food, and potential toxin production. Cooked foods must be cooled and refrigerated rapidly to 7.2 °C (45 °F) or below to minimise the growth of *Staph. aureus* and its toxin production. The growth of *Staph. aureus* and its toxin in fermented sausages such as Lebanon bologna, summer sausages and pepperoni can be controlled by a rapid drop in pH (<4.6) during fermentation. Addition of solutes or drying foods to lower the a_w (a_w 0.85) of the meat product will inhibit the growth of *Staph. aureus* and enterotoxin production.

2.6.9 Trichinosis

The causative agent of infection known as trichinosis is a small nematode *T. spiralis* that is only 1.5 - 5.0 mm in length in the adult form. Humans may become infected by eating the larvae in raw or partially cooked pork products and game animals. Another concern is cross contamination resulting from equipment like sausage grinders, which may mix raw pork into non-pork foods. The only sure safeguard against trichinosis is the cooking of pork until it reaches an internal temperature of 65.6 or 76.7 °C if cooked in a microwave oven. Freezing will also kill larvae found in pork products that are less than 6 inches thick when they are stored at -15 °C for 30 days, -34.4 °C for 20 days, and -134 °C for 12 days. Equipment such as sausage grinders and utensils should be washed, rinsed and sanitised after working with raw pork.

Cervelat, summer sausage, Thuringer and Lebanon bologna are semi-dry sausages. These sausages contain 50% moisture and are usually heated to an internal temperature of 60 - 67.78 °C (140 - 154 °F) during smoking, which is enough to destroy *T. spiralis* larvae. However, a sausage-associated outbreak of trichinosis occurred in Illinois, and 23 persons became ill through eating improperly prepared home-made fermented sausage, where a cooler smoking temperature was used (52).

2.7 Published Microbiological Criteria

Table 2.VII
Published microbiological criteria for pre-cooked turkey rolls (53)

Test	Result (counts/g)
Total count	$<10^3$
Coliforms	$<10^2$
Enterococci	$<10^2$
Salmonella	Absent
C. perfringens	Absent

Table 2.VIII
Published microbiological criteria for ready-to-eat meats (54)

Food Category	Microbiological quality (cfu/g)			
	Satisfactory	Acceptable	Unsatisfactory	Unacceptable /potentially Hazardous*
	Aerobic plate count †30 °C/48 hours			
1	$<10^3$	10^3 - $<10^4$	$\geq 10^4$	NA
2	$<10^4$	10^4 - $<10^5$	$\geq 10^5$	NA
1	$<10^5$	10^5 - $<10^6$	$\geq 10^6$	NA
4	$<10^6$	10^6 - $<10^7$	$\geq 10^7$	NA
5	NA	NA	NA	NA
1-5	Indicator organisms‡			
Enterobacteriaceae	<100	100 - 10^4	$\geq 10^4$	NA
E. coli (total)	<20	20 - <100	≥100	NA
Listeria spp. (total)	<20	20 - <100	≥100	NA
1-5	Pathogens			
Salmonella	ND in 25 g			Detected in 25 g
Campylobacter spp.	ND in 25 g			Detected in 25 g
E. coli O157 and other VTEC	ND in 25 g			Detected in 25 g
V. cholera	ND in 25 g			Detected in 25 g
L. monocytogenes	<20**	20 - <100	NA	≥100
Staph. aureus	<20	20 - <100	100 - $<10^4$	$\geq 10^4$
C. perfringens	<20	20 - <100	100 - $<10^4$	$\geq 10^4$
B. cereus and other pathogenic *Bacillus* spp.#	$<10^3$	10^3 - $<10^4$	10^4 - $<10^5$	$\geq 10^5$

*Prosecution based solely on high colony counts and / or indicator organisms in the absence of other criteria of unacceptability is unlikely to be successful
†Guidelines for aerobic colony counts may not apply to certain fermented foods – for example, salami. These foods fall into category 5. Acceptability is based on appearance, smell, texture, and the levels or absence of indicator organism or pathogens
‡On occasion some strains may be pathogenic
If *Bacillus* counts exceed 10^4 cfu/g, the organism should be identified
**Not detected in 25 g for certain long shelf life products under refrigeration
NA – Not applicable

1 Meat products covered include beef burgers, meat pies (steak and kidney, pasty), pork pies, sausage rolls, scotch eggs and pasties
2 faggots, kebabs, meat meals (shepherds/cottage pie, casseroles), poultry (unsliced), sausages (British)
3 Sliced meats - beef, haslet, pork, poultry
4 brawn, sliced meat (cooked ham, tongue), tripe and other offal
5 ham – raw (Parma/ country style), salami and fermented meat products, sausage (smoked)

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3. DRIED MEATS, POULTRY AND RELATED PRODUCTS

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3.1 Historical Data

Drying is considered to be the oldest and most important method of food preservation employed by man. Preservation of meat by drying results in the reduction of available water (moisture content) to levels at which the growth of microorganisms or the activity of the enzymes responsible for the breakdown of meat is inhibited. The art of drying meat (mostly sun-drying) originally was from necessity, so that meat could be held for extended periods for later consumption. It has been suggested that sun-drying of meat dates back several centuries in the Middle East and Southern Asia where higher temperatures prevail and refrigeration was unknown (1). Scientific principles of drying meat were not applied until the early part of the twentieth century when ways to improve quality and extend shelf life of meat products were first introduced.

3.2 Types and Classification of Dried Meats

Dried meats can be defined as whole muscle and/or ground-and-formed meat products that have been subjected to dehydration (i.e. removal of water), which results in unique sensory properties and increased stability. Depending on the degree of dehydration, dried meats can be conveniently classified as

(i) *low moisture meats* - products that contain less than 25% moisture and have a water activity (a_w) level of <0.60

(ii) *intermediate moisture meats* - products that contain less than 50% moisture and have a a_w level of 0.60 to 0.85

As the production of dried meats is often combined with other processing technologies such as curing (i.e. addition of salt or curing salts) and/or fermentation, several different types of meat products can be classified as dried

meats (Table 3.I). Moreover, variations in the type of meat used, as well as in the processing conditions applied give rise to a large diversity of products (2, 3, 4, 5). This chapter will mainly focus on the technology and the microbiological quality and safety of low, and intermediate moisture 'whole muscle' meat snacks such as jerky, while more detailed information on cured (i.e. ham) and fermented meats, are to be found in other chapters.

Table 3.I
Types and classification of dried meats

Dried-meats	Process involved	Typical products
Dry and semi-dry sausages (mainly fermented)	Fermentation and ripening	Pepperoni, Genoa, Italian & Greek salami, summer sausage, Lebon bologna, etc.
Dried whole muscle products (mainly dry-cured)	Curing (i.e. addition of salt, nitrite etc.) and drying	Parma & country hams, Prosciutto, Pancetta, Cappocola, etc.
'Whole muscle' meat snacks	Drying (occasionally marinated)	Jerky, biltong, pastruma or bastruma, droëwors, tasajo, pemmican, meat (mainly beef) nuggets, steak tenders, etc.

3.3 Initial Microflora

Dried whole muscle meat snacks are commonly manufactured from lean meat (i.e. skeletal muscle tissue) of beef; however; almost any meat, including pork, poultry, camel, water buffalo, game (deer, antelopes), venison, elk, caribou, and kangaroo can be used for the production of high quality products (2, 3, 5). The use of lean meat in the manufacture of high quality products (i.e. jerky) is important as a high fat content (>35%) significantly reduces the drying rate of the meats and causes detrimental effects (i.e. melting, blocking pores in the dried part of meat) on the quality of the final products (6). Moreover, the microbiological quality of the raw meat used significantly influences the microbial quality and stability of the finished product (7). The initial microbial population of raw meat is highly variable (see Chapter 1: Chilled and Frozen Raw Meat, Poultry and their Products, in this book), and strongly depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature, and other conditions of storage (8). During drying most of the initial microbial population of raw meat will be inhibited; however, since the process is not lethal *per se* (9), many microorganisms may be recovered from dried meats, especially if low-quality raw materials are used, and if the manufacturing process is not properly controlled (7).

3.4 Processing and its Effects on the Microflora

3.4.1 Traditional methods

3.4.1.1 Sun-drying

Sun-drying is the traditional method of preserving meats and is characterised by direct solar radiation and natural air circulation on the product. This type of preservation was probably developed in dry climates to produce products such as *charqui* and *came secca* in South America, and *biltong* in South Africa, where dried meats were the major source of animal protein. Large quantities of meat are still sun-dried in many areas (of Africa) using traditional techniques or improved approaches such as *solar-drying* (Figure 3.1). Solar-drying is the improved version of the sun drying technique and is based on indirect solar radiation (10). Specifically, solar-drying is performed in closed systems (consisting of a solar collector and a meat drying chamber) with a specific micro-climate (controlled air circulation; temperature of 30 to 55 °C; air humidity of 20 to 60%) that favours the fast evaporation of moisture from meat (10, 11).

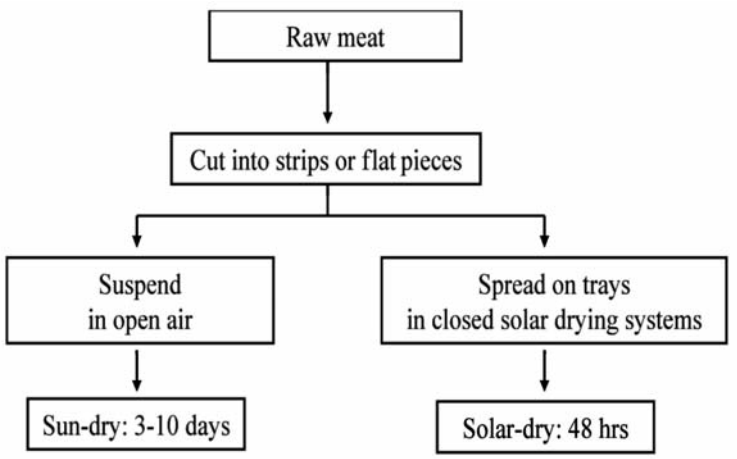

Fig 3.1. Typical traditional and improved techniques for the production of dried meats

Typically, for the production of dried meats using these traditional methods (sun-drying and solar-drying), the raw meat is usually cut into narrow strips or flat pieces that are then either suspended in the open air (for sun-drying) or spread on drying trays and placed in drying chambers (for solar-drying) (Figure 3.1). The duration of the drying process ranges from 2 days for solar-drying to up to 10 days for meats drying in the open air; it is highly dependent on temperature, humidity and the size of the pieces of meat. In particular, the use of thin and well separated

pieces of meat accelerates the drying process, whereas the use of large or closely packed meats increases the duration of drying and increases the likelihood of formation of wet spots. By the end of drying process the meat has a typical moisture content of 12 to 18% (a_w 0.5 - 0.6) and is ready for consumption.

3.4.1.2 Modern methods

In industrialised countries, substantial quantities of dried meat are produced with the use of specifically designed dehydrators that operate under well controlled conditions (12). Cabinet dryers, otherwise called *air-dryers* and *freeze-dryers* are among the most common dehydrators used for the production of dried meat (12). Each process uses a different technique: air-drying is based on evaporation, whereas freeze-drying is based on sublimation. More specifically, during evaporation the water content of raw meat is reduced as the heat applied converts water from its liquid phase to the gaseous phase (vapour). During sublimation, reduction of water content occurs on frozen meat by the direct transfer of the solid phase of water (ice) to vapour.

The differences in the principles of the applied techniques obviously result in differences in the production processes (Figure 3.2). Regardless of the drying technology, the first step in the production process of dried meats is to defat the meat. In most industrialised countries, the meat is then cooked in order to minimise case hardening, thus improving quality (6). The extent of cooking is important, since overcooking degrades the connective tissue while undercooking decreases the rate of dehydration. Moreover, the United States Department of Agriculture (USDA) has proposed cooking/heating as a lethal performance standard for the production of certain ready-to-eat (RTE) products, including dried meats such as jerky (13, 14). Such standards require that the time and temperature used in making jerky ensure the safety of the product (13, 14, 15).

In the case of air-drying, the cooked meat can either be minced into small cubes and transferred direct to the air-dryer, or cooled and refrigerated before drying (Figure 3.2). Mincing of meat in small pieces provides a large surface area to volume ratio that results in the reduction of the drying duration. Drying temperature is known to significantly influence the quality of dried products (6). Drying of meat in systems that operate under low temperatures (<50 °C) is generally less costly, but requires more time and can cause damage when the water content of meat is low. High-temperature (>80 °C) drying systems are generally more conventional, as the drying time is reduced to approximately 2 hours.

Before packaging, air-dried meats with a final moisture content of 4 to 8% can sometimes be compressed in order to reduce volume and facilitate handling. This is usually achieved by pressing (*ca*. 20,000 kPa) the meat into cans or moulds to form blocks. Dried meats must be then packaged with moisture-impermeable films, in order to prevent moisture uptake or high humidity during storage.

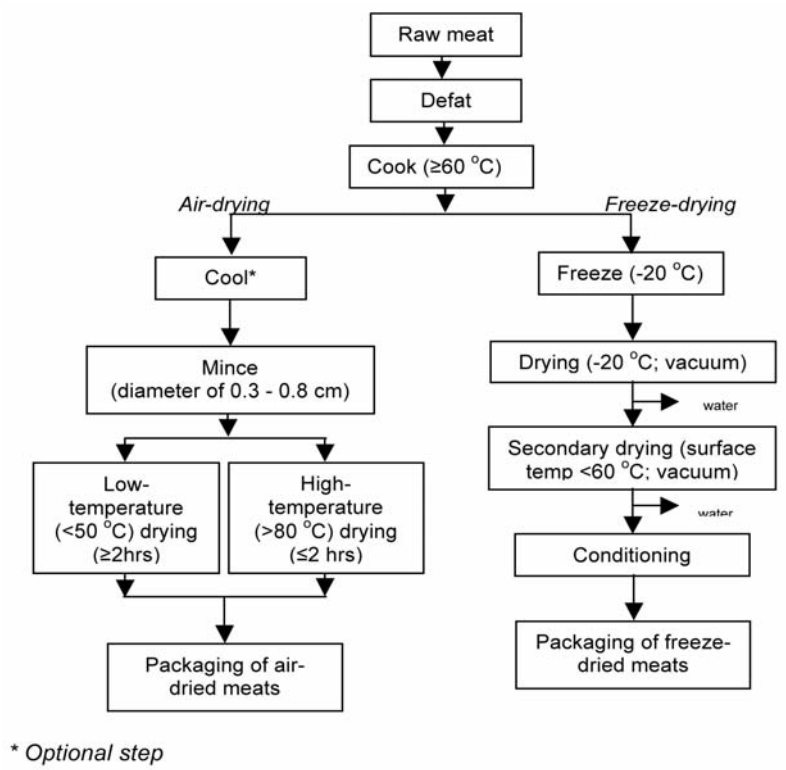

Fig. 3.2. Industrial production of dried meats

During the production of freeze-dried meats, the pre-cooked meat is drained and cooled prior to freezing in a wind tunnel or a blast freezer. Freezing of meat is usually achieved at -20 °C in order to ensure that the majority of water is converted to ice (Figure 3.2). Frozen meat is then placed into a chamber under vacuum where the temperature is raised (>60 °C) prior to extraction of frozen water. Residual water (unfrozen) is removed either by desorption or secondary drying. This step completes the separation of water from the meat and results in drying. This process can take several hours (even days) to complete as gradual drying is desired to maintain the structure of the meat. After drying, the pressure is equalised with nitrogen and the meat is conditioned to achieve an even distribution of the residual water (Figure 3.2). During freeze-drying, sub-zero temperatures must be maintained until the reduction of water content. As this process is not lethal (9), the microbiological quality of the final product is highly dependent on the initial microbial flora of the meat and on any bacterial post-process (after drying) contamination. As with air-dried meats, the final products must be packaged with moisture-impermeable films to avoid moisture uptake during extended storage.

3.4.1.3 Production of dried meats with combined processing technologies

Apart from air-dried and freeze-dried meats, a large variety of dried meats are produced with combined or modified processing technologies. Such modifications have been made either to improve the quality and safety as well as the nutritional status of the product, or to increase consumer acceptance (16). Modifications include (i) the pre-treatment (i.e. seasoning, dry-curing, marinating) of meat and (ii) the addition of humectants (i.e. salt, sugar, glycerol etc.) in combination with a decrease in drying time (intermediate-moisture meats). For example, for the production of biltong, a well known RTE dried meat originating from Africa, the raw meat is cured for several hours (<12 hours) in a salt/pepper mixture, and then dipped in a vinegar solution prior to drying. Production of pastruma, another RTE dried meat product originating from Asia, is characterised by a series and repeated lengthy (2 - 3 days) applications of salting, drying and spicing, prior to final drying. Moreover, processing of jerky is known to include marinating (salt, sugar and flavouring ingredients), heating, and drying; however, some interventions, mainly heat and antimicrobial, have been shown to increase safety (2, 3, 4, 16, 17, 18, 19).

3.5 Spoilage

Spoilage of dried meats is unusual and is mainly due to insufficiencies or defects in the manufacturing processes. For example, prolonged drying of large pieces of meat may result in the formation of wet spots in which microbial growth of Enterobacteriaceae and staphylococci is likely to occur. Moreover, undercooking of the meat decreases the rate of dehydration, thus resulting in the possible occurrence of wet spots. In addition, packaging of dried meat in high-permeability films will result in the uptake of water and the subsequent growth of moulds and yeasts during storage (20). Hence, the microbiological quality and stability of the dried meat depend entirely on the removal of moisture during the drying step, and on the a_w of the final product and the relative humidity (RH) at which the dried meat is stored. It is therefore essential to ensure that drying of meat is done at a constant rate, and that the moisture content of dried meat at any stage during processing and storage is below the minimum levels for microbial growth (Table 3.II).

Table 3.II
Minimum a_w levels for the growth of microorganisms frequently isolated from meat, poultry and related products (Based on: 7, 21, 22)

Organisms		Water activity (a_w)
Bacteria	*Aeromonas hydrophila*	0.97
	Bacillus cereus	0.93
	Campylobacter jejuni	0.98
	Clostridium botulinum A & B	0.94
	Clostridium botulinum E	0.97
	Clostridium perfringens	0.97
	Escherichia coli	0.95
	Listeria monocytogenes	0.92
	Pseudomonas spp.	0.97
	Salmonella spp.	0.94
	Staphylococcus aureus	0.86
Yeasts	*Candida zeylanoides*	0.90
	Debaryomyces hansenii	0.83
	Saccharomyces cerevisiae	0.90
	Zygosaccharomyces bacilli	0.80
	Zygosaccharomyces rouxii	0.62
Moulds		
	Aspergillus flavus	0.80
	Aspergillus niger	0.77
	Aspergillus ochraceus	0.77
	Aspergillus parasiticus	0.80
	Aspergillus restrictus	0.71
	Penicillium expansum	0.83
	Eurotium amstelodami	0.70
	Eurotium repens	0.71

Indeed, it has been well established that a moisture content of 20% inhibits most bacteria, yeasts, and moulds, but a level of 15% is needed to inhibit xerotolerant species of fungi (20). Moreover, meat with a high fat content (40%) must be dried to about 9% moisture in order to ensure inhibition of mould growth, since higher levels of moisture (12%) may result in the growth of mould after several weeks (20). It has also been shown that storage (at 20, 28 and 37 °C) of dried meats at high RH (90%) results in the faster growth (5 - 6 days) of mould compared to storage at 75% RH (36 - 40 days) (20).

In general, the main spoilage organisms associated with low-moisture dried meats are moulds, and in particular those capable of growing at low a_w (e.g. *Eurotium* spp). Spoiled intermediate moisture meats are often dominated by moulds, yeasts, lactobacilli, as well as by staphylococci. Studies on the microflora of biltong have shown that xerophilic moulds, for example *E. amstelodami,*

E. repens, Eurotium chevalieri, Eurotium rubrum, Aspergillus versicolor and *Aspergillus sydowii* are the main spoilage species of this type of dried meat (23). In addition, several yeasts including *D. hansenii, C. zeylanoidese,* and *Trichosporon cutaneum* are also frequently isolated from intermediate moisture meats such as biltong, and cabanossi (23, 24). Other studies have shown that staphylococci and micrococci are often present in intermediate moisture meats such as pastrima and cecina (25, 26, 27).

The ability of chemical inhibitors to inhibit the growth of *A. niger* and *Eurotium* spp. has been evaluated. Results have shown that the inhibitory effect increased with decreasing a_w levels (0.85>0.88); a combination of potassium sorbate and calcium propionate was found to be more effective as it inhibited the growth of the moulds for 25 weeks.

Production of low and/or intermediate moisture dried meats of high microbiological quality should be formulated correctly in order for deficiencies in processing to be avoided. It is also essential that the preservative system and temperature control should remain stable during storage, as moisture migration could lead to areas of localised high a_w levels, which in turn result in a heterogeneous structure.

3.6 Pathogens: Growth and Survival

During the manufacture of dried meats, the rate at which the water content of meat is reduced, as well as the a_w of the dried meat, will significantly affect the survival and proliferation of foodborne microorganisms. Many pathogenic bacteria, such as *Salmonella, Staph. aureus*, clostridia etc., that originate from raw meat or are contaminants during preparation may survive the drying process. A recent report indicated that for the period of 1990 - 1999, the cumulative prevalence of *Salmonella* and *L. monocytogenes* in dried meats (jerky) produced in federally inspected plants in the United States of America (USA) was 0.31 and 0.52 %, respectively (29). In addition, documented outbreaks due to the consumption of dried meats (Table 3.III) including jerky, pastruma, and biltong (29, 30, 31) increases the concern over pathogen growth and survival in dried meats.

In an outbreak of salmonellosis associated with the consumption of beef jerky, the investigation found that the producer used a dry oven set at 82 °C to dry the jerky to a crumbling state (approximate a_w 0.3). However, the actual temperature of the oven was found to be only 30 °C, suggesting that *Salmonella* was able to become very resistant to dry heat (19). In response to this event, the USDA's Food Safety and Inspection Service (FSIS), based on research experiments, issued lethality compliance guidelines for manufacturers in order to ensure the safety of the product (14, 15). Subsequently, a significant body of research has been initiated in order to evaluate the efficacy of drying procedures to inactivate or

control pathogenic bacteria. *Salmonella*, enterohaemorrhagic *Escherichia coli* (EHEC), *Staph. aureus* and *L. monocytogenes*, are among the pathogens that have been extensively studied; thus in the following paragraphs, a more detailed description of the risks associated with their survival on dried meats is provided.

Table 3.III
Gastrointestinal outbreaks in the USA associated with the consumption of dried jerky (Based on: 29, 30, 31, 33)

Causative agent	Year	Confirmed cases
Salmonella thompson	1966	39
C. botulinum type F	1972	5
Staph. aureus	1982	15
Salmonella cerro	1985	44
Salmonella montevideo	1986	5
Salmonella newport	1987	4
S. newport	1988	23
Salmonella spp.	1995	93
Staph. aureus	1995	5
Escherichia coli O157:H7	1995	11
Trichinellosis	1995	10
Salmonella kiambu	2003	6

3.6.1 Salmonella spp.

Under sufficiently rigorous drying conditions, growth of Enterobacteriaceae is inhibited and inactivation is also likely if the a_w of dried meat is reduced below the growth-limiting levels. However, several published reports on foodborne outbreaks involving dried meats in the USA (Table 3.III) and around the world (31) are indicative of the survival potential of *Salmonella* in dried meats (29, 30, 31).

Early research on the effects of time and temperature on *Salmonella* reduction in jerky recipes revealed that drying of whole beef strips for 10 hours at 60 °C (the traditional drying process) reduces the levels of *Salmonella* from 5 log to undetectable levels (17). However, this traditional drying process (10 hours/60 °C) is insufficient for pathogen destruction in jerky made from ground meats; it has been concluded that ground-meat jerky should be oven heated to 71 °C prior to drying to ensure microbiological safety (34). It has also been found that oven heating jerky strips to 71 °C after drying enhances both safety and consumer acceptance (16). Recently, it has been shown that drying for 1.5, 2.5, or 3.5 hours to a target temperature of 80.7 °C with constant smoke, at an initial average RH

of 63.1% to a final average RH of 20.9%, resulted in >7.3 log cfu/ per strip (>6.9 log cfu/g) reduction in levels of *Salmonella* (35).

Calicioglu *et al*. (36) studied the effects of acid adaptation and organism variation in marinade composition on the survival of *Salmonella* used to contaminate beef jerky during storage. The results showed that the modified marinades and low a_w of the products provided antimicrobial effects against *Salmonella*, and that previous acid adaptation of the inoculum did not increase survival of the pathogen.

The fate of acid-adapted *Salmonella* serovars has also been evaluated during the manufacture and vacuum-packaged storage (7 days at 20 - 22 °C) of biltong and droëwors. It has been shown that the highest reduction occurred during storage, ranging from >2 to <4 log for droëwors and biltong, respectively (37). A higher level (4.7 - 5.1 log) of destruction was observed when the fate of acid-adapted *Salmonella* serovars was evaluated during the manufacturing process of basturma (38). It was speculated that the dry-curing and rinsing steps employed in making basturma increased the process lethality relative to that for biltong and droëwors (37).

3.6.2 Staphylococcus spp.

Although the presence of *Staph. aureus* in dried meats has been considered to be of no significance (6), dried meats have been implicated as a cause of staphylococcal intoxication (Table 3.III). This has been attributed to the ability of *Staph. aureus* to outgrow indigenous bacteria during the manufacture of dried meats and produce heat-stable enterotoxin (6), probably due to its tolerance of salt and reduced a_w. Therefore, studies have been conducted to evaluate the fate of *Staph. aureus* during the manufacture processes of dried meats.

Studies on the effect of time and temperature on *Staph. aureus* reduction in jerky have revealed that drying of whole-strip beef jerky for 8 hours at high temperatures (ranging from 60 to 68.3 °C) significantly reduces pathogen levels compared to drying at lower temperatures of 53 °C (for 4hours), and 48 °C (for 4 hours) (39, 40). Despite recovering viable *Staph. aureus*, it has been concluded that drying of jerky at higher temperatures is safe as long as wholesome meat is rapidly dried (39). Recent evidence shows that the drying process used in making biltong and droëwors caused a significant decrease in numbers of *Staph. aureus*. It has been suggested, however, that additional intervention treatments and/or raw material pathogen testing should be incorporated in order for producers to meet USDA requirements for process lethality (37).

In addition, challenge testing studies on the survival of *Staph. aureus* during the storage of RTE non-acidified jerky (a_w 0.82; moisture:protein ratio of 0.8) have

shown the reduction in the levels of *Staph. aureus* during vacuum-storage (21 °C for 4 weeks) of beef jerky (38, 41). However, these studies also suggested that factors other than a_w, such as presence or amounts of spices, smoke compounds, or other ingredients, may affect the survival of *Staph. aureus* on non-acidified jerky (38).

3.6.3 Listeria monocytogenes

Despite the ability of *L. monocytogenes* to grow at low a_w (0.92), growth of *L. monocytogenes* is not expected in dried meats. However, the cumulative prevalence of *L. monocytogenes* in dried meats (jerky) produced in federally inspected plants has been found to be 0.52% (28) suggesting that the pathogen can survive the more moderate drying temperatures (approximately 60 °C) used by commercial jerky manufacturers.

In general, studies have shown that conditions controlling salmonellae and *Staph. aureus* in jerky also prevent growth of listeriae. More specifically, is has been shown that drying of whole beef strips for 10 hours at 60 °C (traditional drying process) reduces the levels of *L. monocytogenes* from at least 5 log to undetectable levels after drying (17), whereas oven heating to 71 °C prior to drying ensures the microbiological safety of ground-meat jerky (34). Furthermore, it has been shown that the use of antimicrobial chemicals or preservatives in jerky marinades improved the effectiveness of drying at moderate (60 °C) temperatures in inactivating *L. monocytogenes* (18, 35, 42). Additional studies have shown that using pre-dried marinades together with drying to a low a_w provided sufficient antimicrobial effects against possible post-contamination of acid-adaptated *L. monocytogenes* (3). It has also been shown that the number of *L. monocytogenes* is reduced (37) or even eliminated (3) during the storage of dried meats.

However, FSIS applies a ‘‘zero-tolerance’’ policy for *L. monocytogenes* in RTE meat products including dried meats such as jerky. Products that are not in compliance with this policy are considered adulterated under the provisions of the United States Federal Meat Inspection Act (United States Code). According to the new EC Regulation 2073/2005 on microbiological criteria for foodstuffs, acceptable RTE meat products are considered those that contain ≤100 cfu/g of *L. monocytogenes.*

3.6.4 Escherichia coli O157:H7

Although growth of *E. coli* O157:H7 is also not expected in dried meats, the outbreak in the mid-1990s associated with *E. coli* O157:H7 in jerky (Table 3.III) prompted federal action mandating that manufacturers of such products demonstrate a 5 log reduction of *E. coli* O157:H7 during processing.

Studies on the effect of processing conditions on the fate of *E. coli* O157:H7 in dried jerky products (2, 16, 17, 32, 34) have shown that the survival of the pathogen is highly dependent on the type (beef vs. venison), form (whole muscle vs. ground) and shape (thick strips vs. thin slices) of meat used in the jerky formulation as well as on the processing conditions applied (temperature, drying time). Specifically, it has been shown that traditional marinading of beef jerky followed by drying for 10 hours at 62.5 °C reduced the levels of *E. coli* O157:H7 by more than 5 log cfu/g (16, 17). On the other hand, Keene *et al*. (32) concluded that drying whole venison strip jerky for 10 hours at 62.8 °C was an unreliable means of eradicating *E. coli* O157:H7, whereas Faith *et al*. (2) reported that only two of eight jerky preparation procedures evaluated achieved a 5 log reduction by the time the product was judged visually dry. Similarly, it has been shown that marinating whole beef strip jerky is inadequate to destroy *E. coli* O157:H7 after drying strips for 8 hours at 62.5 or 68.3 °C (43). It has been found that marinating alone does not enhance the reduction of *E. coli* O157:H7 during drying (43, 44); however, the use of an acidified marinade may enhance bacterial destruction during drying (19). The inclusion of a pre-marination treatment with acetic acid may enhance the destruction of *E. coli* O157:H7 during drying of jerky even at high levels of contamination (45). Although discrepancies in the results of these studies may have been attributed to differences in the heat resistance of pathogen strains, these conflicting results are indicative of the need for additional studies to evaluate procedures for achieving adequate (>5 log cfu/g) reductions of *E. coli* O157:H7 during the manufacturing processing of dried meats.

3.6.5 Clostridium spp.

Apart from the above 4 major microbiological hazards, clostridia originally present on raw meat or as contaminants during preparation may survive the drying process of meats. Specifically, clostridia spores can survive cooking, freezing, and drying processes of dried meats. An early outbreak of botulism has been linked to the consumption of beef jerky contaminated with *C. botulinum*, type F (Table 3.III). It has been suggested that proper drying is required to minimise any risks posed by non-proteolytic *C. botulinum* spores (19).

3.6.6 Parasites

Trichinellosis, also called *trichinosis*, is caused by eating contaminated raw or undercooked meat of pigs and other animals infected with the nematode *Trichinella spiralis*. In 1995, an outbreak of *Trichinellosis* was linked to the consumption of home-made jerky contaminated with *T. spiralis* (Table 3.III) that survived the drying process. In the United States, pork is not generally inspected for the absence of *Trichinella;* therefore regulations stipulate that dried meats must be made from meat that has been stored frozen, or be heated to 58.3 °C (137 °F).

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4. CURED MEATS AND POULTRY, INCLUDING COOKED CURED MEATS

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4.1 Definitions

The term *cured meats* refers to meat products that have been treated with sodium chloride (NaCl) and nitrate or nitrite. The addition of the former takes place in order to enhance the flavour, and to solubilise the myofibrillar protein fraction thus improving the yield and texture of the product. Addition of nitrate or nitrite not only provides the characteristic bright red colour of uncooked cured meats and the typical pink colour of cooked cured meats, but in combination with NaCl exerts certain bacteriostatic effects against both aerobic and anaerobic bacteria.

The above-mentioned ingredients were historically the first to be used for the production of cured meats and are still the basic ones. A wide range of other ingredients, such as sweeteners, reducing agents, seasonings, flavours and polyphosphates are also used.

The taxonomy of cured meats is mainly based on the intensity of the thermal treatment that they receive; they are accordingly divided into uncooked cured meats and cooked cured meats. The latter can be further subdivided on the basis of their stability into shelf-stable cooked cured meats and perishable cooked cured meats. Cured meats can also be classified as packed sliced cured meat products; these could be either uncooked or cooked cured products.

Uncooked cured meats are whole meat cuts that are cured, drained, matured (optional) and possibly packed before distribution. The most important products in this category include bacon products and dry cured hams.

Shelf-stable cooked cured meats are shelf-stable products, where the heat treatment is the most important factor for safety and stability. After manufacturing, the products are packed in hermetically sealed containers (most often metal cans) and processed to a F_0 above 3. Mild heat treatment (max. centre temperature around 104 °C) is performed to preserve the sensory quality of the products. The most important products in this category include hams, shoulders, luncheon meats and chopped pork.

Perishable cooked cured meats (keep refrigerated) are products that are manufactured in a similar way to that for shelf-stable cooked cured meats; however, the processing temperature of these products is moderate (70 - 75 °C in order to give rise to a centre temperature of min. 68 °C). Traditionally, the products are packed in cans, but moulds and casings of nylon and polyethylene are now used to a great extent. The most important products in this category include hams, luncheon meats and frankfurters.

Packed sliced cured meat products are products that are often vacuum packed (VP) or packed in a modified atmosphere (MAP), e.g. combinations of carbon dioxide (CO_2) and nitrogen (N_2), because the colour of cured meat products will fade if oxygen (O_2) is present, which is further accelerated upon light exposure normally used during display conditions (1). Many of the above-mentioned products are sold as sliced cured meat products from chill cabinets in supermarkets. Consequently, this category of products includes both uncooked cured products (bacon, dry cured ham) and cooked cured products (ham, Bologna-type sausages, etc.).

4.2 Initial Microflora

Despite the wide variety of products obtained by curing and mixing different cuts of an animal with flavours, aromas and additives of technological importance, they share common characteristics from a microbiological point of view. As a result, the microbial ecosystem of cured meat products, whether cooked or not, and concomitantly their quality and shelf life, are related to the microbiological quality of the raw materials, product formulation, hygienic conditions of the processing plant, and storage conditions including packaging and temperature.

The microbiological quality of meat, i.e. the type and extent of contamination, is determined by the conditions under which the animals are reared and slaughtered. The abiotic environment in contact with the animal, the animal itself, in terms of hides, intestinal tract and faeces, as well as the processing equipment, including utensils and humans, are the possible sources of contamination. As a consequence, carcasses are dominated by Gram-negative rods (mostly pseudomonads) and micrococci (mainly *Kocuria* spp. and *Staphylococcus* spp.). Furthermore, Gram-negative bacteria such as *Acinetobacter* spp., *Alcaligenes*

spp., *Moraxella* spp. and members of the Enterobacteriaceae family along with other Gram-positive species including spore forming bacteria, lactic acid-producing bacteria, *Brochothrix thermosphacta*, as well as yeasts and moulds may also be present (1).

Spices may also contribute to the initial microbiota of the product mixture. More accurately, spices may confer moulds, spore formers such as *Bacillus cereus* and *Clostridium perfringens*, members of the Enterobacteriaceae family, and *Staphylococcus aureus*. Contamination with *Escherichia coli, Salmonella* spp. and *Shigella* spp. may be regarded as less likely to occur (2, 3, 4).

Curing brines, when applied, may also contribute to the initial flora of cured meats. Freshly prepared brines generally contain as many microorganisms as have managed to contaminate the constituents of the brine and the utensils used. After injection into the product or immersion of the product in them, brines become enriched with microorganisms as defined by the microbiological quality of the meat. Thus, in order to avoid cross contamination, the repeated use of either 'run-off' injection or immersion brines should be limited.

The substitution of NaCl and fat, and concomitantly the re-formulation of the respective recipes have been studied to some extent, especially in dry fermented sausages. Given their specific role in the manufacture of these products, the experimental substitution of NaCl by potassium chloride, potassium lactate, glycine, manganese chloride, calcium chloride and calcium ascorbate (5, 6, 7, 8, 9, 10, 11), and fat by inulin, dietary fibre, olive oil and soy oil (12, 13, 14, 15, 16, 17) have provided promising results regarding their application in cured meat products as well.

4.3 Processing and its Effects on the Microflora

Due to the diversity of cured meats, no common processing stage exists in the processing of all types. The processing of cured meats can most appropriately be divided into uncooked, cured meats and cooked, cured meats; this division is used in the descriptions below.

4.3.1 Uncooked cured meat products

This category embraces products such as bacon, dry-cured hams and raw sausages. These products are usually pork based and the common feature that accounts for their preservation is addition of nitrite and salt, and in some cases air drying and smoking.

4.3.1.1 Bacon

On the basis of the thermal treatment that may be applied during the production procedure, bacon products may be further subdivided into traditional bacon products, such as Wiltshire bacon, and cooked bacon products, such as Australian bacon. However, all types of bacon, for reasons of text integrity, are presented in the current paragraph.

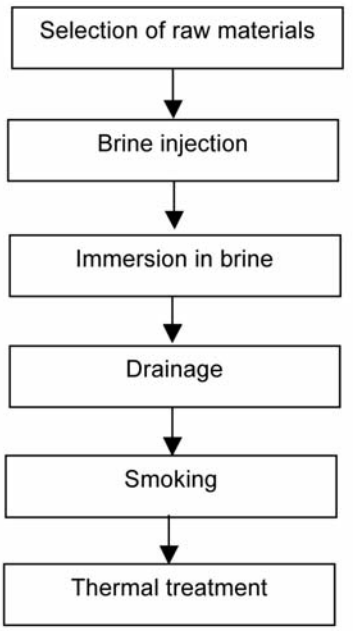

Fig. 4.1. Basic steps for bacon production

Figure 4.1 outlines the basic steps for bacon production. The production procedure begins with the selection of raw materials, the pork cut, which may vary from only the belly with the loin attached, to all cuts. Injection of the brine then takes place; the amount may range from 11% (USA bacon) to 90% (New Zealand bacon). The additives that are applied with the injection brine include NaCl, nitrite, nitrate, colour and flavour enhancers, sugars, phosphates, etc. Immersion in brine is the next step that in some bacon types is optional, in other types omitted, but in Wiltshire bacon production it is a critical step. In this case, the immersion brine contains a certain microbial consortium that not only assists in conversion of nitrate to nitrite, but it also produces desirable flavours. When this stage is applied, immersion normally takes place for 24 - 48 hours; for Wiltshire bacon production immersion takes place for 3 - 5 days at 3 - 5 °C. The meat is then dried; drying takes place at 60 - 70 °C with subsequent or simultaneous smoking, usually at the same or slightly elevated temperature. A thermal treatment usually

follows, until the core temperature reaches 45 - 76 °C depending on the type of bacon. Finally, the product is cooled to temperatures below 0 °C in order to assist slicing.

Bacon is generally characterised by slightly acidic pH values (pH 5 - 6) and a low salt level, usually below 3 - 4%. Given the high nutritional quality of meat itself, the utilisation of additional hurdles, in order to expand the shelf life and to ensure safety, is inevitable. Thus, hurdles such as refrigerated storage, VP or MAP, or even utilisation of bacteriocinogenic lactic acid bacteria (LAB) strains have been successfully applied (18). Effective inhibition of microbial growth along with retention of the proper colour are normally achieved by addition of small amount of nitrite (50 ppm) and storage at temperatures below 10 °C, given the low salt concentration (3 - 4%). Despite this, a wide range of microorganisms including *Bacillus* spp., *E. coli, C. perfringens, Clostridium botulinum, Staph. aureus* and even *Listeria monocytogenes* can be found in bacon either VP or MAP (19, 20, 21, 22). Recently, the cooling stage that follows smoking has been identified as a critical control point (CCP) in bacon production due to non-compliance with FSIS guidelines for cooling heat-treated meat and poultry products (23). Taormina and Bartholomew (24) studied the behaviour of S*taph. aureus* and *C. perfringens* during smoking and cooling of bacon and concluded that cooling of smoked bacon from 48.9 to 7.2 °C within 15 hours renders the product safe for consumption, at least as far as these two pathogens are concerned.

4.3.1.2 Dry-cured ham

The term ‘dry-cured ham’ refers to a wide variety of products that undergo lengthy drying that contributes to their preservation. Products such as Parma ham or prosciutto di Parma, Parma coppa, Black Forest ham and Serrano ham are included in this category. Figure 4.2 illustrates the basic steps for the production of dry-cured ham.

Selection of the raw materials is of vital importance; a wide range of studies exploring the properties of meat, fat, and curing agents critical for obtaining a final product of high quality (25, 26) have been conducted. The term salting refers to the application of a mixture of NaCl and nitrate, in the form of a curing salt. It may consist of one or two phases, excluding the pre-salting step that is usually applied for the production of Spanish Serrano and Iberian hams. Salting takes place at a low temperature (0 - 4 °C), at 75 - 95% relative humidity (RH), for a period ranging from 5 - 6 days (Parma ham) to 40 - 50 days (Country-style ham). A second salting phase is required for Parma and Bayonne hams; this takes place at a slightly lower RH, and requires an additionally salting period of 21 and 14 days, respectively. This is followed by the post-salting step in which the residual salt from the surface is removed by water. Rinsing is followed by drying of the exterior and the simultaneous homogeneous distribution of curing salt in the interior of the ham. This step takes place at a temperature ranging from 1 - 4 °C

(Parma ham) to 10 - 12 °C (Country-style ham), at RH ranging from 50 - 60% (Parma ham) to 70 - 95% (Serrano and Iberian hams), and for a period of 15 days (Country-style ham) to nearly 3 months (Parma ham). The smoking step that follows is usually omitted and is only applied to certain types of ham such as the American country-style, despite the fact that it has been correlated with reduced biogenic amine content (27). During the final step of production, namely ripening or drying, the characteristic flavour and texture of each ham type develops. Ripening may take place for more than a year and may be either completed at constant conditions (Parma and country-style hams) or in various phases depending on the type (28, 29, 30, 31, 32).

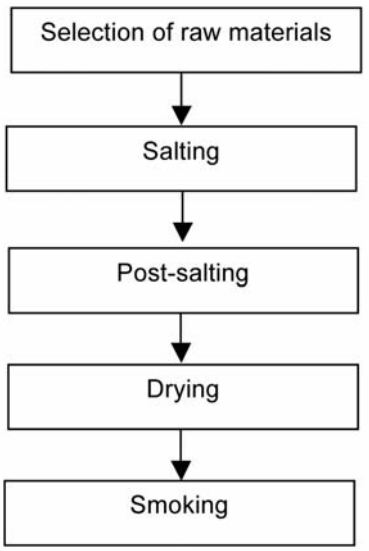

Fig. 4.2. Basic steps for dry-cured ham production

Microbial control in dry-cured hams is achieved mainly by the low a_w (<0.90), resulting from the high salt content and the lengthy drying process; and from the presence of nitrites. Dry-cured hams may be sold as whole hams or sliced, with slicing taking place either at the retail point or at the processing plant. The percentage of hams that are sliced and VP is increasing. Slicing may result in microbial contamination of the product. *Staph. aureus, Salmonella* spp. and *L. monocytogenes* have been detected in such products (33, 34) with a *Salmonella typhimurium* outbreak being associated with the consumption of cured ham (35). The fate of *E. coli* O157:H7, *L. monocytogenes, Staph. aureus*, and a mixture of *Salmonella* spp. inoculated on ham from 6 different manufacturers that was VP and stored at 2 and 25 °C was examined by Ng *et al*. (33). Despite a decrease in the population of the pathogens being observed during storage, survival was evident, suggesting that additional cooking prior to consumption may be required.

High pressure processing (HPP), intended for microbial inactivation, is among the emerging technologies that have been successfully applied in the food industry (36). In the case of dry-cured hams, a reduction of the population of spoilage microbiota along with the reduction of the safety risks associated with *Salmonella* spp. and *L. monocytogenes* has been achieved (37) especially when combined with the application of a bacteriocin such as nisin (38). Moreover, the survival of *L. monocytogenes* Scott A in sliced VP Iberian and Serrano cured hams after high pressure treatment has been studied by Morales *et al*. (39) concluding that a reduction in the population could be achieved without detrimental effects of the organoleptic quality of the product. The latter was also verified by Rivas-Canedo *et al*. (40) who studied the effect of high pressure treatment on the volatile compounds of dry-cured Serrano ham.

4.3.1.3 Raw sausages

The term non-fermented salami refers to those products that are neither acidified nor heat treated, and may be stored either at room or at refrigeration temperatures. The production procedure of such products consists of 5 steps i.e. selection of raw material, surface curing, stuffing, smoking, and storage (Figure 4.3).

Selection of the raw materials: This type of product is made of pork. The basic ingredients are coarse-minced particles of lean meat and fat, with the size varying according to local traditions.

Surface curing: Depending on the type of product, the base emulsion that acts as a binder between these particles is then manufactured. In Greek ‘Nouboulo’ sausage no base emulsion is used, instead, a curing mixture enriched with spices is spread on the surface of lean pork meat.

In Slovenian ‘Kransky’, and eastern European ‘Kabanos’ sausage, the base emulsion makes up 30 - 35% of the whole recipe.The base emulsion consists of comminuted meat, water, and additives; the percentage usage depends on the final product.

Stuffing: The meat is then stuffed into casings.

Smoking: In the case of Nouboulo, smoking occurs at at 60 - 70 °C and lasts for 5 days. On the other hand, for ‘Kransky’ and ‘Kabanos’ drying and smoking steps follow, both of which take place for nearly half an hour at 60 - 70 °C.

Storage: Nouboulo sausage can be stored at room temperature, but only for a limited time and preferably during winter as excessive drying of the product can make it unpleasant to eat. Kransky and Kabanos sausages are then subjected to a mild thermal treatment, usually with steam, until a core temperature of 70 °C is reached.

No literature is currently available concerning the microbiological properties of these products probably due to the fact that they have not been linked to any foodborne disease, and probably because of their comparative limited economic importance.

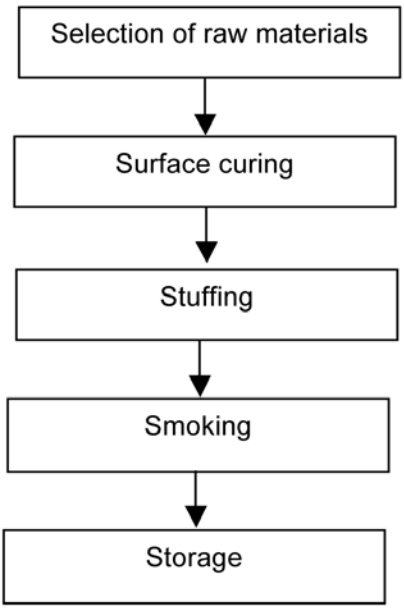

Fig. 4.3. Basic steps for the production of Nouboulo sausage

4.3.2 Cooked, cured meat products

The term 'cooked, cured meat products' refers to products that are subjected to a heat treatment that will eliminate all vegetative forms of microorganisms. Preservation of these products is generally achieved by the combined action of the heat treatment, curing agents, packaging, and storage temperature. On the basis of the heat treatment, they can be further subdivided into shelf-stable cooked cured, and perishable cooked cured meat products (Figure 4.4). After selecting the raw materials and depending upon the type of product, i.e. from whole or comminuted meat; curing and tumbling, or comminuting and mixing takes place, respectively. Canning and sterilisation will lead to the production of shelf-stable cooked cured meat products, whereas casing and cooking will lead to the production of perishable cooked cured meat products.

4.3.2.1 Shelf-stable cooked cured meat products

In this category, all canned meat products are included. These products require sterilisation due to the limited presence of other intrinsic preservation determinants. The minimum heat treatment generally applied is at least equivalent to F_0 value above 3, considered sufficient to reduce the numbers of *C. botulinum* by 12-D.

4.3.2.2 Perishable cooked cured meat products

Under the term 'perishable cooked cured meat products' the majority of cured meat products are found. The average pH of these products is around 6.0 and a_w is usually higher than 0.945; they are therefore not shelf-stable. Their common aspects are the limited shelf life (usually less than two months) and the need for refrigerated storage.

The thermal treatment that is applied to these products is sufficient to eliminate all vegetative microbial cells, but the subsequent exposure to the environment, due to post-thermal treatment operations such as peeling, slicing, and repackaging leads to the contamination of the products. As a consequence, additional preventive actions are taken to ensure safety and quality. Such measures include the incorporation of antimicrobials in the formula, VP or MAP, and refrigeration. However, quite frequently refrigeration is the only barrier for these types of products, and temperature abuse at any point of the supply chain from the processing plants to the consumer's refrigerator, could accelerate growth of pathogens, especially *L. monocytogenes*. Taking this perspective into account, the European microbiological criteria for foodstuffs lays down food safety criteria for certain important foodborne pathogenic bacteria, not only when products are placed on the market, but also during their entire shelf life (41).

4.4 Spoilage

Microbial spoilage of cured meats has been extensively studied. The specific spoilage microorganisms, their catabolic end products along with additional preventing measures are briefly discussed below.

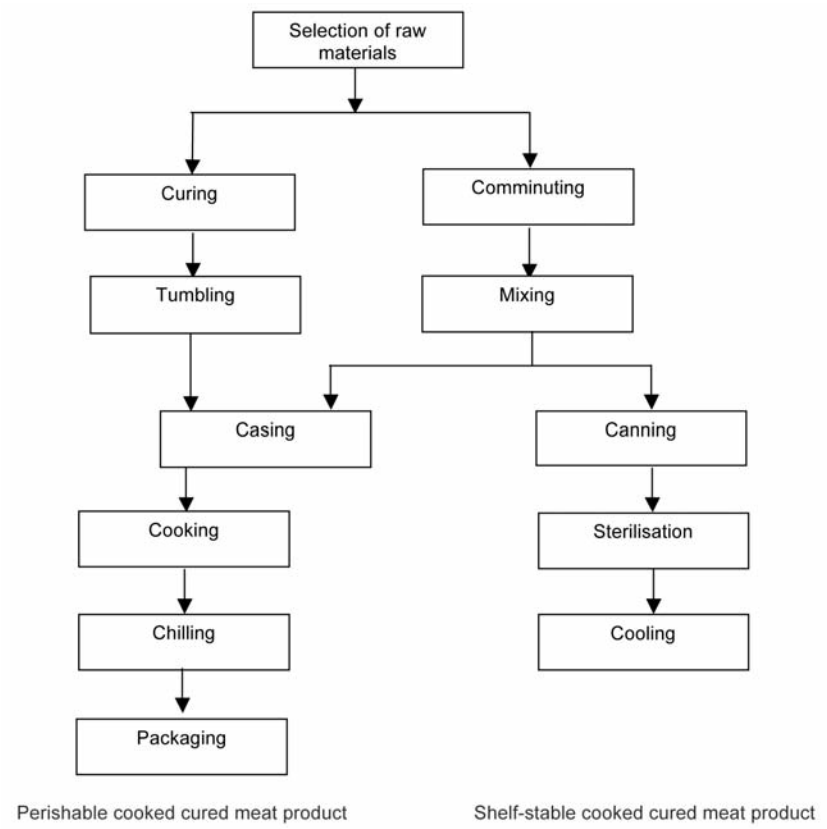

Fig. 4.4. Basic steps for the production of cooked cured meat products

4.4.1 Uncooked cured meat products

4.4.1.1 Bacon

Storage temperature, water activity (a_w) and pH of the product determines the type of microbial growth as well as spoilage perception. Souring is observed when LAB dominates the spoilage microbiota; it occurs when the pH value of the product is below 5.4 and when storage temperature does not exceed 20 °C (42). On the other hand, when Enterobacteriaceae dominate the spoilage microbiota, which occurs when the pH value of the product is above 5.5 - 5.6 or the storage temperature is 30 °C, the product becomes putrid (43). Moreover, yeasts, despite the fact that their spoilage potential is not considered to be important (44), have also been isolated from bacon and may also cause spoilage through lipolytic and proteolytic activity (45, 46).

4.4.1.2 Dry-cured ham

An important defect of microbiological nature is the so-called 'bone taint' or 'deep spoilage' that may even lead to the loss of the entire ham. This type of spoilage is mainly caused by growth of Enterobacteriaceae and Gram-positive catalase-positive cocci. Growth leads to proteolytic activity that causes a pasty texture; also generating peptides, amino acids, amines, ammonia, sulfides, alcohols, aldehydes, ketones, and organic acids with unpleasant and unacceptable off-flavours (47, 48, 49). Thus, special attention should be given to prevent contamination and growth of these microbial groups.

Finally, uncontrolled mould growth on the surface of the ham during production might cause sensory issues through proteolysis and lipolysis; it might also pose a danger for health through the production of mycotoxins. Mould growth on the surface of dry cured hams has been reported for a variety of dry cured meat products (50, 51, 52, 53, 54). Furthermore, detection of ochratoxin A (55, 56, 57) underlines the need for further study and improved control, to avoid mould growth.

4.4.2 Cooked cured meat products

4.4.2.1 Shelf-stable cooked cured meat products

These products do not usually cause any specific microbiological problems, provided that pack integrity, hygienic post-sterilisation handling, and pack damage avoidance are ensured.

4.4.2.2 Perishable cooked cured meat products

Investigation of specific spoilage organisms of perishable cooked cured meat products have been carried out by both culture dependent and independent techniques. It has mainly focused on the predominant microbiota along with the spoilage microbiota under various storage conditions; partially on the succession phenomena taking place due to various trophic relationships arising; and sometimes on any strain-specific characters that may occur. Results have shown that psychrotrophic LAB, *B. thermosphacta, Aeromonas* spp., *Shewanella putrefaciens*, members of the Enterobacteriaceae family, and occasionally yeasts make up the contaminating microbiota of chilled, sliced cooked meat products stored under VP or MAP (58, 59, 60, 61, 62, 63). *Lactobacillus sakei, Lactobacillus curvatus* and *Leuconostoc mesenteroides* are the most frequently isolated LAB species, followed by various other lactobacilli, leuconostocs, *Weissella viridescens* and *Carnobacterium divergens* (64, 65, 66, 67).

Generally, spoilage of meat products is attributed to bacterial growth. However, new processing and storage techniques aim to eliminate, or at least, inhibit bacterial growth. This might allow yeasts to grow and even prevail, thereby becoming the key spoilage agent. This is quite interesting not only from a microbiological point of view but from a food safety perspective as well, since many yeast species may utilise organic acids as carbon sources and even sodium nitrite as a nitrogen source. This could mean a loss of these hurdles that control safety and quality. Few studies have been conducted with regard to the spoilage potential of yeast in cooked cured meat products. Although a variety of yeasts have been identified across the production line of cooked ham, including *Candida zeylanoides, Cryptococcus carnescens, Cryptococcus victoriae, Debaryomyces hansenii, Galactomyces geotrichum, Leucossporidium scotti, Rhodotorula glutinis* and *Rhodotorula larynges*, none of them were detected in the final product, or even at the end of shelf life (46).

Perception of spoilage depends upon storage conditions, and the causative agent, with off-flavours, discolouration, gas production, and ropy slime production being the most frequently encountered spoilage symptoms. Normally, in VP or MAP conditions, acidity will develop mainly due to lactic acid and occasionally acetic acid production. Oxygen diffusion through packaging material promotes the production of a variety of characteristic end products such as acetic acid, hydrogen peroxide, acetoin, diacetyl, 3-methyl butanol and ethanol (68, 69).

4.5 Pathogens: Growth and Survival

4.5.1 Uncooked cured meat products

As thermal treatment is not applied in these type of products' safety is mainly ensured through the proper adjustment of NaCl and nitrite levels. Moreover, the fact that cooking usually precedes consumption renders *Staph. aureus* and *Clostridium* spp. the most important pathogens due to the production of heat stable toxins.

4.5.1.1 Staphylococcus spp.

Staph. aureus originates from the skin and the mucous membranes, especially of the nasopharyngeal region of mammals. Thus, unhygienic food handling constitutes a major risk for contamination. *Staph. aureus* is a salt tolerant microorganism capable of producing heat-stable enterotoxins and it seems to be a typical part of the microbiota of this type of product. (70). The fate of this microorganism has been studied in both bacon and dry-cured ham products. In bacon, it has been shown that it is capable of producing enterotoxins in temperature-abused precooked bacon (71, 72) whereas the cooling of smoked bacon from 48.9 to 7.2 °C within 15 hours eliminates the possibility of a food

safety hazard (24). Ng *et al*. (33) studied the fate of *Staph. aureus* in VP dry-cured (Country-style) ham slices stored at 2 and 25 °C. Although the microorganism managed to survive and in some cases grow, no enterotoxin was detected. Despite this, survival of *Staph. aureus* may pose a safety risk to consumers.

4.5.1.2 Clostridium spp.

Clostridium spp. spores may contaminate the product by a variety of sources, including raw materials, utensils and personnel. Although both *C. perfringens* and *C. botulinum* can routinely be found in both bacon and dry-cured hams (19, 33), salt and nitrite levels provide adequate control, which was also verified during storage of VP bacon at 15, 20 and 25 °C (19). Moreover, it has been shown, as in the case of *Staph. aureus*, that cooling of smoked bacon from 48.9 to 7.2 °C within 15 hours eliminates the possibility of a food safety hazard (24).

4.5.2 Cooked cured meat products

The thermal treatment that is applied during production of this type of product is adequate to eliminate all vegetative forms of microorganisms; further processing steps, such as slicing, or product mishandling would be responsible for re-contamination. Safety and shelf life of cooked cured meat products may be improved by proper post-processing handling, careful control of storage temperature and MAP. The pathogens most commonly associated with foodborne diseases from perishable cooked cured meat products are *Salmonella* spp. and *L. monocytogenes.*

4.5.2.1 Salmonella spp.

Salmonella spp. may contaminate meat during slaughter, as it is commonly carried in poultry and livestock (73). It has been implicated in several outbreaks associated with cooked cured meat products (74). *Salmonella* spp. growth on frankfurters has been studied in detail with or without the addition of various antimicrobials, including bacteriophage (75, 76, 77, 78). Consequently a variety of models for predicting growth of various *Salmonella* spp. strains have been developed (79, 80, 81, 82, 83).

4.5.2.2 Listeria monocytogenes

Despite the decline of foodborne diseases linked to these products over the last decade, mainly due to the effectiveness of the thermal treatment and a variety of additional preventive measures, listeriosis still remains a significant risk for human health linked to the consumption of cooked cured meat products, especially frankfurters, poultry-based hot dogs and deli meats (84, 85, 86, 87, 88).

Control of pathogen growth, especially *L. monocytogenes*, has been the subject of intensive research over the last decade. Growth of this microorganism during storage of cooked cured meat products has been extensively studied resulting in the development of a wide variety of quantitative mathematical models that are used not only to describe growth, but also as tools in predictive microbiology. Thus, among others, the Gompertz, Baranyi and Richards models are under constant evaluation for their robustness, fitting capacity, and ease of use for predictive purposes (89, 90, 91, 92, 93, 94, 95). The increased interest in developing safety-based shelf life date labels (96) has led to the development of modelling approaches different from the traditional ones; these new models take into consideration the time a pathogen is detected or a certain regulatory action level is reached rather than the deterioration of quality indices. Thus different handling scenarios involving various *L. monocytogenes* strains and antimicrobials are continuously under evaluation (97, 98).

Due to the importance of *L. monocytogenes* elimination from the food matrix, several physical, chemical or biological interventions have been studied, and their effect on *L. monocytogenes* growth has been demonstrated (99, 100, 101, 102, 103, 104). Among others, the efficiency of biodegradable packaging containing bacteriocins (105) as well as dipping of the product in solutions of various antimicrobials such as sodium lactate, sodium diacetate and potassium benzoate (106, 107) with or without hydrodynamic pressure processing (HDP) (108) have been exhibited.

4.5.3 Advances in pathogen control

Nowadays, research has been focussed mainly on the exploitation of natural antimicrobials in combination either with antimicrobial packaging, or HPP. As far as antimicrobial packaging is concerned, the antimicrobial effect of several plant extracts, such as cloves (109, 110), rosemary (111), sage (112), coriander (113), grape seed (114), hops (115), satureja (116, 117), thyme (118) and oregano (119) have been examined either *in vitro* or *in situ*. Results obtained were very promising, and only in some cases adjustment of the applied quantity seemed necessary in a few cases due to negative effects on the organoleptic properties of the end product. Utilisation of bacteriocins has also been extensively studied as they have been proven quite effective in controlling *L. monocytogenes* growth in meat products despite the variations in solubility, stability, and uneven distribution or even inactivation by food components (120, 121, 122).

It might be more useful to incorporate bacteriocins in a packaging film. This technology is based on the fact that contamination occurs on the surface of the products, and the incorporation of the antimicrobial into the packaging material leads to a direct contact with the food surface (123, 124). Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* that exerts rapid bactericidal effects against Gram-positive bacteria, especially against strains of *L. monocytogenes*. It

has been successfully incorporated in chitosan-coated plastic films, considerably enhancing its effectiveness against *L. monocytogenes* (125). The incorporation of enterocins with action against *L. monocytogenes* has been successfully applied and tested during refrigerated storage of cooked ham packed with biodegradable films. It has been shown that the most effective treatment for controlling *L. monocytogenes* stored at 6 °C is VP with alginate films containing 2000 AU/cm^2 of enterocins (105). Apart from the incorporation of bacteriocins, several other antimicrobials such as sodium lactate, sodium diacetate, potassium sorbate, and sodium benzoate have been successfully incorporated into packaging materials, and their effectiveness in controlling *L. monocytogenes* in meat products has been well documented (103, 107, 126, 127, 128).

A variety of processing technologies have also been examined. Among others, HDP has been shown to be capable of reducing the numbers of microorganisms in food (129). The application of a 600 MPa treatment effectively inactivated most of the microorganisms (including *L. monocytogenes, Salmonella enterica, Staph. aureus, Yersinia enterocolitica, Campylobacter jejuni,* LAB, *E. coli* and *D. hansenii*) that were inoculated on slices of cooked ham, dry cured ham, and marinated beef loin and stored at 4 °C (130).

The effectiveness of HPP has been improved by the application of purified bacteriocins. The effect of the combination of enterocin, nisin, sakacin, and pediocin with HPP (400 MPa) against *E. coli, Salmonella* spp., *L. monocytogenes, Staph. aureus, L. sakei* and *Lactobacillus carnosum* in meat homogenates (131), and the combination HPP (400 MPa) with nisin against *Salmonella* spp. and *L. monocytogenes* in sliced cooked ham (132) have been studied. The benefits of combining nisin and HPP in chicken meat at 350 - 450 MPa and in sausages at 600 MPa have also been exhibited (133, 134). Other antimicrobials, for example potassium lactate in cooked ham, have also shown a synergistic effect when combined with HPP at 400 MPa during refrigerated storage (132). The combination of nisin, potassium lactate and HPP was successfully used for controlling *Salmonella* spp. and *L. monocytogenes* growth during storage of cooked ham for three months. Effective control of HPP-resistant *Staph. aureus* took place when pressurisation was combined with low temperature and nisin (135). On the other hand, during evaluation of the listericidal activity of sodium diacetate and pediocin on frankfurters in combination with HDP, no synergistic effect between pediocin and HDP for inhibition of *L. monocytogenes* was reported, thus indicating the need for further evaluation of other antimicrobials intented to be used in conjunction with HDP treatment for synergistic inhibition of *L. monocytogenes* during storage at 4 °C (108).

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5. FERMENTED MEATS

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5.1 Historical Data

Fermentation and drying are probably the most ancient ways of meat preservation. Indeed, the first document referring to meat curing is considered to be that of Homer. In Odyssey (about c.1000 – 900 BC) he describes a rather primitive type of sausage as "...smoked goat paunches filled with blood and fat offered as a hors d'oeuvre..." By the fifth century BC production of salted or cured meat products had become commonplace and the Romans had small meat shops operating under strict sanitary control. The manufacturing methods were - and indeed still are in some countries and areas - a craft.

5.2 Types of Fermented Meats

Fermented meats can be defined as meat (e.g. beef, pork, chicken) products that have been subjected to the action of specific microorganisms (mainly lactic acid bacteria (LAB) and staphylococci) or tissue enzymes that results in unique and distinctive meat properties such as flavour, palatability, colour, microbiological safety, and tenderness. Generally, fermented meats are further classified into:
(i) *fermented sausages* - products made from comminuted meat stuffed into casings, and
(ii) *unground fermented meats* - products made from entire meat cuts (i.e. ham).

The microbiological stability and organoleptic properties of fermented sausages are mainly due to fermentation carried out by microorganisms, whereas the characteristic sensory properties of unground meats are due to the action of salt, curing agents and proteolytic muscle enzymes only (1). This chapter will mainly focus on the technology, and the microbiological quality and safety of

fermented sausages, while more detailed information on unground meat products are to be found in previous chapters (see Chapter 4. Cured Meats).

Fermented sausages are meat products that are manufactured with chopped and/or minced meat and fat, mixed with salt, spices and - in most cases - curing agents (nitrite, nitrate, ascorbate), and carbohydrates, filled into casings then ripened, cured, and in some cases smoked. These products, due to bacterial action, reach a pH of 5.3 or less and the drying process removes 15 - 50% of the moisture resulting in a moisture to protein ratio (M/P) of no greater than 2.3:1.0. Although their manufacture is usually considered to entail three main steps - formulation, fermentation, and ripening/drying (2) - variations in the type and amount of raw materials, manufacturing conditions (temperature, duration of fermentation, and ripening period) give rise to a wide range of products.

Consumer eating habits and product costs are among the factors that account for the selection of the meat species used in the production of fermented sausages. Thus, a mixture of pork and beef is usually used in some countries (e.g. Germany and Greece), whereas in other countries (e.g. Hungary and Italy) sausages are manufactured only from pork (3). Turkish 'soudjouk' are made from beef only, often in conjunction with fatty tissue from selected sheep breeds. In high-quality fermented sausages, firm pork back fat is frequently used in preference to other fatty tissues which might produce less organoleptically acceptable products. Addition of carbohydrates to the mixture allows sufficient pH reductions, however, the amount added may vary according to consumer preference, and the desired characteristics of the final product. Generally, southern European technology produces sausages with higher pH (5.2 - 5.8) values than sausages from northern European or the United States of America (USA) which have a pH between 4.8 - 4.9 (3).

5.2.1 Classification of fermented sausages

Classification of fermented sausages may vary depending on the kind of product and their country of origin (4). Fermented sausages are usually arranged into several groups based on the main preservation practices of fermentation and drying. Based on the degree of drying, fermented sausages are often subdivided into: dry, semi-dry and undried. A classification in which the time for fermentation and ripening is considered has also been proposed (5). Classification criteria have also been based on moisture content, protein content, M/P ratio, weight loss, etc. Additional criteria for classification include the casing diameter, the degree of comminution of the ingredients, the spices, seasonings, and other non-meat ingredients used. Surface treatment (e.g. smoking, mould growth) is also used for the classification of fermented sausages (6). In the USA, fermented sausages are divided into two categories: dry and semi-dry or 'new condition' sausages (7). Originally these were defined in terms of the weight loss that occurred during processing, or the final water content of the product; however, since the initial

water content of the sausage preparation will vary according to the type and quantity of meat tissues used, protein ratio as an alternative system of classification is used.

From a food microbiologist's point of view, fermented sausages are best subdivided on the basis of water activity (a_w), and the microbiological stability of the products (Table 5.I) (1). Specifically, low-acid long-ripened products with a_w of 0.90 and below that are microbiologically stable without refrigeration are called *dry sausages*. Short ripened products that have a a_w of 0.95 - 0.91 are termed semi-dry sausages and depending on the product type, should be stored below 10 - 15 °C. Products that have not been ripened or dried to any significant extent are termed *undried sausages* and have a limited shelf life even under appropriate refrigeration.

5.3 Initial Microflora

The appropriate choice of raw meat materials and other non-meat ingredients (i.e. curing salts, nitrite, carbohydrates, spices, and starter cultures) will aid in the selection of the proper microbiota of the final products and is thus critical for its quality and safety.

5.3.1 Raw meat materials

Lean meat that is mammalian (sometimes poultry) skeletal muscle tissue, and firm porcine back fat are the raw materials most frequently used for the production of fermented sausages. Most fermented sausages are produced using porcine meat, however; bovine meat has been frequently used as a supplement in many fermented meat products (8).

Chilled meat (tempered to *ca*. -4 °C) and frozen (≤18 °C) fats are usually mixed in a ratio of 2:1 (9). The selection of raw meat materials is based on the microbiological quality, visual fat content, and the pH (<5.8). Moreover, fat with a high melting point and a low content of unsaturated fatty acids (<12% polyunsaturated fatty acids) and peroxides (indicating a oxidation status) are needed for the production of dry fermented sausages that will be stored for long term (preventing the diffusion of fat from sausage skin).

Table 5.I
Classification of fermented sausages (6, 11, 63, 102, 104)

Type of fermented sausages	Geographic area	Sausages	Processing parameters			Product characteristics[a]	Storage characteristics
			Fermentation	Ripening/Drying	Smoking / Cooking		
Dry	Southern Europe	Italian salamis (Milanese, Calebrese), Saucissons secs, Spanish chorizos	18 - 24 °C; 3 days	12 - 15 °C; 4 - 6 weeks	Yes / No	pH 5.1 - 5.5 a_w 0.85 - 0.86 M/P[b]:1.6 - 1.9	<25 °C
		Greek salamis (aeros)	20 - 24 °C; 7 days	14 - 16 °C; 3 - 7 weeks	Yes / No	pH <4.8 a_w 0.85 - 0.87 M/P:1.6 - 1.9	
	Northern Europe	German salamis, Danish salamis	20 - 30 °C; 18 - 48 h	12 - 15 °C; 1 - 3 weeks	Yes / No	pH 4.6 - 5.1 a_w 0.92 - 0.94 M/P: 2.0 - 2.3	
		Hungarian salamis, Nordic salamis,	20 - 32 °C; 2 - 5 days	12 - 15 °C; 2 - 3 weeks	Yes / No	pH 4.6 - 5.1 a_w 0.92 - 0.94 M/P: 2.0 - 2.3	
	US	US-style pepperoni	35 - 43.3 °C; 8 - 12 h	10 - 12.8 °C; 12 - 14 days	No / Yes (53.3 °C;1h)	pH < 5.0 a_w 0.82 - 0.85 M/P: ≤1.6	
Semidry	Europe	German cervelat	20 - 32 °C; 18 - 48h	10 °C; 10 - 25 days	Yes / Yes	pH 4.4 - 5.0 a_w 0.93 - 0.95 M/P: 2.3 - 3.7	<15 °C
	US	Summer sausage, Lebanon bologna, thuringer	27 - 41°C; 15 - 20 h	10 °C; 2 - 3 days	Yes / Yes (71.1 - 73.9 °C)	pH 4.4 - 5.0 a_w 0.93 - 0.95 M/P: 2.3 - 3.7	
Undried	Europe	GermanTeewurst, Mettwust, Spanish sobrasada	20 - 25 °C; 2 - 3 days	<15 °C; < 3days	Usually / No	pH < 5.3 a_w 0.95 - 0.97 M/P: 2.0 - 3.7	<7 °C

[a]: Not critical but typical
[b]: Moisture to Protein ratio

The initial microbial population of sausage mince (batter) is highly variable and depends on the microbial load and the storage conditions of the raw materials, particularly meat. The physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution are among the factors that determine the microbiological quality of meat (10). It has been well established that Gram-negative psychrotrophic bacteria such as *Pseudomonas* spp. (e.g. *Pseudomonas fragi, Pseudomonas fluorescens,* and *Pseudomonas lundensis*) and Enterobacteriaceae (e.g. *Hafnia alvei, Serratia liquefaciens* and *Enterobacter agglomerans*) accumulate on the meat and prevail under aerobic chilled storage conditions (10, 11, 12, 13, 14). Gram-positive bacteria such as LAB and *Brochothrix thermosphacta* are known to accumulate on meat packed under vacuum- or modified-atmospheres. The psychrotrophic species *Lactobacillus sakei* and *Lactobacillus curvatus* predominate in sausages fermented without the addition of starters. These species are also found on chilled meats stored at low-oxygen partial pressure. Some of these microorganisms will be derived from the animal's intestinal tract and others from the environment with which the animal had contact some time before or during slaughter. Studies on the origin of the contaminants have shown that the source of Enterobacteriaceae on meat is associated with the work surfaces and not by direct faecal contamination. Psychrotrophic bacteria are recovered from hides and work surfaces within an abattoir as well as from carcasses and butchered meat at all stages of processing. Therefore, the quality of the raw material (lean and fatty tissue) should be controlled by continuously monitoring the temperature during meat handling and transport, by visual inspection of shipments, and by selecting suppliers following Good Hygienic Practice (GHP) in slaughtering and butchering.

5.3.2 Non-meat ingredients

Salt is the major ingredient of fermented sausages that contributes to the technological and sensory quality of the products. It is usually added at 2 - 3% (w/v) which is sufficient for the desired bind, and will not retard fermentation. Addition of salt in the first steps of processing causes a slight decrease of a_w and thus suppresses the growth of both spoilage and pathogenic bacteria.

Nitrite is an essential additive for sausage preservation due to its antimicrobial, colour forming, antioxidant, and flavouring properties. Sodium nitrite ($NaNO_2$) is formulated in a curing salt and is used at levels between 0.4 - 0.6% (w/v), normally in combination with 300 - 500 mg sodium ascorbate/kg. In production of long-ripened dry sausages, potassium nitrate (KNO_3, 300 mg/kg) may also be used in the sausage mixture as it acts as a reservoir of nitrite (reduced to nitrite by nitrate reductase activity of bacteria). Generally, nitrite inhibits bacterial growth (i.e. Enterobacteriaceae) early in fermentation and contributes to the development of colour and aroma during the ripening phase of fermented sausages.

Carbohydrates (glucose and occasionally lactose or sucrose; 0.3 - 0.8%) are commonly added into the sausage mixture as the fermentation substrate for LAB. The rate and the extent of acidification are known to be influenced by both the nature and the quantity of carbohydrates used (15). This acidification has a preservative effect due to inhibition of most pathogenic and spoilage bacteria, and it contributes to the development of the typical organoleptic characteristics of the fermented sausages (16).

A wide variety of spices (e.g. ground pepper, paprika, cinnamon, mustard, garlic) in various combinations are often included in sausage mixtures. Spices can be used either in a natural form or as flavouring extracts (i.e. essential oils). Apart from their effects on flavour and aroma, a variety of spices (e.g. paprika and garlic) have been found to accelerate lactic acid production, due to their manganese content (17, 18, 19). Some spices also contain powerful antioxidants that can extend the shelf life of dry fermented sausages (20, 21).

5.3.3 Starter cultures

Traditionally, meat fermentation was based on the selective development of the natural microflora of raw materials, sometimes enriched with 'back-slopping' (i.e. the use of a fermented sausage from the previous batch to inoculate the following batch); such practices resulted in products with heterogeneous quality. Thus, in the mid 1950s, standardised processing and safety assurance measures were enforced, and the commercial use of microbial starter cultures in industrial sausage fermentations was implemented. Suitable starter cultures must be able to grow at fermentation temperatures and to be sufficiently active under low a_w conditions (0.93 - 0.96). Starter cultures also must have a good enzyme profile for the generation of the desired products (e.g. lactic acid for acidification, volatile compounds for aroma development, and nitrate reduction for colour development) (22). Nowadays, most commercial preparations contain a combination of LAB strains, mainly of *Lactobacillus* or *Pediococcus* genus, with coagulase-negative staphylococci and members of Micrococcaceae.

The major role of LAB is to metabolise glucose or other carbohydrates through either homo- (e.g. *Pediococcus pentosaceus, Pediococcus acidilactici*) or hetero- (e.g. *L. sakei, L. curvatus, Lactobacillus plantarum*) - fermentative pathways and generate lactic acid (23). Acidification rates are highly dependent on the species used as well as on the processing conditions; e.g. strains of *L. sakei* tend to have faster rates than other lactobacilli and are thus commonly used at lower fermentation temperatures (18 - 25 °C), whereas strains of *P. acidilactici* better adapt at higher temperatures (35 - 40 °C) (6).

Members of the genera *Staphylococcus* and *Kocuria* (formerly *Micrococcus*) are often used as starter cultures because of their ability to reduce nitrate and produce catalase (15, 24, 25). Specifically, strains of *Staphylococcus carnosus,*

Staphylococcus xylosus and *Kocuria varians* are commercially available. Addition of sufficiently high levels (10^6 - 10^7/g) of these microrganisms is recommended as their population levels are reduced during fermentation, due to acidification (9).

The presence of moulds on the outer surface of sausages is typical in certain types of Mediterranean dry fermented sausages. Strains of *Penicillium nalgiovense* and *Penicillium chrysogenum* are used as starter cultures to prevent the growth of other mycotoxin producing moulds and to give the characteristic white coating on the surface.

5.4 Processing and its Effects on the Microflora

As already mentioned, the manufacture of fermented sausages is considered to entail three main steps: formulation, fermentation, and ripening/drying (Figure 5.1). Each step is known to consist of several intermediate phases that are crucial for the production of high quality fermented sausages.

5.4.1 Formulation

During this stage, the raw meat materials (meat and fat) are ground or minced to a specific size, depending on the type of product. Comminution of meat and fat is usually performed in a meat grinder or cutter at low temperatures (between -5 and 0 °C). Application of low temperatures at this stage prevents the release of intramuscular fat that may cause changes in the colour and the drying process during ripening (26). Comminution of meat and fat is followed by the addition of starter culture, curing salts (salt, nitrite and/or nitrates), and other ingredients. The sausage mixture (batter) is placed in a stuffing machine (kneader) and sausages are made by vacuum-filling of natural or synthetic casing. Exclusion of oxygen from the matrix is important because oxygen may lead to abnormal fermentations and the formation of undesirable colour and flavours.

5.4.2 Fermentation

After filling the sausages are sorted and placed in ripening cabinets under conditions of controlled temperature, relative humidity (RH), and air flow. Fermentation conditions are quite variable in industrial productions, but generally higher fermentation temperatures result in faster acidification. Specifically, most European-type sausages are fermented at temperatures ranging from 18 to 30 °C, with lower temperatures used in the Mediterranean area (Table 5.I). On the other hand, USA products are usually fermented for shorter periods at much higher temperatures (up to 43 °C) (Table 5.I); however, special precautions are required to suppress pathogens such as Salmonellae and *Staphylococcus aureus* under these conditions. In general, lower fermentation temperatures should be applied in dry

fermented sausages with a long shelf life in order to restrict acid formation and to enhance the activity of nitrate-reducing microorganisms.

The two basic microbiological reactions that proceed simultaneously and influence one another take place during fermentation. These consist of (i) the formation of nitric oxide by nitrate- and nitrite-reducing bacteria and (ii) the production of lactic acid by LAB. Immediately after stuffing, meat enzymes consume the residual oxygen and oxygenated myoglobin is turned into brownish metmyoglobin. Subsequently, glucose is metabolised by LAB and the formation of lactic acid begins. Nitrate is reduced to nitrite, which then reacts with metmyoglobin to form pink nitric oxide myoglobin. Lactic acid decreases the pH of the product from 5.7 to its lowest value, which could vary from 5.3 to below 4.6 depending on the sausage type (Table 5.I). By the end of fermentation, most acid-sensitive bacteria are inhibited, and the water-binding capacity of the product is minimal.

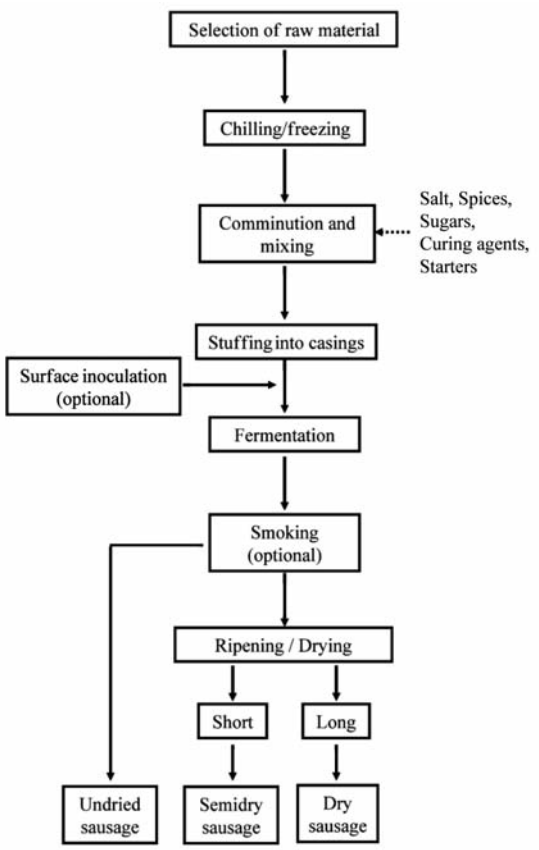

Fig. 5.1. Flow diagram of the manufacture of fermented sausages

5.4.3 Ripening

Application of smoke after fermentation is optional and highly dependent on tradition and product type (Table 5.I). Application of smoke is either achieved by the controlled combustion of wood, generating smoke temperatures as high as 80 °C, or by spraying smoke flavouring on the surface of the sausage. This flavouring is a liquid produced by distillation and subsequent condensation of volatile compounds from smoke (27). Apart from the characteristic flavour, smoking gives a darker external colour to the product. Smoking also contributes to preservation as the phenolic portion of the smoke exerts antioxidant and antimicrobial properties (4).

Most dry fermented sausages are usually ripened at temperatures between 12 - 15 °C (Table 5.I). During this phase the RH in the ripening cabinet is gradually lowered to about 75 - 80%, and air velocity is adjusted to about 0.1 m/s in order to ensure a slow but steady removal of moisture from the sausages. Controlled conditions will also prevent undesirable mould growth on the surface while avoiding uneven drying of the product, and undesirable changes in appearance and texture (6). In general, most dry fermented sausages are dried to moisture contents of 35% or less (a_w 0.90; weight loss of >25%) while semi-dry sausages are dried to moisture contents of about 40% (a_w 0.93; weight loss of 18%) (Table 5.I).

During ripening, the flavour and aroma characteristics for fermented sausages are formed and stabilised. As already mentioned, the nature of the starter cultures has a great influence on the volatile composition and sensory characteristics contributing to dry sausage flavour. Lipids are precursors of many unbranched aldehydes, 2-alkanones and short-chain unbranched fatty acids. This process involves tissue lipases, autoxidation reactions, and the transformation of the reaction products by microorganisms. Tissue proteases split proteins into peptides, which are subsequently transformed by microorganisms into amino acids and branched-chain volatile fatty acids. Ethyl esters also contribute to sausage aroma (28). Sufficient activity of catalase-positive cocci is important for the development and stabilisation of sausage aroma. Use of curing agents results in partial inhibition of oxidative changes of lipids, and into a shift in the reaction products.

5.5 Spoilage

The occurrence of spoiled fermented sausages is relatively uncommon, and can be the result of processing defects in which the hurdles (e.g. acidification and drying) are insufficient (Table 5.II). For example, the delay in acid formation not only increases the potential growth of pathogens, but also leads to elevated numbers of Enterobacteriaceae causing off-odours and off-flavours.

Table 5.II
Main spoilage defects in fermented sausages

Defect	Cause	Prevention measure
Sticky or 'Slimy' sausages	Overgrowth (biofilm formation) of microorganisms on casing due to: • High temperature inside fermentation chamber • Visible film of liquid water on the surface • Low temperature during ripening • Water condensation in the pack	• Temperature during ripening higher than 12 °C • Increase air velocity inside ripening chamber • Packaging of finished products under similar temperatures (12 °C)
Mouldy sausages	Growth of moulds on casing	• Elimination of the moulds by cleaning and brushing • Inoculation of the casings with white moulds and yeasts • Increasing air velocity in the fermentation and ripening chamber • Controlling RH in ripening chamber and application of smoking • Treatment with natamycin or other anti-mould products
Souring	Acidic taste due to: • Domination of hetero-fermentative lactobacilli	• Selecting starters that degrade the sugars rapidly • Increase the salt content of the sausage mixture
Rotten sausages	Rare defect in industrial productions. Usually due to: • Use of low quality raw materials • Lack of hygiene during processing • Weak acidification inside fermentation chamber • High pH (>6.2) of the final product	• Choice of high-quality raw materials • GMP
Discolored sausages	Discoloration due to: • Defective fermentation processes • Peroxide production by greening LAB	Decreasing the a_w of the sausage mixture and decreasing the temperature inside fermentation chamber
Hollow sausages	• Hollows tend to be rancid due to microbial activity	• Elimination by hanging large diameter sausages inside nets
Crusty sausages	Zone discoloration in sliced sausages, due to: • Fast dehydration Overgrowth of moulds that increase the speed of dehydration	• Ensure proper dehydration of the products

Similarly, the undesired metabolic activity of lactobacilli by adding excessive amounts of fermentable sugars in conjunction with insufficient drying and elevated ageing and/or storage temperature, results in high levels of lactic and acetic acids, sometimes even in pore formation and swollen packs, due to production of CO_2 by hetero-fermentative lactobacilli.

It is well known that many lactobacilli may also contribute to spoilage by forming H_2O_2 (29, 30, 31).The lactobacilli are divided in two groups. One possess a flavin-containing l-lactate oxidase using O_2 as electron acceptor, the other requires an acceptor methylene blue (MB) to perform lactate oxidation at significant rates. In group I (*L. curvatus, L. sakei, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus lactis*) lactate oxidation with O_2 yields hydrogen peroxide (H_2O_2) and pyruvate. Group II strains (*L. plantarum, Lactobacillus casei, Lactobacillus coryniformis*) exhibit only a very low rate of lactate oxidation to acetate and CO_2, which increases tenfold by the addition of MB when H_2O_2 and pyruvate are produced. The accumulation of the latter in both cases leads to spontaneous breakdown of the acid (29). The H_2O_2, which attacks haem-compounds and polyunsaturated fatty acids, is formed by the oxidation of lactate (32). The production of H_2O_2 leads to grey or greenish discolorations and flavour defects, particularly if soft, improperly stored fatty tissue has been used. Hence, LAB that are suitable as starters should not be able to accumulate H_2O_2, i.e. they should either not have H_2O_2 forming oxidases, or be able to form a functional catalase (by using haem from the meat) or a haem-independent 'pseudocatalase'. Oxygen should also be excluded from raw sausage mixtures as far as possible, and enough peroxide-destroying micrococci and staphylococci should persist throughout the fermentation.

After fermentation, undesired mould growth and/or oxidative changes in the lipid fraction (rancidity) limit the shelf life of most semi-dry or dry sausages.

5.6 Pathogens: Growth and Survival

Pathogen survival and proliferation during manufacturing of fermented sausages is dependent on the rate of drop in pH and a_w, as well as the final levels of these two factors. Given that these are further dependent on the amount of carbohydrate added in the batter, the presence of nitrates (which can inhibit starter cultures), the type and level of starter cultures, and the variability in the manufacturing process etc. these combine to result in variable risk for pathogen growth and survival. Of the potential pathogens, *Salmonella*, enterohaemorrhagic *Escherichia coli* (EHEC)*, Staph. aureus*, and to a lesser extent, *Listeria monocytogenes*, have been implicated in food outbreaks associated with consumption of fermented sausages. In the following paragraphs a more detailed description of their epidemiological

significance and the risks associated with their survival in the ecosystem of fermented sausages is provided.

5.6.1 Salmonella enteritica

Proper sausage fermentation prohibits growth of Enterobacteriaceae, and if the pH and/or a_w drop below the growth-limiting levels, inactivation is also likely. The risk for Enterobacteriaceae growth or extended survival, and potential *Salmonella* infection increases if there is high initial water activity and/or high initial pH value (33). This also depends on the concentration of fermentable carbohydrates; if they are low/ insufficient to support a large pH reduction (e.g. below 5.0). Other factors that may contribute to the increase of *Salmonella* survival include the low number of lactobacilli in the fresh sausage mixture, the use of nitrate or of very low levels of nitrite as the curing agent (34), and high ripening temperatures.

Fermented meat products that have been linked with salmonellosis include dry salami, Lebanon bologna sausages, and fresh sausages (35, 36, 37, 38). In Germany, fresh spreadable sausages have been involved in some outbreaks of salmonellosis. This was due to the high water activity of such sausages in order to retain their 'fresh' consistency and 'meaty' taste, Furthermore, these specific characteristics are achieved by use of little, or no, sugar or acidulant (e.g. glucono-delta-lactone (GdL)), and a short (e.g. 1 - 2 day) fermentation at 20 - 25 °C, which is sufficient for curing colour formation but does not always suppress the development of Enterobacteriaceae. Increasing the amount of added salt to at least 2.5% and addition of appropriate LAB along with some fermentable carbohydrate or small amounts of GdL was most effective in the control of Enterobacteriaceae including salmonellae (39, 40). Outbreaks of salmonellosis from semi-dry or dry sausages have also been reported. In one of them (41), a very low input of nitrite (which is common in Italian-type sausage formulations) was combined with a fermentation temperature above 25 °C (as common for US-style summer sausages). Another outbreak affected consumers (mainly children) that had consumed dry 'salami sticks' (42). It is unlikely that the salmonellae had grown during the ripening process, but apparently, the infective dose was small because the products were eaten as snacks rather than as part of a full meal.

The United States Department of Agriculture (USDA) has proposed lethality performance standards for ready-to-eat (RTE) products. Such standards require a 6.5 log cfu/g reduction of viable *Salmonella* populations for finished products (43). However, concern has been expressed for the survival of post-processing *Salmonella* contamination especially on sliced fermented meats, such as Lebanon bologna, as well as in naturally fermented meat products. Thus, validation of manufacturing processes and challenge testing on finished products for survival of *Salmonella* is essential. Reported rates for *Salmonella* destruction during sausage ripening vary from less than 1 to 3 - 4 log cycles (44). Assuming a reduction of salmonellae by one or two log cycles in European-type smoked products is in

agreement with the observation that salmonellae occur much less frequently in dry sausages than in ground meat or fresh, and only slightly in fermented raw sausages (6, 38, 40).

Ellajosyula *et al*. demonstrated a 5 $\log_{10}$ cfu reduction of *Salmonella typhimurium* in cooked Lebanon bologna sausages (45). Chikthimmah and Knabel showed, in the same products, that *S. typhimurium* exhibited the most rapid reduction compared to *Escherichia coli* O157:H7 and *L. monocytogenes* inoculated both pre- and post-fermentation (46). During manufacture and storage of Norwegian dry salami, *Salmonella kentucky* was the most sensitive pathogen in comparison to the above two, which reduced to undetectable levels after 46 days of storage both at ambient and cold temperatures. Similar reports exist for Italian-style salami of pH 4.4 - 4.8 and a_w of 0.869 - 0.935 (47). However, a cooking step at 60 °C could eliminate the pathogen from the product (48).

5.6.2 Enterohaemorrhagic Escherichia coli

Strains of the bacterium *E. coli* capable of producing certain cytotoxins are reported as verotoxigenic *E. coli* (VTEC). EHEC are a subset of the VTEC harbouring additional pathogenic factors. More than 150 different serotypes of VTEC have been associated with human illness; however, the majority of reported outbreaks and sporadic cases of VTEC infections have been attributed to serotype O157. There is a wide spectrum of symptoms associated with VTEC infections ranging from mild to bloody diarrhoea, often accompanied by severe abdominal cramps but usually without fever. VTEC infection can also result in haemolytic uraemic syndrome (HUS). HUS is characterised by acute renal failure, anaemia and lowered platelet counts. HUS develops in up to 10% of patients infected with VTEC O157 and is the leading cause of acute renal failure in young children.

Verotoxigenic strains of *E. coli* O157:H7 have been associated with the following outbreaks

- Washington D.C., dry-fermented salami (49)
- Australia, 21-cases including 1 death, semi-dry fermented sausages (mettwurst) incriminated
- Canada, 39 cases, Genoa salami associated with this outbreak
- Canada, 143 cases, salami was also the suspected food

In contrast to salmonellae, EHEC have a generally low infective dose and no multiplication during sausage fermentation is required to cause disease. The presence of *E. coli* O157:H7 in final products can be eliminated in sausages fermented and dried according to good manufacturing practice (GMP); recontamination and growth is also inhibited due to the combined effect of low pH, low temperature, and low a_w (50). *E. coli* O157:H7 was implicated in an outbreak involving consumption of pre-sliced dry fermented salami (51) with an

estimated dose of less than 50 cells (49); a year later, another *E. coli* outbreak was linked to mettwurst, an uncooked semi-dry fermented sausage in Australia (52). Furthermore, due to its higher acid resistance, EHEC dies slowly during sausage ripening (53), especially if an acid adaptive response has taken place due to gradual reduction of pH during fermentation (46, 54).

The above two outbreaks prompted US food authorities to develop guidelines through which fermented sausage manufacturers were required to show a 5 log unit reduction in EHEC levels during processing (55, 56). Such guidelines were further established as lethality performance standards (43). Among the predominant alternatives was a heating step at 63 °C for 4 minutes as well as combined antimicrobial treatments (56). Fermentation of Lebanon bologna to pH 4.7 followed by gradual heating to 48.9 °C in 10.5 hours is sufficient to destroy 5 $\log_{10}$ cfu of *E. coli* O157:H7 (45). The traditional character of some dry fermented sausages, which are known as raw products, may be compromised due to cooking; furthermore, in some cases cooking to an internal temperature of 63 °C could lead to unacceptable products (57, 58). Therefore, it is important that the fermentation and ripening process modulate the pH and a_w of the product in such way that storage of the final products may also contribute to the inactivation of a pre- or post-process contamination. For instance, *E. coli* O157:H7 that had survived the manufacturing process (without cooking) was further reduced by 1 to 4 logs within 28 days of storage, while higher reduction was achieved at 21 compared to 4 °C (58, 59). Higher log reductions of *E. coli* O157:H7 during storage occur in sausages fermented with starter cultures compared to naturally fermented sausages, apparently due to the lower pH and a_w sustained with starters (58, 59). However, bacteriocin-producing starters do not influence the Gram-negative EHEC, as they do against Gram-positive pathogens (58, 59, 60). Furthermore, it is likely that *E. coli* O157:H7 contamination after fermentation is reduced more easily than when contamination occurs at the initial batter. In the latter case the fermentation process may allow for the development of acid adaptation (46, 61), except that pH and a_w are rapidly reduced so that the pathogen has become acid sensitive (62, 63).

Fortunately, the prevalence of EHECs in meat appears to be low and restricted to products made using beef and lamb (64). However, unless effective control measures on the farm and meat inspection levels exist, the use of beef- or lamb-containing raw sausages with short ripening times should be limited in the diet of small children. The latter constitute the main risk group for haemorrhagic colitis and its life-threatening sequela disease, HUS.

5.6.3 Staphylococcus aureus

Staphylococcal food poisoning is an intoxication caused by the consumption of fermented sausages containing enterotoxin produced by *Staph. aureus*. This

pathogen is frequently found in raw meat and fermented sausages, but generally at low levels. For example, in the US during the period 1975 - 1979, 540 food poisoning outbreaks were reported; *Staph. aureus* was responsible for 28% (153 outbreaks). Misuse of foods in food service operations seems to be the major cause of outbreaks, followed by mishandling in the home. Illness results from the ingestion of water soluble, heat stable enterotoxins secreted by the staphylococcal cells. Secretion of these enterotoxins occurs at different periods of the growth phase, either as primary or secondary metabolites. Only a few outbreaks appear to be directly attributed to contamination during food processing. The occurrence of *Staph. aureus* in fermented sausages has been reported on many occasions at various stages of sausage production as well as in finished products in retail shops. Bad practices during manufacture, especially poor conditions of fermentation and poor conditions of storage, have led to food poisoning outbreaks from products, especially those that are consumed raw. When compared with other foods, fermented sausages have a good safety record. However, delayed fermentation can allow the growth of pathogens. The occasional outbreaks of *Staph. aureus* food poisoning which appear to be more common in the USA and Canada than in Europe have led to studies of the ability of *Staph. aureus* to grow in fermented sausages. Different types of fermented sausages have been implicated in staphylococcal food poisoning outbreaks (15). *Staph. aureus* may be commonly found on industrial equipment surfaces and its growth and enterotoxin production may occur during the initial stage of fermentation (65). Both growth and enterotoxin production of *Staph. aureus* are highly dependent on the starter cultures used and the fermentation conditions, such as duration, temperature and aeration (65, 66, 67, 68). The combination of lower fermentation temperature and activity of starter cultures may restrict growth and toxin production (65). However, *Staph. aureus* is expected to be significantly reduced throughout ripening. Smith and Palumbo (66) addressed the importance of starters and the addition of glucose in meat fermentations in order to enhance the injury of *Staph. aureus* by the metabolic activity of starter cultures. Prevalence studies and thorough validation of separate processes are required to ensure minimisation of risk by staphylococcal food poisoning due to consumption of fermented sausages.

5.6.4 Listeria monocytogenes

The genus *Listeria* comprises six species, but human cases are almost exclusively caused by the species *L. monocytogenes*. In humans, infections most often affect the pregnant uterus, the central nervous system or the bloodstream. Symptoms vary, ranging from mild flu-like symptoms and diarrhoea to life threatening infections characterised by septicaemia and meningoencephalitis. In pregnant women, the infection spreads to the foetus, which will either be born severely ill or die in the uterus resulting in abortion. Although human infections are rare, it is worth noting that it is considered to be of high mortality.

Because of its ability to grow at low temperatures and relatively low a_w (0.93), *L. monocytogenes* may be expected to represent a hazard to the consumers of fermented sausages. Sausage ripening causes slow inactivation of *Listeria*. Using lactobacilli as starters that form a listeriocidal bacteriocin may enhance inactivation of *Listeria* early in fermentation by about one log (69, 70, 71). Meat products (e.g. sausages) that are contaminated with more than 100 *L. monocytogenes* bacteria/ gram, and that are to be consumed without further heat treatment are considered to form a direct risk to human health. These food categories (RTE meat products) have been typically identified as risk products for contamination with *Listeria spp*. Member States in the European Community have conducted a report on the presence of *Listeria*; so far, the comparison between data from different studies is difficult due to differences in sample sizes and testing protocols (72).

Although prevalence of *L. monocytogenes* in fermented sausages is reportedly up to 30% (73, 74) critical listeriosis cases have yet to be linked to the consumption of fermented sausages contaminated with *L. monocytogenes* (47). So far, there is only one epidemiological association of *L. monocytogenes* with salami in Philadelphia, US, but no confirmed outbreak due to the consumption of such products with *Listeria* has been reported (75). Likewise, little if any growth is likely during sausage fermentation under conditions resembling commercial practice (76), even in undried sausages (69) and in mould-ripened salamis (77, 78). Conditions controlling *Salmonella* and *Staph. aureus* were found also to prevent growth of *Listeria spp*.. However, to make sure that *Listeria* did not grow on the surface of mould-ripened sausages a lower ageing temperature of 8 - 10 °C, for Italian-type salami, was recommended (77). USDA has published rules and guidelines to control the specific pathogen in fermented meats regarded as RTE foods (79, 80, 81). For example, the reduction of pH due to the fermentation process, the reduction of a_w via drying, and the deposition of inhibitory compounds via smoking are considered antimicrobial processes. These processes, should not allow more than 1 log increase of *L. monocytogenes* throughout the shelf life of the products. Furthermore, it is stated that a_w <0.92 or pH <4.39 (a marginal pH achieved after fermentation depending on the use of fermentable carbohydrates and starter cultures) are the lowest levels permitting growth of *L. monocytogenes* based on scientific evidence. Moore (75) summarised the guidelines for microbiological quality of fermented meats, and for *L. monocytogenes*, acceptable fermented meat products were those that contained ≤100 cfu/g of *L. monocytogenes* consistently, with the new EC regulation 2073/2005 for microbiological criteria in foods.

Given that *L. monocytogenes* is ubiquitous in the processing environment and hence post-process contamination may occur, it is important that the sausages cause inactivation (e.g. >5 log units) of the pathogen during storage. This has been demonstrated for various dry-fermented sausages with pH ranging from 4.2 - 4.8 and a_w from 0.8 to 0.9 (47, 72, 81).

5.6.5 Miscellaneous pathogenic bacteria

Apart from the above 4 major pathogens with high prevalence in fermented sausages, there is no epidemiological evidence that *Yersinia enterocolitica* infections have been caused by consumption of fermented sausages. It may be postulated that pathogenic yersiniae are controlled in a way similar to salmonellae. Growth potential of *Y. enterocolitica* during sausage ripening is small, and inactivation rates reported vary between zero and 5 log cycles, mainly depending on the rate and extent of acid formation and drying (82). Likewise, fermented sausages do not support growth of *Clostridium botulinum* (83) or any other species of *Clostridium* and *Bacillus*, due to their low pH and a_w (84); their growth is also inhibited during fermentation. There is no report of food poisoning due to growth of these bacteria in fermented sausages having a low pH.

5.6.6 Microorganisms forming biogenic amines

For the formation of biogenic amines, a specific amino acid decarboxylation is required. To be achieved, microbial enzymes (decarboxylases), which are commonly found in fermented meat, should be present in bacterial species. If this is the case, fermented sausage species can be found able to produce biogenic amines e.g. histamine, tyramine, tryptamine, and cadaverine. The key decarboxylation enzymes are histidine decarboxylase for histamine, arginase for the formation of ornithine from arginine, ornithine decarboxylase for putrescine, lysine decarboxylase for cadaverine, arginine decarboxylase for agmatine, agmatinase for the formation of putrescine from agmatine and seprmidine, and spermine synthase for the formation of spermidine and spermine from putrescine (85).

In general, decarboxylases have been found in species of the genera *Pseudomonas* (86) as well as in genera of the families (i) Enterobacteriaceae, such as *Citrobacter, Klebsiella, Escherichia, Proteus, Salmonella,*and *Shigella*; (ii) Micrococcaceae, such as *Staphylococcus, Micrococcus* and *Kocuria*. Furthermore many LAB, including *Lactobacillus, Enterococcus, Carnobacterium, Pediococcus, Lactococcus* and *Leuconostoc* are able to decarboxylate amino acids (87 - 97).

It should be noted, however, that not all the strains of these species are amine-positive. Some strains have a rather wide spectrum and are able to decarboxylate many amino acids, whereas others have only strictly substrate specific decarboxylases.

Protein degradation by tissue enzymes during prolonged storage of raw meat, in conjunction with growth of psychrotrophic LAB capable of histidine decarboxylation, in particular, certain strains of *Carnobacterium* spp. and of *L. curvatus*, appear to be the main risk factors for histamine formation (98, 99).

5.6.7 Toxigenic moulds

During drying and storage of sausages at 15 °C or below, Penicillia predominate. Among the *Penicillium* toxins, small amounts of cyclopiazonic acid and verrucosidin, and possibly citreoviridin and rugulosin may be formed in the outer layer of the sausage under commercial ripening conditions (100, 101). In general, the levels found in experimentally inoculated sausages were small and restricted to no more than a few millimetres below the surface. Nonetheless, growth of all undesired moulds on fermented sausages should be prevented, not only to eliminate the risk of mycotoxin and antibiotic formation, but also to avoid defects in appearance and flavour. *Aspergillus flavus*, an aflatoxin-producing mould, is rarely found on the surface of ripened salami (102). Mould growth may be inhibited by appropriate control of temperature and RH (e.g. <75%) during ageing, storage and distribution, and/or by surface treatment with antimicrobial agent, such as smoke, permitted antifungal agents or waxes. If the sausage is dried to a_w below 0.82 and packaged under nitrogen, e.g. 'snack salamis', growth of moulds, even at ambient temperature, will not occur; rancidity is also delayed (103). Products to be mould-ripened should be inoculated with a suitable non-toxic mould strain and dried at temperatures below 15 °C.

5.6.8 Viruses and parasites

Viruses present in livestock during slaughtering normally do not affect humans. However, since these viruses are slowly inactivated during sausage ripening, fermented meats should not be exported from areas where viral diseases of meat animals prevail. The infectivity of pathogenic viruses for humans is also retained during sausage fermentation; scrupulous GHP must therefore be employed at all stages of production.

Parasites (protozoa, nematodes, tapeworms) that may occasionally escape detection during inspection of the animals and their meat at the slaughterhouse are susceptible to low a_w values of dried sausages. Thus, they are usually absent from meats with a_w levels of <0.90. Parasites are also unlikely to survive a_w and pH values typical for most semi-dry sausages. However, in the US pork is not generally inspected for the absence of *Trichinella*, and regulations stipulate that semi-dry and undried fermented sausages must be made from meat that has been stored frozen, or be heated to 58.3 °C (137 °F).

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6. EGGS

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6.1 Definitions

Egg for the purposes of this chapter means an egg laid by a hen. A hen egg consists of 12.8 - 13.4% protein, 10.5 - 11.8% lipid, 0.3 - 1.0% carbohydrate, and 0.8 - 1.0% ash. However, these components are not distributed evenly between the albumen and the yolk. The yolk would typically consist of 15.7 - 16.6% protein (*cf*. 9.7 - 10.6% in albumen), 31.8 - 35.5% lipid (*cf*. 0.03% in albumen), 0.2 - 1.0% carbohydrate (*cf*. 0.4 - 0.9% in albumen) and 1.1% ash (*cf*. 0.5 - 0.6% in albumen) (1). According to Annex XIV of Regulation European Commission (EC) No. 1234/2007 regarding marketing standards for eggs, eggs are graded by quality into Class A (fresh) and Class B (those that do not meet the quality characteristics of Class A). Class B eggs can only be used by the food and non-food industry. It also defines *industrial eggs* as those eggs not intended for human consumption.

Egg product means products obtained from eggs (components or mixtures thereof) once the shell and membranes have been removed. Egg products may be liquid, concentrated, dried, crystallised, frozen, quick-frozen, or coagulated. Egg products may be partially supplemented by other foodstuffs or additives. Egg products are used widely for the commercial production of confectionery and bakery products, and added-value meals, and are found increasingly in the home.

Whole egg is considered to be the liquid content of the egg in natural proportions of albumen and yolk. Whole egg blends, with lower or higher solids content, contain additional albumen or yolk, respectively. Whole egg may be mixed with sugar or salt to improve its stability.

Liquid egg contains 23 - 25% solids and has a pH of 7.0 - 7.6. Liquid egg albumen normally contains 10.5% solids and has a pH of about 9.0, but because the pH depends on the amount of carbon dioxide (CO_2) that has diffused out of the egg,

the value varies between 7.6 and 9.5. Therefore the pH of liquid albumen changes during storage. This is not the case for liquid yolk, which consistently has a pH of approximately 6. The solids content of yolk varies from 46 - 48%, depending on the amount of egg albumen adhering to the yolk. Liquid egg products may include additives such as stabilisers (e.g. salt, benzoate, sorbate, and for yolk colour in particular, phosphates). Other additives may include emulsifiers and thickeners, anti-coagulants (vegetable oils) and acids or bases (to modify pH). Sugar may be added to retain the functional (whipping) properties of egg.

Frozen egg products, that contain less than 75% water, are formed at between -24 and -40 °C, and should be stored below -12 °C, or if deep-frozen, at -18 °C. If liquid yolk is to be frozen it is usual to add either salt or sugar (10% w/v) to prevent gelation. Boric acid has been used as a preservative in frozen egg products.

Dried whole egg has a minimum of 95% solids, 30% fat, and no more than 3.5% of the free fatty acids should be oleic acid. Dried yolk has 95% minimum solids (31 - 32.5% protein, and 60 - 64% lipids), and generally contains 3 - 4% moisture. Pan- and spray-dried albumen has a minimum 84% and 92% solids, respectively, and may contain 5 - 7% moisture. The moisture content of fresh samples (whole egg) should not exceed 5%, although this may rise during storage to 9%. Before liquid egg is spray-dried it is usual to reduce the glucose level in it to less than 0.01%. This prevents the occurrence of the "Maillard reaction" during manufacture or storage. Anti-caking agents may be incorporated into dried egg mixes.

6.2 Properties of the Egg

The egg has certain important properties that protect it from microbial attack for a period of days, consisting of: a cuticle, inner membrane, shell, and outer membrane, in decreasing order of importance (2). The cuticle covers the shell, and makes the shell resistant to water entry. Each bird species has egg characteristics that make it more, or less resistant to microbial penetration e.g. the cuticle is relatively thin on duck and chicken's eggs, compared with guinea fowl eggs. Damage to the cuticle is therefore more important for the former two species. Abrasive cleaning of eggs can damage the cuticle, rendering it more susceptible to microbial ingress. The cuticle offers resistance to microbes for up to four days, becoming less effective as it dries out (3). The contamination of eggs with *Pseudomonas* and the Enterobacteriaceae tends to increase with the age of the chickens in a flock, perhaps due to the decreased quality of the cuticle as flocks age (2).

The egg shell is porous, with up 10,000 pores on the surface of a chicken's egg (4); these increase with flock age. Shells with fewer pores (and higher specific gravity), are more resistant to bacterial ingress than shells with more pores. For

example, spoilage may be delayed for 10 - 12 days if the specific gravity is high, compared with 3 days for low specific gravity eggs.

The egg albumen is the last antimicrobial barrier of an egg. Several antimicrobial factors contribute to its effectiveness: lysozyme, conalbumin, avidin, ovoinhibitor, ovomucoid, and the alkaline pH; lysozyme is the most effective (2).

6.3 Initial Microflora

There are two routes of contamination of shell eggs: transovarian, or trans shell transmission. The former will be covered later in the section on *Salmonella*.

Before it emerges from the bird, the egg contents and the porous calcitic shell that envelops the contents are normally free of contaminants. However, as the egg passes through the cloaca and comes into contact with the cage floor/nest box, the surface of the shell acquires a heterogeneous bacterial flora. Although an average of approximately 100,000 bacteria per shell are often cited, a number of surveys have highlighted the wide range in the level of contamination of egg shells, with populations varying from a few hundred to tens of millions of bacteria per shell (5). It is notable that, while the number of organisms isolated from a shell may vary, the genera remain remarkably constant. Contaminants identified in two studies (5, 6) ranged from *Micrococcus,* which was always isolated, to occasional organisms such as *Aeromonas*, *Proteus*, *Sarcina*, *Serratia*, and *Streptococcus*. Organisms found on most eggs included *Achromobacter*, *Acinetobacter*, *Aerobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Cytophaga*, *Escherichia*, *Flavobacterium*, *Pseudomonas*, and *Staphylococcus.* Most contaminants come from dust, soil and faeces, with the dry conditions on the shell favouring Gram-positive organisms. Wet and dirty shells in combination with a fall in temperature allow entry of bacteria (2). A fall in temperature causes the egg sac to contract, leading to a negative internal pressure, drawing in moisture.

The moulds *Penicillium*, *Aspergillus*, *Cladosporium*, *Rhizopus*, and *Mucor* have been isolated from eggshells, as has the yeast *Rhodotorula*.

6.4 Processing and its Effects on the Microflora

6.4.1 Shell eggs

Shell eggs are collected, packed, stored, and distributed; some categories of eggs may be washed. In the European Union (EU), washing of grade A eggs is not permitted, for example, but they can be cleaned if they will be further processed into egg products (2).

6.4.1.1 Shell eggs – effects of processing

A storage temperature below 8 °C after collection, inhibits the growth of *Salmonella* and other mesophilic pathogens. At temperatures above 18 °C, the egg's natural barriers break down more quickly. In the United States (US), shell eggs are held under 7.2 °C once in the distribution chain (2). Eggs should be kept dry in storage, since eggs with wet shells are more likely to allow microbial ingress.

Eggs can be cleaned dry, or wet. Dry cleaning may remove the cuticle, allowing microbial penetration. Eggs must be dried after wet cleaning to reduce the risk that bacteria remaining on the surface of the egg are aspirated into the egg as it cools to ambient temperature (2). The water used for wet cleaning should be at least 12 °C higher than the egg temperature, or water can be drawn into the egg, potentially bringing microorganisms with it. Washing must be carried out hygienically using potable water, with low levels of iron, as iron can decrease the antimicrobial effects of conalbumin. Poor quality or dirty eggs are more likely to spoil than undamaged eggs. Eggs must be dried as soon as practical after washing, to minimise the risk of microbial ingress (2).

6.4.2 Processed eggs

Eggs may be processed into refrigerated liquid egg products, frozen egg products, dried egg products, and speciality egg products. Apart from some of the speciality products, the first stage in the manufacture of processed egg products is the production of liquid egg.

6.4.2.1 Liquid egg

The production of liquid egg is covered by EC Regulations 852/2004 and 853/2004, which are enforced in the United Kingdom (UK) by the Food Hygiene Regulations 2006 (Statutory Instrument 2006 No. 14). Most producers in the UK also conform to standards set by the British Egg Products Association (BEPA). Liquid egg may be produced from three categories of egg: i) eggs produced specifically for this purpose, ii) Grade A eggs that, owing to overproduction, for example, cannot be sold, and iii) ungraded eggs or eggs that have been downgraded (owing to shell faults, faecal contamination, etc.) at the packing station. Incubated eggs cannot be used for egg products.

BEPA standards (7) require that eggs only be held at ambient temperature for 14 days before breaking. However, eggs held continuously between 5 and 12 °C may be kept for 28 days, and this increases to a maximum of 8 weeks where eggs are held continuously between 0 and 5 °C.

Before the eggs are broken, they are candled to remove dirty or contaminated eggs (Figure 6.1). Dirty eggs are washed in a room separate from the breaking machine, air-dried and then returned to the candling machine. Cracked eggs may be used provided that they are delivered directly from the packing stations, or the farm where they were produced. BEPA standards demand that eggs are rejected if: i) the shell and underlying membrane are broken, ii) the egg is mouldy, or iii) the egg has been washed other than at the processing site. Liquid egg albumen, yolk, or whole egg are produced with an egg-breaking machine, which uses a cup and ring device to separate the albumen from the yolk. An automatic breaking and separating machine will process up to 20,000 eggs per hour. After breaking, the egg products should be processed within 48 hours, during which time the liquid egg should be held either frozen, or at a temperature of not more than 4 °C. Ingredients that will be desugared do not have to conform to these criteria.

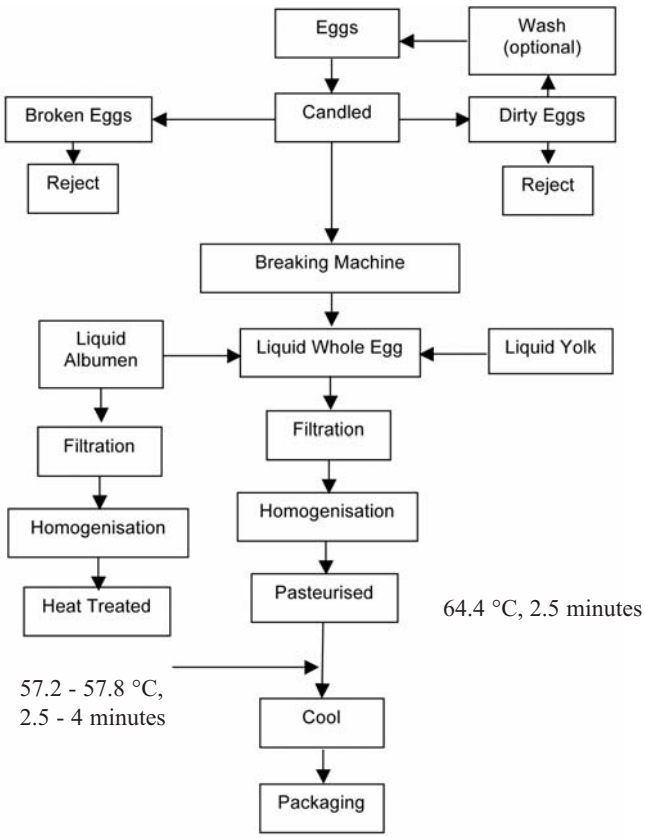

Fig. 6.1. Production of liquid egg

Egg production was formerly regulated by The Egg Products Regulations 1993. This stipulated that whole egg and yolk must be held at a temperature of 64.4 °C for at least 2½ minutes, and then cooled immediately to below 4 °C. If salt or sugar is to be added, the product must be cooled to below 4 °C immediately following the addition. Automatic flow diversion, and means of recording heat treatment and cooling must be supplied; also, BEPA requires its members to test automatic flow diversion valves daily, and whenever pasteurisation temperatures change. The Egg Products Regulations prescribed the method of sampling and examination by the α-amylase test. If pasteurisation has been carried out correctly, the enzyme α-amylase, which is normally present in the yolk of eggs, will be inactivated. Because albumen coagulates at a lower temperature, it is processed at 57.2 - 57.8 °C for 2½ minutes. A higher temperature may be used if the albumen is stabilised with, for example, added salt.

Egg products may be pasteurised using a high temperature-short time (HTST) pasteurisation system. This technique relies upon the counter-current flow of product, and either the heating or cooling medium through a stainless-steel plate heat exchanger or a "triple tube". A triple tube consists of an outer space that contains the heating or cooling medium, while the liquid to be pasteurised flows (counter-current) between the intermediate tube, and the inner tube. Unlike the traditional plate heat exchanger, the triple tube units can be suspended from the ceiling to make better use of floor space.

The storage temperature for chilled, frozen and deep-frozen products should not exceed 4, -12 and -18 °C, respectively.

6.4.2.1.1 Liquid egg - effect on microflora

Candling is a useful process to identify contaminated eggs; a single egg could potentially contaminate an entire batch of liquid egg. Automatic breaking machines tend to preclude the use of candling, due to the speed of the operation. The use of clean, unbroken eggs should minimise the risk of contamination during the breaking process. Eggs should be processed rapidly after homogenisation, or quickly chilled to 4 °C (2).

Current commercial practice should produce unpasteurised liquid egg with an initial bacterial count of 10^4 to 10^6 bacteria per ml; however, there may be considerable variation between sites. For example, when samples of unpasteurised liquid egg were taken over a 52 week period in the United States of America (USA), *Salmonella enterica* Serovar Enteritidis was recovered from 20% of plants in the northern region, while plants in the south-east, central and western regions produced samples that were positive 10, 15 and 6% of the time, respectively (8). In a separate 5-month study (9), 173 samples of unpasteurised whole egg products were examined, of which 72% were found to contain *Listeria*. However, the only organisms isolated were relatively small numbers (one organism/ml) of *Listeria*

innocua (62.6%) and *Listeria monocytogenes* (37.8%). In a separate study (10), liquid egg from 11 processing plants was sampled before pasteurisation over an 8 month period. Forty-five *Listeria* isolates were obtained from 36% of the samples. The most frequently isolated species was *L. innocua*, which was found in all samples, while *L. monocytogenes* occurred in 5% of the samples.

The time-temperature combination specified for pasteurisation was chosen after trials with the most heat-resistant member of the genus *Salmonella*, *Salmonella senftenberg*. The pasteurisation of liquid egg means that there are now few hazards from *Salmonella*, and other pathogens in liquid egg products. Current pasteurisation conditions from different countries reduce the *Salmonella* counts by 10^3- to 10^4-fold (2). Initial flora counts are therefore very important in ensuring that pasteurisation produces a safe product. Additives may, however, partly protect pathogens during processing. For example, adding salt or sugar to liquid yolk affects the D-value for *Salmonella*. The D-value for *Salmonella* in egg yolk at 64.4 °C is typically less than 0.2 minutes, but it increased to 6.4 minutes when 10% salt was added (11). *Salmonella* will not grow in products with 10% salt, however, and will die off after a few weeks of storage (2).

The authors obtained similar results for *Listeria* spp. These findings were supported by other studies that indicated that current HTST pasteurisation conditions for liquid egg would not ensure a *Listeria*-free product, particularly if the products were supplemented with salt (12) and if initial populations were large (13). Minimal (USA) pasteurisation parameters (60 °C for 3½ minutes for liquid whole egg) normally reduce populations of *L. monocytogenes* by 2 - 3 log cycles (14). In practice, tests on pasteurised liquid egg proved to be negative for *Listeria*, providing an indication of the quality of the unpasteurised liquid, and the efficacy of the pasteurisation process (9). Each country has its own regulations for pasteurisation of liquid eggs (2).

Mesophilic aerobic bacteria may be present in liquid egg at levels of $<10^5$/g. In one study (15), *Alcaligenes*, *Bacillus*, *Escherichia*, *Flavobacterium* spp., and Gram-positive cocci were recovered from pasteurised liquid egg, and in another study (16), *Micrococcus*, *Streptococcus*, and *Bacillus* were isolated, as were low numbers of faecal streptococci. Similar results were obtained when liquid egg was pasteurised at 65 °C for 3 minutes (17); organisms related to *Microbacterium lacticum*, *Bacillus* spp., and unidentified cocci and coccibacilli being identified. When liquid egg products were examined (18) for 4 months in three processing plants, at no time was enterohaemorrhagic *Escherichia coli* (EHEC) detected, suggesting that this organism is unlikely to be present in pasteurised eggs. *Pseudomonas* and *Serratia* spp. have, however, been isolated from pasteurised whole eggs although adding salt to egg yolk may reduce the *Serratia* population. Yeasts have been detected in low numbers in liquid egg products.

6.4.2.2 Dried egg products

While most dried egg products are produced by spray drying, processes such as pan drying or freeze drying may also be used.

6.4.2.2.1 Dried egg products – effect of processing

Spray drying

To produce dried egg products, the liquid egg is homogenised. The chalazae and contaminants such as membranes and shell are then filtered out before the liquid is pasteurised (Figure 6.2). The liquid egg product is then pumped through an atomising device (pressure nozzle or a centrifugal atomiser) into a hot (145 - 200 °C) air stream. The product is dried in vertical or horizontal chambers in which air flows either co- or counter-currently with the atomised product. Screw conveyors, drags, air sweeps, etc. remove the powder that separates from the air in the drying chamber, while a secondary collection system (e.g. cyclone-type) removes most of the remaining product. Excessive heat damage will occur if the product is not removed quickly from the air stream. Therefore, the powder is removed and cooled rapidly by mixing cooling air with the powder, or adding coolants, such as CO_2, or cooling by direct contact with a cold surface. Dried egg products may be packed in fibre drums lined with polythene, polythene bags, cartons, or tins.

While whole egg, and yolk powder are normally produced by drying, the pasteurised liquid product, dried albumen, may be produced from liquid albumen in which the level of glucose has been reduced to less than 1% using either glucose oxidase or a bacteria or yeast fermentation. This prevents discoloration and unpleasant odours due to the Maillard reaction.

Dried whole egg has a shelf life of about one month at room temperature (RT), and about a year if the product is refrigerated. In contrast, in dry storage conditions, dried albumen (glucose <1 %) products can be kept almost indefinitely without noticeable deterioration. Dried yolk has a shelf life of about 3 months at RT and, when refrigerated, a year. However, as with albumen, removing the glucose from the egg yolk extends its shelf life to approximately 8 months at RT, and more than a year if refrigerated.

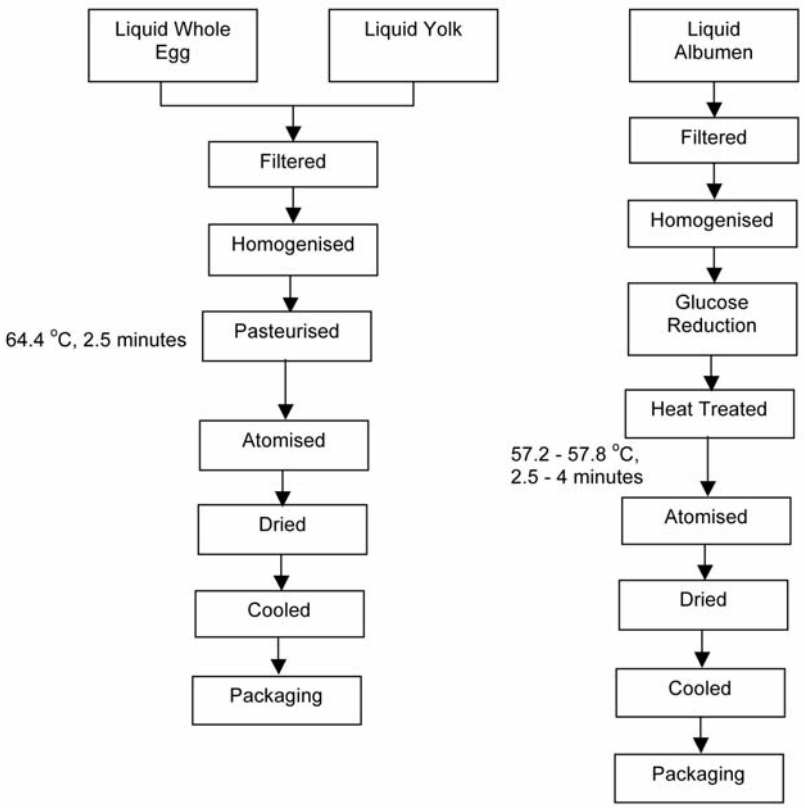

Fig. 6.2. Production of spray-dried egg

Pan drying

Pan drying may be used to produce dried, and in particular, flake-type albumen products. Although both air ovens, and water-jacketed pans (water flows beneath the pan) are used for this process, the latter gives better temperature control. This is important because albumen coagulates at temperatures exceeding 54 °C. Drying to a moisture level of 12 - 16% will produce a flake-type material, and if required, this may be milled to a fine powder.

Freeze drying

While freeze drying is a relatively simple procedure, it is expensive. For this reason, its use for egg products tends to be restricted to the production of added-value meals such as pre-cooked scrambled egg.

6.4.2.2.2 Dried egg - effect on microflora

Drying will significantly reduce the total bacterial load, but because some bacteria are resistant to drying it does not necessarily produce a sterile or even pasteurised product. It is therefore a requirement in the UK that dried egg products be produced from pasteurised liquid egg. This ensures very low numbers of bacteria in the final product. No *Salmonella* or *Shigella* strains were isolated in a Polish study of 610 yolk powder samples (19); however, *E. coli* was found in 13.9% of the samples, coagulase-positive staphylococci in 4.1%, enterococci in 10.5% and *Proteus* spp. in 9.0% of the samples. Overall, the contamination with aerobic bacteria was 8.5 x 10^2/g, with 2.6% of samples showing the highest rate of contamination. Anaerobic sporogenic bacilli were found in 2.6% of samples, and moulds or yeast in 13.2% of samples. In another study (16), *Micrococcus* spp., *Staphylococcus* spp., and *Bacillus* spp. were the predominant flora in dried egg (average count of 6,900 bacteria/g).

6.4.2.3 Frozen egg

Sugar and salt (10% w/v) may be added to inhibit frozen egg yolk gelling (Figure 6.3). Liquid egg must be pasteurised, for example, before a blast freezer is used to freeze the product.

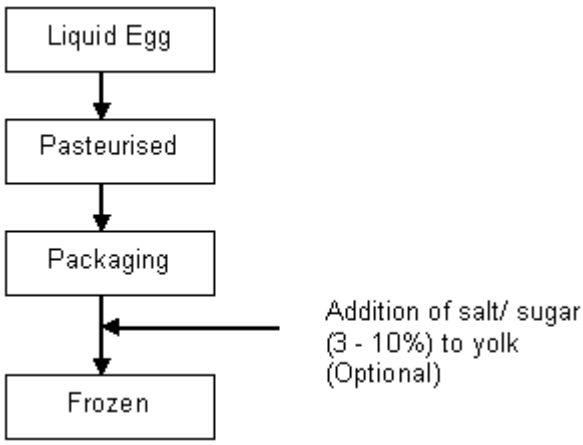

Fig. 6.3. Production of frozen egg

6.4.2.3.1 Frozen egg - effect on microflora

Freezing unpasteurised egg will kill up to 99% of the bacteria present. One report (15) suggests that when liquid egg is pasteurised prior to freezing, *Bacillus* dominates (83.5%). Another study (16) indicated, however, that *Micrococcus* spp.,

Staphylococcus spp., *Pseudomonas* spp. and *Streptococcus* spp. were present at levels ranging from 30 - 1,500 bacteria/g (average 410 bacteria/g) in frozen pasteurised liquid egg. In a survey (16) of 44 pasteurised frozen egg products, it was reported that the total viable counts (TVCs) for albumen were significantly lower (220 counts per g) than those for yolk and whole egg, which had 500 and 630 counts per g, respectively.

6.4.2.4 Hard-cooked eggs

Hard-cooked eggs are produced by submerging the eggs for 20 - 30 minutes in potable water heated to approximately 90 °C. Following rapid cooling, the shell is removed, either by hand, or by machine. Hygienic practices are paramount for whichever method is used to remove the shell. Following shelling, the eggs are packed in containers with an organic acid (e.g. citric), and 0.1% sodium benzoate or potassium sorbate (mould inhibitor), and then refrigerated. Eggs can be further processed by being placed in retortable pouches or jars, etc., and then heated at 121 °C for 15 minutes. Following heating, these packages can be held at RT for more than 5 months (20). Hard-cooked eggs have many uses, including pickling, and as diced eggs for egg salad.

6.4.2.4.1 Hard-cooked eggs - effects on microflora

A study (21) of hard-cooked eggs pickled in 3% acetic acid found low numbers of *Staphylococcus*, aerobic, and anaerobic spores, but no coagulase-positive organisms. It has been reported (22) that yeast and mould growth has occurred in packages of hard-cooked eggs packed in diluted citric acid following one week's storage, although no bacterial cells were detected.

6.5 Spoilage

6.5.1 Shell eggs

Most contaminants detected in the egg penetrated the shell through some of the *ca* 10,000 tube-like pores (*ca* 10 μm in diameter), through which the embryo breathes. As noted above, the egg remains relatively free from microorganisms for 10 - 20 days, given good conditions of hygiene and storage (2). The movement of contaminants from the shell to the egg contents may be active, for example micro-fungi, or passive, as is the case for bacteria. For example, when stored in humid conditions, micro-fungi, such as *Cladosporium herbarum*, may grow on the proteinaceous cuticle that envelops the shell. The hyphae may penetrate the pore canals and eventually grow throughout the albumen (23). While the dry environment of the shell favours Gram-positive bacteria, Gram-negative bacteria dominate the flora in the contents of contaminated eggs. The change from what is predominantly a Gram-positive flora on the shell to a Gram-negative flora in the

egg occurs in the shell membranes. Organisms that thrive in the egg tend to have relatively simple nutritional requirements, and some are able to grow and multiply at low temperatures. The bacteria that cause taints in eggs, as opposed to those that cause rots, are unable to digest proteins, form hydrogen sulphide, split lecithin, or produce pigment (5).

At ambient temperature, some 10 to 20 days elapse between bacterial infection of the shell and membranes with bacteria, and the appearance of large numbers of organisms in the albumen or yolk. Board (24) concluded that this lag period ended either when the yolk, which floats towards the top of the egg during storage, made contact with the contaminated membranes or when bacteria migrating through the albumen reached the yolk. It has also been postulated (25) that quiescent cells in the albumen multiply when they come into contact with micronutrients that diffuse out from the yolk. Although there are different explanations for the eventual growth of bacteria in the albumen, most agree that it is the antimicrobial proteins found in albumen that limit the initial growth of contaminants. From a historical perspective, lysozyme was considered to be one of the more important antimicrobial proteins. However, recent studies (26) have highlighted the role of arguably a more important protein, the iron-chelating compound, ovotransferrin. Other antimicrobial proteins isolated (5) from albumen include avidin and ovoflavoprotein, which bind biotin and riboflavin, respectively, making it unavailable to bacteria. In addition, several proteins inhibit enzyme activity: ovomucoid and ovoinhibitor inhibit trypsin, while ovoinhibitor also inhibits a-chymotrypsin, subtilisin and fungal proteinase. Albumen also contains a Ficin-papain inhibitor. Furthermore, the rapid rise in albumen pH from near neutrality to 9.5, which occurs following laying, is itself an inimical factor.

While there are variable numbers of organisms on a shell, it is notable that the most common contaminants occur on eggs throughout the world, and from a range of production systems. Species of *Pseudomonas* are the main spoilage organisms of shell eggs, and egg products. When the inner membrane of hens' eggs were inoculated with *Pseudomonas putida*, *Staphylococcus xylosus*, *Enterococcus faecalis*, and *E. coli*, it was *Ps. putida* that eventually dominated the albumen of eggs stored at 4, 15 and 20 °C (27). Although *Pseudomonas* dominates the microflora of addled eggs, other common spoilage organisms include *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Citrobacter*, *Cloaca*, *Escherichia*, *Hafnia*, *Proteus* and *Serratia* (5). Egg-spoilage organisms frequently change the colour and appearance of the yolk and albumen. The following colour changes have been described:

1. *Black rot* (*Proteus* and *Aeromonas*): The egg contents appear dark brown (*Proteus* spp.), or the yolk may be gelatinous and blackened with a grey, watery albumen (*Aeromonas liquefaciens*). This type of rot occurs more frequently at RT than under refrigeration.

2. *White rot* (*Citrobacter*, *Salmonella* and *Alcaligenes*): The egg yolk has a crusted appearance, and there may be a fruity odour.

3. *Fluorescent green rot* (*Pseudomonas*): *Ps. putida* and *Pseudomonas fluorescens* cause fluorescent green albumen.

4. *Fluorescent blue rot* (*Pseudomonas*): *Pseudomonas aeruginosa* causes fluorescent blue albumen.

5. *Red rot* (*Serratia*): *Serratia marcescens* may cause a red discoloration of the albumen, and surround the yolk with custard-like material.

6. *Custard rot* (*Enterobacter spp*.): The yolk, which may be flecked with olive-green pigment, is encrusted with custard-like material. The albumen is watery, may have an orange tint, and can have a putrid odour. *Citrobacter*, and *Proteus vulgaris* have been associated with this type of spoilage. *Bacillus cereus* produces lecithinase, which breaks down the lecithin, resulting in a "custard yolk."

7. *Rust red rot* (*Proteus*): This is caused by the growth of *P. vulgaris*.

6.5.2 Liquid egg

Initial contaminants in liquid egg reflect those found in the shell egg. While pasteurising liquid egg reduces the number of bacteria by 99%, the final product may still contain *Pseudomonas* spp., *Serratia* spp., and salt-tolerant micrococci (16, 28). Before the introduction of the pasteurisation regulations, *Pseudomonas*, *Flavobacterium*, and *Chromobacterium* caused fishy odours in liquid egg, while other off-odours were attributed to *Alcaligenes*, *Bacillus*, *Escherichia*, *Proteus*, *Pseudomonas*, and *Flavobacterium*. Pasteurised liquid egg typically has bacterial counts of <100 cfu/g (2).

6.5.3 Dried egg and frozen egg

The introduction of pasteurisation has significantly reduced the number of contaminants in these products. Those organisms that do occur are those that are found in liquid egg, e.g. *Pseudomonas* spp. have been recovered from frozen egg (16).

6.6 Pathogens: Growth and Survival

6.6.1 Shell eggs

Approximately 90%, and possibly more newly laid eggs are free from microorganisms. Therefore, most contamination must occur because pathogens pass through the shell. Organisms on the shell can also contaminate the egg contents when the egg is broken.

6.6.1.1 Salmonella spp.

Note that the more common shortened form of nomenclature *Salmonella* Enteritidis, for example as opposed to the full name, *S. enterica* Serovar Enteritidis is used here.

For many years there was a relatively low incidence of *Salmonella* poisoning associated with eggs (except duck eggs). This is understandable because, although a wide range of *Salmonella* serotypes have been isolated from shells (29), including *Salmonella* Bareilly, *Salmonella* Derby, *Salmonella* Essen, *Salmonella* Thompson, *Salmonella* Typhimurium, *Salmonella* Worthington, *Salmonella* Montevideo, and *Salmonella* Oranienburg, salmonellae on eggshells tend to die rapidly. A survey (30) of eggs passing through an egg-processing plant showed that approximately 1% of the shells were contaminated with either *Salmonella* Heidelberg, or *S.* Montevideo.

In the late 1980s the incidence of salmonellosis associated with eggs increased, and in particular outbreaks due to *S.* Enteritidis. Research suggested that *S.* Enteritidis was capable of infecting a bird's ovaries, and thereby contaminating eggs before oviposition. Prior to this finding it was assumed that the host-specific *Salmonella* Gallinarum, and *Salmonella* Pullorum were the main salmonellae capable of infecting the ovaries. There is still a lot of debate about the proportion of eggs infected by the ovarian route or in the oviduct, versus those contaminated by the organism passing across the shell. There is evidence that particular serotypes of *S.* Enteritidis, such as PT4 (31), may be better adapted to multiplying in the ovaries, whereas *S.* Enteritidis PT13A is usually associated with faecal contamination of the shell.

There is no clear correlation between the level of *Salmonella* infection detected in a flock, and the number of contaminated eggs. For example, results from 22 flocks that were positive for *S.* Enteritidis PT4 showed that between 0.1 and 10% of the eggs were contaminated (mean of 0.6%) (31). Similarly, the number of bacteria isolated from the egg varies. Differences in the time that elapsed between the eggs becoming contaminated, and the time of sampling may account for some of this variation. It has been shown that there is a positive relationship between the age of an egg, and the number of *S.* Enteritidis recovered from its contents (25).

Although storage temperature affects the time taken for bacteria to multiply in the egg, there is evidence that the hostile environment within the egg tends to limit the growth of *Salmonella*, and other bacteria for up to 20 days. The importance of the yolk as a source of nutrients is shown by a study in which an initial inoculum of <5 cells established growth of *S.* Enteritidis in the yolk (25), a finding consistent with studies with other *Salmonella*. If the initial inoculum is not placed directly into the yolk, the survival and growth of *S.* Enteritidis depends on the pH of the albumen, the size and position (relative to the yolk) of the inoculum, the age of the egg, and the storage temperature. It has been shown (25) that following an inoculum of <10 cells *S.* Enteritidis will survive and grow only if the organisms are placed adjacent to the yolk of eggs that have been stored for three weeks or more at 20 °C. Temperature may also influence the type of contaminant that flourishes in the egg contents. Thus, inoculation of the inner membrane of hens' eggs with *Ps. putida*, *Staph. xylosus*, *E. faecalis*, *E. coli* and *S.* Enteritidis resulted in *Ps. putida* eventually dominating the albumen of eggs stored at 4, 15 and 20 °C, while *S.* Enteritidis became dominant at 37 °C (27).

In the light of continued problems with the contamination of chickens and eggs by *Salmonella*, the UK government instigated a vaccination policy for UK breeding flocks in 1993; vaccination of breeding chickens began in 1994, and vaccination of commercial laying hens in 1997. This lead to a dramatic reduction in the number of *Salmonella* cases in humans from a peak of over 35,000 cases in 1997, down to 11,529 in 2005 (32). Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified foodborne zoonotic agents, is now in place and prohibits the use of eggs for direct human consumption, as table eggs, unless they originate from a commercial flock of laying hens subjected to a national control programme, and are not under official restriction.

Compared with the yolk or albumen of hens' eggs, that of ducks' eggs is more frequently infected with *Salmonella*. This may be a reflection of the greater porosity of the shell of ducks' eggs, but probably also involves the frequently wet, and muddy environment that many ducks inhabit.

Food poisoning outbreaks associated with *Salmonella* in eggs have been reviewed recently (33). In summary, outbreaks tend to be associated with products such as mayonnaise, home-made ice cream, and milk drinks that contain raw eggs. An example of such an outbreak was reported in 1991 (34). The 15 restaurant patrons who were affected had all consumed Caesar salad. In addition, because they had either handled, or consumed the raw egg, 29% of the restaurant's employees also developed gastroenteritis caused by *S.* Enteritidis. Phage typing identified the organisms as *S.* Enteritidis type 8.

A recent outbreak, in 2004, in the UK associated with caterers was found to be caused by eggs imported from Spain (35). Spain does not employ a vaccination policy against *Salmonella*, to reduce the levels of *Salmonella* in breeding or laying

flocks. It was found that some caterers in the North West of England were using eggs imported from Spain in recipes that did not ensure adequate kill of the pathogen. A Food Standards Agency (FSA) survey of almost 1700 boxes of eggs imported from outside the UK identified that two thirds of the eggs that were tested came from Spain (36). Of the 1700 boxes tested, 157 boxes were contaminated with *Salmonella*, at an estimated incidence of 3.3%. 154 of the 157 contaminated samples came from Spain. PT1 was the most commonly isolated serovar, with 120 isolates. This is not a common serovar in the UK; PT4 is more commonly isolated.

6.6.1.2 Campylobacter spp.

Campylobacter jejuni has been isolated on the shells of table eggs, and also, occasionally, from the inner shell membrane of refrigerated eggs. *Campylobacter* spp. has not been implicated, however, in any food-poisoning outbreaks related to eggs.

6.6.1.3 Listeria spp.

L. monocytogenes has been isolated from the surfaces of egg shells, but not from the egg contents.

6.6.1.4 Aeromonas spp.

Aeromonas spp. have been cultured from eggs (37). They multiply rapidly at 4 °C, and therefore, although no reports exist in the literature, represent a potential threat when eggs are stored in refrigerators (37).

6.6.2 Liquid egg

6.6.2.1 Salmonella spp.

It was the increase in the incidence of salmonellosis associated with spray-dried, and bulk liquid egg in the 1940s and '50s that led to the introduction of the Liquid Egg (Pasteurisation) Regulation, 1963, later replaced by the Egg Products Regulations 1993. Following the introduction of the 1963 regulations the reported number of outbreaks of human salmonellosis in England and Wales fell by 25%.

Although it has been suggested that *S.* Enteritidis PT4 may be more resistant to heating than some other salmonellae commonly associated with poultry products, there have been no outbreaks associated with products made from pasteurised egg.

6.6.2.2 Listeria spp.

A pasteurisation temperature of 60 °C for 3½ minutes will reduce *L. monocytogenes* populations by only 2 - 3 log cycles. This accounts, in part, for why *L. monocytogenes* has been identified in pasteurised liquid egg *L. monocytogenes* can survive in liquid albumen and liquid whole egg, and will multiply in liquid yolk, when held at 4 °C (38).

6.6.2.3 Staphylococcus aureus

Staph. aureus can grow in liquid egg if it is held above 15.6 °C (2). They could potentially grow in salted liquid egg since they are relatively tolerant of low water activity (a_w), and high levels of salt.

6.6.3 Dried egg

6.6.3.1 Salmonella spp.

Contamination of spray-dried whole egg with salmonellae first occurred during the years 1939 - 45; however, since the introduction of pasteurisation, outbreaks of salmonellosis due to dried egg have ceased in the UK.

6.6.3.2 Campylobacter spp.

Campylobacter is susceptible to high temperatures, dry environments, and the presence of oxygen, and consequently is unlikely to survive in egg powder.

6.6.3.3 Listeria spp.

L. monocytogenes can survive spray drying (39); however, provided that pasteurised egg is used for the production of dried egg, it is unlikely that *L. monocytogenes* will survive both processes.

6.6.4 Frozen egg

Outbreaks of enteric fever and salmonellosis, due to *Salmonella*, including paratyphoid bacilli, have been attributed (40) to the use of Chinese frozen whole eggs for cakes in bakeries. However, these cases pre-date the requirement to produce frozen egg from pasteurised liquid egg.

6.6.5 Speciality egg

6.6.5.1 Salmonella spp.

Outbreaks of *S.* Enteritidis PT4 infection (33) in the late 1980s and early 1990s were found to be associated with the consumption of products containing raw eggs, and in particular, mayonnaise, mousse, and lightly cooked, but not soft-boiled eggs.

The storage (23.9 °C for up to 2 weeks) of cholesterol-free reduced-calorie mayonnaise, and reduced-calorie mayonnaise that had been inoculated with *Salmonella* spp. at the level of 10^6 cfu/g resulted in the death of all *Salmonella* within 48 hours when the mayonnaise was made with 0.7% acetic acid. However, when the mayonnaise was made with 0.3% acetic acid it required 14 days storage before *Salmonella* was not detectable (41). Another study that examined sandwich spread, real mayonnaise, reduced-calorie mayonnaise, and cholesterol-free reduced-calorie mayonnaise produced similar results. The survival of *Salmonella* spp. in home-made mayonnaise is influenced by the pH of the mayonnaise, and the acidulant used. It is recommended that mayonnaise is prepared with acetic acid to a pH of 4.1, and not citric acid, which is less efficacious (42). Storage of mayonnaise at refrigeration temperatures protects *Salmonella* spp. from acidulants, and a holding time of 24 hours at 18 - 20 °C is therefore advised before refrigeration. As in liquid egg, if salt is added to mayonnaise, this can have a protective effect, for example, on *S.* Enteritidis.

6.6.5.2 Listeria spp.

Although *L. monocytogenes* has been shown to be more resistant to the effects of the acidulants in mayonnaise, even with an initial inoculum of 10^6 cfu/g, no *L. monocytogenes* was detected at 14 or 10 days in mayonnaise samples made with 0.7% acetic acid, and stored at 23.9 °C for up to 2 weeks (41).

L. monocytogenes has been shown to grow in eggnog containing 7% ethanol (43).

6.7 Published Microbiological Criteria

Microbiological criteria (Food Safety Criteria and Process Hygiene Criteria) relating to egg products, and ready-to-eat foods containing raw eggs is covered under EC Regulation 2073/2005 on microbiological criteria for foodstuffs. The legal aspects are considered in more detail in the Legislation chapter (44).

Microbiological criteria were also laid down in The Egg Products Regulation 1993. These could be used as guidance:

Salmonellae: absent in 25 g or 25 ml of egg product
Mesophilic aerobic bacteria: $M^{\dagger} = 10^5$ in 1 g or 1 ml
Enterobacteriaceae: $M = 10^2$ in 1 g or 1 ml
Staph. aureus: absence in 1 g of egg products

where, $M^{\dagger}$ = maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more sample units is M or more.

Source: Statutory Instruments 1993 No. 1520 Food

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7. HACCP IN MEAT AND MEAT PRODUCT MANUFACTURE

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7.1 Introduction

The Hazard Analysis Critical Control Point (HACCP) system is a structured, preventative approach to ensuring food safety. HACCP provides a means to identify and assess potential hazards in food production and establish preventive control procedures for those hazards. A critical control point (CCP) is identified for each significant hazard, where effective control measures can be defined, applied and monitored. The emphasis on prevention of hazards reduces reliance on traditional inspection and quality control procedures, and end-product testing. A properly applied HACCP system is now internationally recognised as an effective means of ensuring food safety.

The HACCP concept can be applied to new or existing products and processes, and throughout the food chain from primary production to consumption. It is compatible with existing standards for quality management systems such as the ISO 9000-2000 series, and HACCP procedures can be fully integrated into such systems. The new ISO 22000 food safety standard formally integrates HACCP within the structure of a quality management system. HACCP is fully integrated into the British Retail Consortium (BRC) Global Standard for Food Safety, and is one of the ‘fundamental’ requirements of that system.

The application of HACCP at all stages of the food supply chain is actively encouraged, and usually required, worldwide. For example, the Codex Alimentarius advises that "the application of HACCP systems can aid inspection by regulatory authorities and promote international trade by increasing confidence in food safety".

In many countries, there is a legal requirement for all food business operators to have some form of hazard analysis based on HACCP as a means of ensuring food safety. For example, within the European Union, Regulations 852/2004 and 853/2004 require a fully operational and maintained HACCP system, according to Codex, to be in place.

7.2 Definitions

*Contro*l (verb) - To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.

Control (noun) - The state wherein correct procedures are followed and criteria are met.

Control measure - An action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Corrective action - An action to be taken when the results of monitoring at the CCP indicate a loss of control.

*Critical Control Poin*t (CCP) - A step at which control can be applied and is essential to prevent or eliminate a food safety hazard, or reduce it to an acceptable level.

Critical limit - A criterion which separates acceptability from unacceptability.

Deviation - Failure to meet a critical limit.

Flow diagram – A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

HACCP - A system which identifies, evaluates, and controls hazards which are significant for food safety.

HACCP Plan – A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for safety in the segment of the food chain under consideration.

Hazard - A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis - The process of collecting and evaluating information on hazards and the conditions leading to their presence to decide which are significant for food safety and therefore should be addressed by the HACCP plan.

Monitoring – The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

Step - A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.

Validation - Obtaining evidence that the elements of the HACCP plan are effective.

Verification - The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

7.3 Stages of a HACCP Study

The HACCP system consists of the following seven basic principles:

1. Conduct a hazard analysis.

2. Determine the CCPs.

3. Establish the critical limit(s).

4. Establish a system to monitoring control of the CCP.

5. Establish the corrective action to be taken when monitoring shows that a CCP is not under control.

6. Establish procedures for verification to confirm that the HACCP system is working effectively.

7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

It is recommended by the Codex Alimentarius that the practical application of the HACCP principles be approached by breaking the seven principles down into a 12-stage logic sequence. Each stage is discussed below in detail. Figure 7.1 is a flow diagram illustrating this 12-stage logic sequence.

7.3.1 Assemble HACCP team

HACCP requires management commitment of resources to the process. An effective HACCP plan is best carried out as a multidisciplinary team exercise to ensure that the appropriate product-specific expertise is available. The team should include members familiar with all aspects of the production process as well

as specialists with expertise in particular areas such as production, hygiene managers, quality assurance or control, ingredient and packaging buyers, food microbiology, food chemistry or engineering. The team should also include personnel who are involved with the variability and limitations of the operations. If expert advice is not available on-site, it may be obtained from external sources.

The scope of the plan should be determined by defining the extent of the production process to be considered and the categories of hazard to be addressed (e.g. biological, chemical and/or physical).

7.3.1.1 Meat industry

Apart from the previously suggested team members, it would be useful to have someone familiar with the slaughter and butchery practices applied at the abattoir. In addition, it is desirable to have close contacts with the farmers or their co-operatives, so that someone is familiar with information concerning the agricultural practices applied from the birth of the animal to its arrival at the abattoir.

The animal itself is a major contributor of both pathogenic and spoilage organisms, and so every stage in the rearing of the animal could comprise a critical control point that should be controlled by application of HACCP. Therefore, companies with integrated operations are better able to control such hazards than those who purchase livestock on the open market.

7.3.2 Describe the product

It is important to have a complete understanding of the product, which should be described in detail. The description should include information such as the product name, composition, physical and chemical structure (including water activity (a_w), pH, etc.), processing conditions (e.g. heat treatment, freezing, fermentation, etc.), packaging, shelf life, storage and distribution conditions, and instructions for use.

7.3.3 Identify intended use

The intended use should be based on the expected uses of the product by the end-user or consumer (e.g. is a cooking process required?). It is also important to identify the consumer target groups. Vulnerable groups of the population, such as children or the elderly, may need to be considered specifically.

7.3.3.1 Meat industry

Meat products may be consumed by high-risk groups, such as the young, the elderly and the immunocompromised. Therefore the instructions for use of these products should receive particular attention.

7.3.4 Construct a flow diagram

The flow diagram should be constructed by the HACCP team and should contain sufficient technical data for the study to progress. It should provide an accurate representation of all steps in the production process from raw materials to the end-product. It may include details of the factory and equipment layout, ingredient specifications, features of equipment design, time/temperature data, cleaning and hygiene procedures, and storage conditions. Ideally, it should also include details of CCP steps, once determined.

7.3.4.1 Meat industry

Many meat-processing lines have simple flow diagrams (refer to the production lines described in the product in previous chapters of this book). These can include steps such as washing, evisceration, chilling, smoking, drying, cooking, and packaging.

Although the flow diagrams may seem simple, the flow of the product and possibilities for re-contamination are not always obvious. Pumps, pipes, valves, scalding or chilling tanks, conveyor belts, air handling and cleaning systems may complicate the safe manufacture of meat products.

7.3.5 On-site confirmation of the flow diagram

The HACCP team should confirm that the flow diagram matches the process that is actually being carried out. The operation should be observed at all stages, and any discrepancies between the flow diagram and normal practice must be recorded and the diagram amended accordingly. It is also important to include observation of production outside normal working hours such as shift patterns and weekend working, and circumstances of any reclaim or rework activity. It is essential that the diagram is accurate, because the hazard analysis and decisions regarding CCPs are based on these data. If HACCP studies are applied to proposed new process lines or products, then any pre-drawn HACCP plans must be reviewed once the lines or products are finalised.

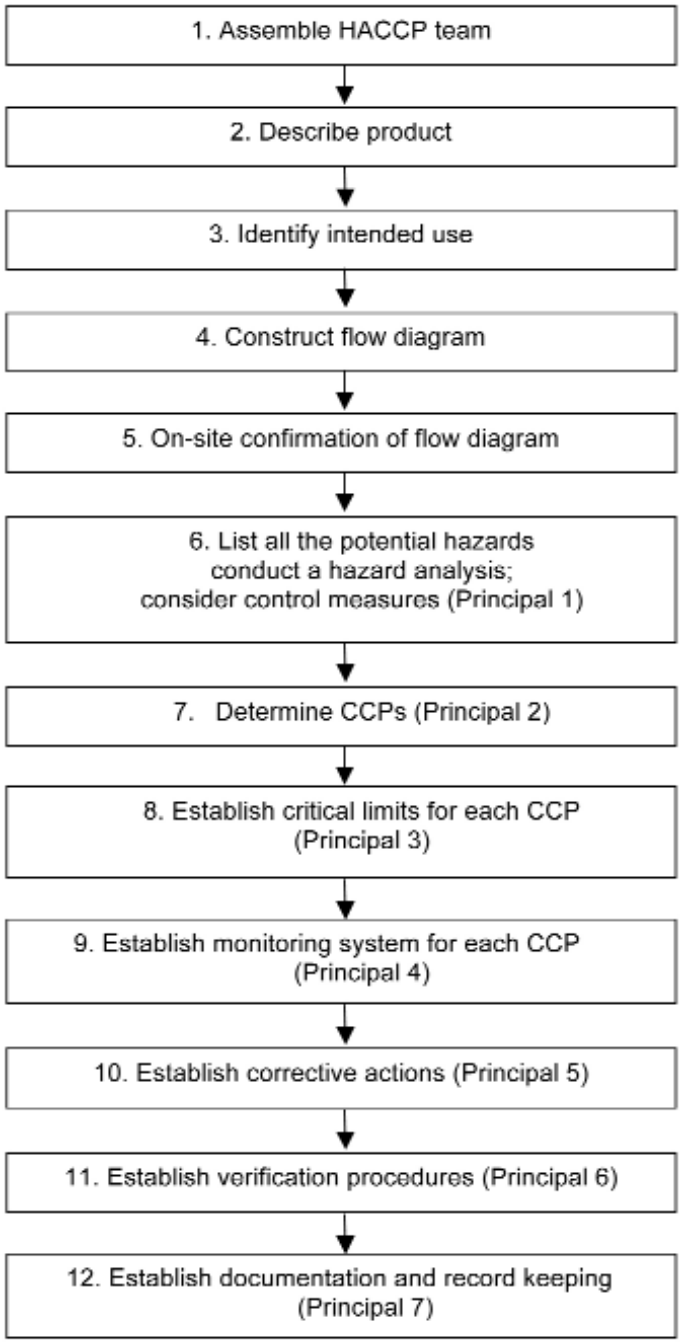

Fig. 7.1. Logic sequence for application of HACCP

7.3.6 List all potential hazards associated with each step; conduct a hazard analysis; and identify any measures to control identified hazards

The HACCP team should list all hazards that may reasonably be expected to occur at each step in the production process.

The team should then conduct a hazard analysis to identify which hazards are of such a nature that their elimination or reduction to an acceptable level is essential to the production of safe food.

The analysis is likely to include consideration of:

- ❖ the likely occurrence of hazards and the severity of their adverse health effects;
- ❖ the qualitative and/or quantitative evaluation of the presence of hazards;
- ❖ survival or multiplication of pathogenic microorganisms;
- ❖ production or persistence of toxins;
- ❖ the hurdle effect;
- ❖ the number of consumers potentially exposed and their vulnerability;
- ❖ any food safety objectives or manufacturer's food safety requirements.

The HACCP team should then determine what control measures exist that can be applied for each hazard.

Some hazards may require more than one control measure for adequate control and a single control measure may act to control more than one hazard. One control measure may be relevant to several process steps, where a hazard is repeated.

Note: it is important at this stage that no attempt is made to identify CCPs, since this may interfere with the analysis.

7.3.6.1 Meat industry

The first step is to determine what likely hazards are associated with the raw materials, i.e. raw meat. Microbiological hazards mainly consist of bacteria and include *Salmonella, Campylobacter, Escherichia coli* O157:H7, *Yersinia enterocolitica*, and spore-forming pathogens such as *Clostridium botulinum, Clostridium perfringens* and *Bacillus cereus*. Also, certain parasites are important such as *Trichinella spiralis* in raw pork.

Chemical contaminants in meat may be due to consumption of contaminated feed (hormones, pesticides), or a result of veterinary therapy (antibiotics), or exposure to environmental pollution (lead, radioactive elements).

Control of these hazards must occur at the farm, and this shows the importance of the agricultural sector being represented on the HACCP team. Good farming practice is the major means of controlling animal disease.

Transporting the animals to the abattoir, and holding them in pens prior to slaughter can result in spread of infection from animal to animal. Therefore, good sanitary precautions are required, and it is preferable that the animals are held for as short a time as possible.

The methods used for the slaughter, and the environmental conditions during slaughter can allow contamination of the meat with microorganisms. Several measures can be taken to minimise this contamination including training workers in the proper use of knives and equipment (i.e. decontaminating knives between animals), providing adequate work space and time to carry out each task, having a plant layout that allows microbial control, using readily cleanable equipment, and using procedures that reduce or destroy organisms (i.e. spraying equipment or utensils with organic acids, or using steam).

The environment of the meat-processing plant may also be a source of hazards. It is important to be aware of the possibilities of contamination. However, since production technologies vary widely, the potential microbiological hazards vary from product to product (refer to the spoilage bacteria and pathogens associated with each different meat and meat product in previous chapters of this book).

Ingredients other than raw meat that are used in meat products also need to be inspected. These may include water, starter cultures, salt, spices, preservatives and gelatine. The quality of these ingredients can affect the end-product; for example, the addition of gelatine to a meat pie carries the risk of contamination with *Salmonella* spp., and so the gelatine must be heated to 90 °C before use.

There is also the possibility of the presence of physical hazards, such as metal, bone, plastic, wood or glass. These hazards are controlled by minimising their introduction into the product via the raw materials and processing conditions.

7.3.7 Determine CCPs

The determination of CCPs in the HACCP system is facilitated by using a decision tree (Figure 7.2) to provide a logical, structured approach to decision making. However, application of the decision tree should be flexible, and its use may not always be appropriate. It is also essential that the HACCP team has access to sufficient technical data to determine the CCPs effectively.

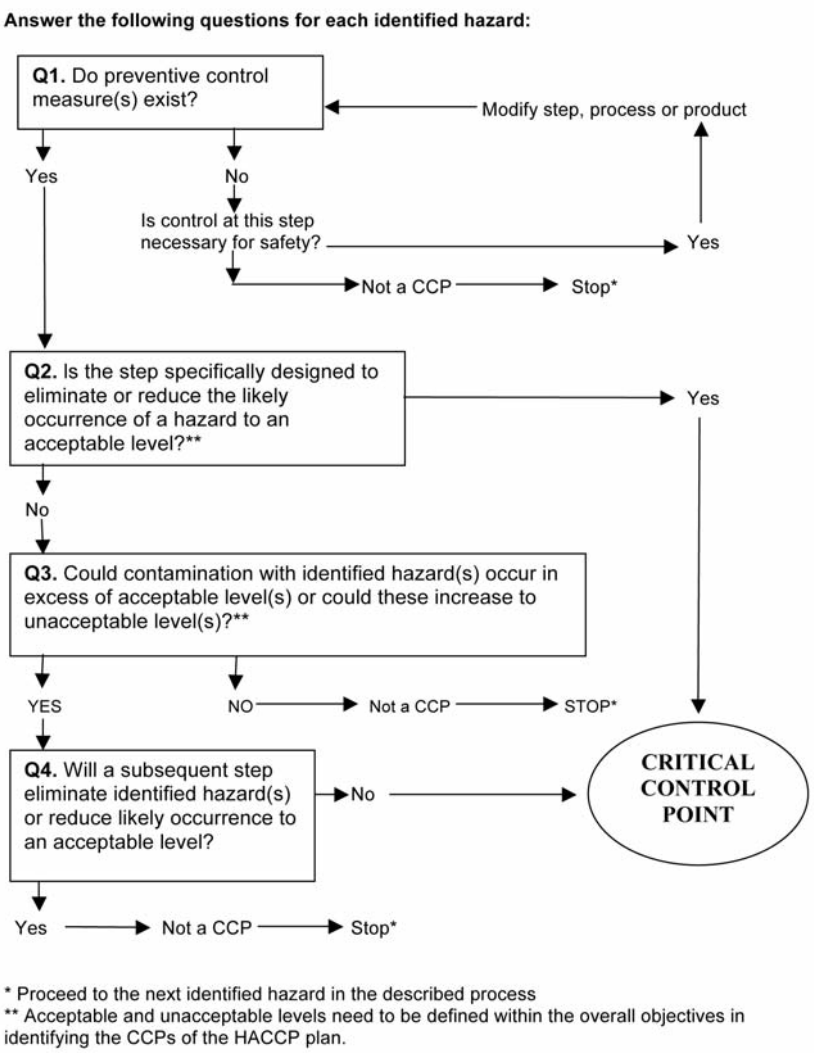

Fig. 7.2. CCP Decision Tree

(Adapted from Codex Alimentarius Commission, 2003)

If a significant hazard has been identified at a step where control is required for safety, but for which no control exists at that step or any other, then the process must be modified to include a control measure.

7.3.7.1 Meat industry

HACCP analyses on the production of raw poultry meat and manufacture of raw fermented meat products can be used to illustrate the principles of step 7.

7.3.7.1.1 Production of raw poultry meat

Figure 7.3 shows the schematic diagram of HACCP analysis of the production of raw poultry meat.

The major hazard in relation to raw poultry is that it can be contaminated with the foodborne pathogens *Salmonella* and *Campylobacter*. These organisms may be present in the breeding flock, and in the case of *Salmonella*, can contaminate the eggs, and lead to the rearing of contaminated birds. Therefore, it is important that the birds are obtained from *Salmonella*-free stock, and are fed *Salmonella*-free feed. This will reduce the hazard but cannot guarantee to eliminate the problem, because poultry may become contaminated from other animals harbouring *Salmonella*, such as rodents and wild birds.

The carcasses of the birds are scalded to facilitate the removal of feathers, and this is usually achieved by immersion of the carcass in a scalding tank. However, such a system allows for spread of pathogens from bird to bird, and the temperature used is not sufficient to kill off all the organisms. This is another case where the hazard can be reduced but not eliminated.

The washing stages are also recognised as CCPs. The spray washing of carcasses removes surface-contaminating material, including some of the surface microflora, but it does not remove all the microflora.

Evisceration often results in contamination of the birds, with cross contamination of the visceral cavity and exterior of the carcass by enteric pathogens. This process can be carried out manually or by automated machinery. In the case of manual evisceration, control of the process is achieved by the use of skilled operators and correct application of the technique. However, manual evisceration is being rapidly replaced by mechanical systems, and, with these, gut breakage occurs with greater frequency. Therefore, it is important to check the correct operation of the mechanical systems frequently in order to control this hazard.

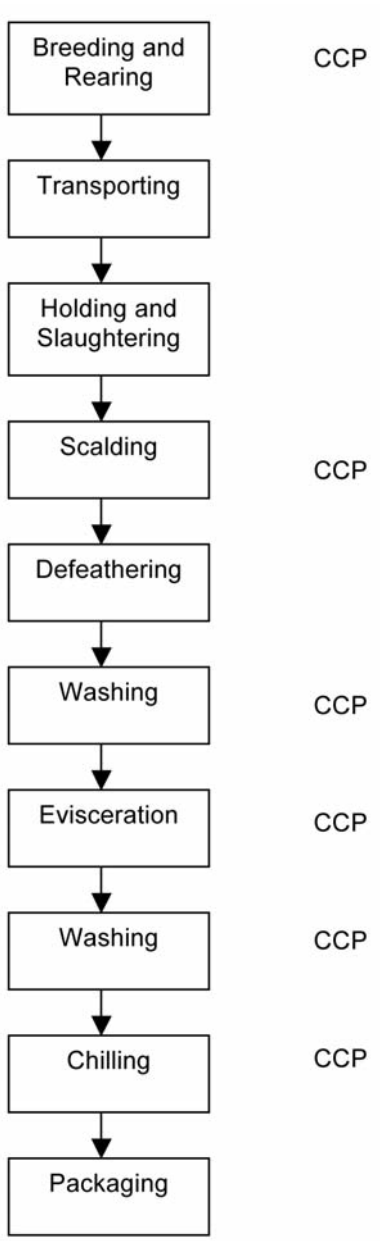

Fig. 7.3. Flow diagram for the production of raw poultry meat

The final CCP for poultry production is chilling. Continuous counter-current immersion chilling, using chlorinated water, is the method of choice. When

properly controlled, the chilling of carcases can prevent the outgrowth of most pathogens, and delay the growth of most spoilage organisms. However, there are organisms that are capable of growing at chill temperatures, and so this is another example of a CCP that reduces the hazard as opposed to eliminating it.

7.3.7.1.2 Raw fermented meat products

Raw fermented meats may be fully dried products (e.g. salamis) or semi-dried products (e.g. cervelat), and the manufacture of these products is depicted in the flow diagram in Figure 7.4.

As previously mentioned, the main hazards associated with raw meats are microbiological, and these are controlled at both the farm and abattoir level. In both cases, it is a process of reduction of the hazard as opposed to elimination.

The addition of curing salts and that of the starter cultures are also critical control points. It is essential that these ingredients are of the highest quality, so that additional contaminants are not introduced into the production process, and that these ingredients function correctly to provide favourable conditions, and then growth resulting in fermentation. The fermentation stage is another important CCP, because the rapid fall in the pH value results in inhibition of the growth of most pathogenic microorganisms. Similarly, the heating and drying stages may reduce the numbers of organisms or inhibit their growth.

It should be noted that none of these CCPs is absolute and so pathogenic microorganisms may be present in the final product.

7.3.8 Establish critical limits for each CCP

Critical limits separate acceptable from unacceptable products. Critical limits must be specified and validated for each CCP. More than one critical limit may be defined for a single step. Thermal critical limits usually have time and temperature parameters. Criteria used to set critical limits must be measurable and may include physical, chemical, biological or sensory parameters.

It is prudent to set stricter limits (often called target or process limits/levels) to ensure that any trend towards a loss of control is noted before the critical limit is exceeded.

7.3.8.1 Meat industry

In the US, the Department of Agriculture has established a minimum time and temperature regime for cooking roast beef. This critical level is designed to achieve a 6.5 log reduction of salmonellae in the coldest part of the joint.

However, this does not take into account the additional thermal destruction that occurs during the full heating and cooling period. Therefore, this allows for a normal variation of the temperature, without endangering product safety.

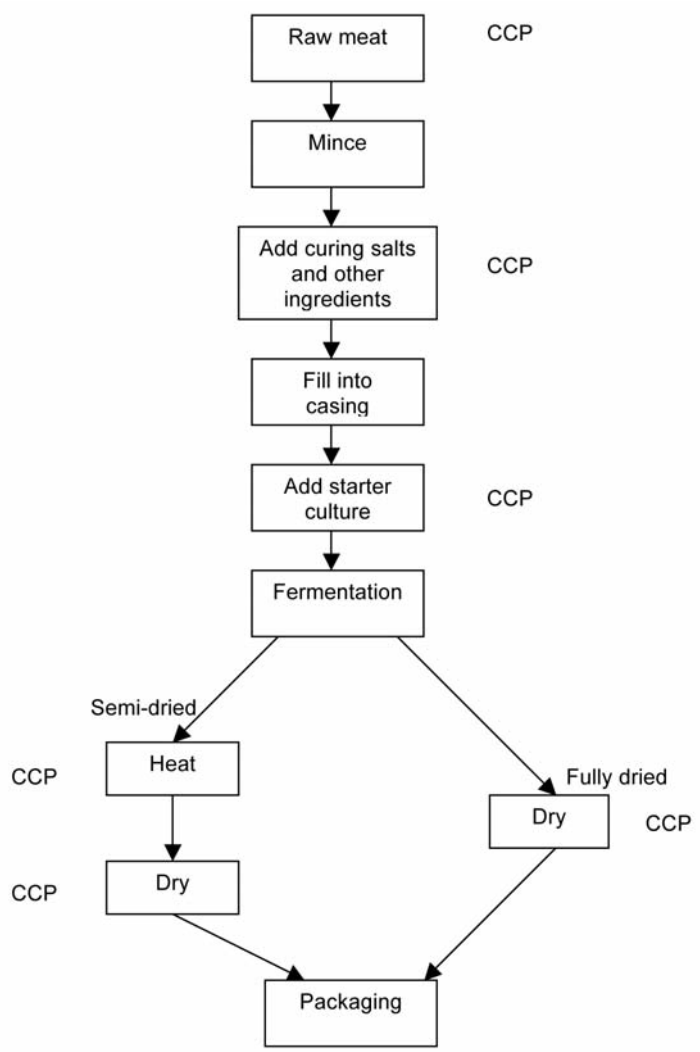

Fig. 7.4. Flow diagram for the production of raw fermented meat

With meat products that result from a fermentation process, acidity or pH may be the parameters necessary to prevent unacceptable growth of potential pathogens. So the critical limit may be that a specific pH level occurs at a specific point in the process, with a stricter pH value for the target level.

7.3.9 Establish a monitoring system for each CCP

Monitoring involves planned measurement or observation of a CCP relative to its critical limits. Monitoring procedures for target levels must be able to detect loss of control of the CCP, and should provide this information with sufficient speed to allow adjustments to be made to the control of the process before the critical limits are violated. Monitoring at critical limits should be able to detect rapidly when the critical limit has been exceeded. Monitoring should therefore either be continuous, or carried out sufficiently frequently to ensure control at the CCP. Therefore, physical and chemical on-line measurements are usually preferred to lengthy microbiological testing. However, certain rapid methods, such as ATP assay by bioluminescence, may be useful for assessment of adequate cleaning, which could be a critical limit for some CCPs, for example pre-start-up hygiene.

Persons engaged in monitoring activities must have sufficient knowledge, training and authority to act effectively on the basis of the data collected. These data should also be properly recorded.

7.3.9.1 Meat industry

A problem common to all industries is what to do with products or semi-finished products that cannot be safely consumed. It may be that the product can be reprocessed; however, this may require a rapid HACCP study because, without previous experience, this product should be regarded as a new raw material.

7.3.10 Establish corrective actions

For each CCP in the HACCP plan, there must be specified corrective actions to be applied if the CCP is not under control. If monitoring indicates a deviation from the critical limit for a CCP, action must be taken that will bring it back under control. Actions taken should also include proper isolation and disposition of the affected product, repair of defective equipment, and an investigation into why the deviation occurred. A further set of corrective actions should relate to the target levels, if process drift is occurring. In this case, only repair of the process defect and investigation of the fault are required. All corrective actions should be properly recorded.

7.3.11 Establish verification procedures

Verification usually involves auditing and testing procedures. Auditing methods, procedures and tests should be used frequently enough to determine whether the HACCP system is being followed, and is effective at controlling the hazards. These may include random sampling and analysis, including microbiological testing. Although microbiological analysis is generally too slow for monitoring

purposes, it can be of great value in verification, since many of the identified hazards are likely to be microbiological.

In addition, reviews of HACCP records are important for verification purposes. These should confirm that CCPs are under control and should indicate the nature of any deviations and the actions that were taken in each case. It is also useful to review customer returns and complaints regularly.

7.3.11.1 Meat industry

In the meat industry, certain tests are used as verification, e.g. microbiological analyses, determination of fat, moisture and protein, and checks on the final internal temperature of cooked products. These tests should not be used for release purposes. The HACCP system should ensure product safety by allowing release only when monitoring results indicate that the line is under control.

7.3.12 Establish documentation and record keeping

Efficient and accurate record keeping is an essential element of a HACCP system. The procedures in the HACCP system should be documented.

- Determination of critical limits
- The completed HACCP plan

Examples of documented procedures include:

- The hazard analysis
- Determination of CCPs

Examples of recorded data include:

- Results of monitoring procedures
- Deviations from critical limits and corrective actions
- Records of certain verification activities, e.g. observations of monitoring activities, and calibration of equipment.

The degree of documentation required will depend partly on the size and complexity of the operation, but it is unlikely to be possible to demonstrate that an effective HACCP system is present without adequate documentation and records. The length of time that records are kept will be as per company policy,

but should not be less than one year beyond the shelf life of the product. Three to five years is typical for many food companies.

7.4 Implementation and Review of the HACCP Plan

The completed plan can only be implemented successfully with the full support and co-operation of management and the workforce. Adequate training is essential and the responsibilities and tasks of the operating personnel at each CCP must be clearly defined.

Finally, it is essential that the HACCP plan be reviewed following any changes to the process, including changes to raw materials, processing conditions or equipment, packaging, cleaning procedures and any other factor that may have an effect on product safety. Even a small alteration to the product or process may invalidate the HACCP plan and introduce potential hazards. Therefore, the implications of any changes to the overall HACCP system must be fully considered and documented, and adjustments made to the procedures as necessary.

Triggered reviews/audits should occur as a result of changes, whereas there should be an annual scheduled review/audit, as a minimum.

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8. EC FOOD HYGIENE LEGISLATION

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8.1 Introduction

Hygiene is a key aspect of ensuring food safety, and one that plays an important role in most countries' food legislation. Hygiene is a general concept that covers a wide subject area, from structural conditions in the factory or process facility, to personnel requirements, final product specifications, conditions of raw materials, including microbiological criteria, transport and delivery vehicles requirements.

Microbiological standards have a useful role and help establish requirements for the microbiological safety and quality of food and raw materials. A number of standards are provided in food legislation; however, the existence of microbiological standards alone cannot fully protect consumer health. It is generally considered that the principles of Good Manufacturing Practice (GMP) and application of Hazard Analysis Critical Control Point (HACCP) systems are of greater importance.

Due to the importance of hygiene and the emphasis on a high level of consumer protection, hygiene rules form a key part of European community legislation. A revised package of European Commission (EC) hygiene measures became applicable on 1 January 2006 to update and consolidate the earlier 17 hygiene directives, with the intention of introducing consistency and clarity throughout the food production chain from primary production, to sale or supply to the final consumer. The general food hygiene Directive 93/43/EEC and other specific Directives on the hygiene of foodstuffs and the health conditions for the production and placing on the market of certain products of animal origin intended for human consumption previously in force, have been replaced by several linked measures on food safety rules and associated animal health controls.

The revised legislation has been designed to establish conditions under which food is produced to optimise public health and to prevent, eliminate or acceptably control pathogen contamination of food. Procedures under the revised legislation are based on risk assessment and management and follow a 'farm to fork' approach to food safety with the inclusion of primary production in food hygiene legislation. Prescribed are detailed measures to ensure the safety and wholesomeness of food during preparation, processing, manufacturing, packaging, storing, transportation, distribution, handling, and offering for sale or supply to the consumer.

8.2 Legislative Structure

From 1 January 2006, the following European Union (EU) hygiene regulations have applied:

- Regulation (EC) No. 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs

- Regulation (EC) No. 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin

- Regulation (EC) No. 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption

- Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs

The general hygiene requirements for all food business operators are laid down in Regulation 852/2004. Regulation 853/2004 supplements Regulation 852/2004 in that it lays down specific requirements for food businesses dealing with foods of animal origin. Regulation 854/2004 relates to the organisation of official controls on products of animal origin, and sets out what those enforcing the provisions have to do.

N.B: A number of more detailed implementing and transitional measures have been adopted at EC level.

The EU hygiene regulations apply to all stages of food production including primary production.

As in the form of regulations, the legislation is directly applicable law and therefore binding in its entirety on all member states from the date of entry into force.

Although the regulations have the force of law, national legislation in the form of a Statutory Instrument (S.I.) in England, and equivalent legislation in Scotland,

Wales and Northern Ireland, is required in the UK to give effect to the EU regulations, e.g. setting offences, penalties and powers of entry, revocation of existing implementing legislation, etc.

The Food Hygiene (England) Regulations 2006 (S.I. 2006 No.14, as amended) came into force on 11 January 2006 (separate but equivalent national legislation also came into force on that day in Scotland, Wales and Northern Ireland). The national legislation in all four UK countries also enforced the provisions of the EU Microbiological Criteria Regulation No. 2073/2005.

Although EU food hygiene regulations are directly applicable in the individual Member States there are some aspects where Member States are required or allowed to adopt certain provisions into their national laws.

8.3 Regulation (EC) No. 852/2004 on the General Hygiene of Foodstuffs

Food business operators must ensure that all stages of production, processing and distribution of food under their control satisfy the relevant hygiene requirements laid down in Regulation (EC) No. 852/2004.

This Regulation lays down general rules for food business operators on the hygiene of foodstuffs, particularly taking into account a number of factors ranging from ensuring food safety throughout the food chain beginning with primary production, right through to the implementation of procedures based on HACCP principles.

There are some exemptions, for example, with primary production, domestic preparation or handling, food storage that is for private or domestic consumption, and also if the producer supplies small amounts of primary product to the final consumer or local retail establishments supplying the final consumer. Likewise Regulation 852/2004 will not apply to collection centres and tanneries meeting the definition of food business because they handle raw material for the production of gelatine or collagen.

The regulation lays down general hygiene provisions with which food business operators carrying out primary production must comply as laid down in Part A of Annex 1. *Primary products* are defined as products of primary production including products of the soil, of stock farming, and of hunting and fishing.

Additionally the requirements of Regulation (EC) No. 853/2004 must be complied with for products of animal origin, which will be covered later in this chapter.

8.3.1 Annex I – Primary production

Annex I (Part A) relates to general hygiene provisions for primary production and associated operations covering:

(a) the transport, storage and handling of primary products at the place of production,

(b) the transport of live animals,

Food business operators have the responsibility to ensure primary products are protected against contamination. Any community and national legislation relating to the control of hazards in primary production such as measures to control contamination resulting from surroundings e.g. air, soil, water etc., and measures relating to animal health and welfare, and plant health that may impact on human health should be complied with.

Requirements for record keeping are also laid down. This relates to animal feed (nature and origin), veterinary medicines administered to animals (date given and withdrawal periods), any diseases, analysis of samples from other animals that might impact on human health, as well as reports on animal checks that have been performed.

Part B of Annex I contains recommendations for guides to good hygiene practice (GHP).

An example of a UK guide that has been published is the 'Guide to Food Hygiene & Other Regulations for the UK Meat Industry' also known as the 'Meat Industry Guide' ('MIG'), which is relevant to those UK food businesses that slaughter animals for human consumption, dress carcases, or cut or process meat, particularly those establishments that are subject to approval and veterinary control.

http://www.food.gov.uk/multimedia/pdfs/migguidefullversion.pdf

8.3.2 Annex II – Stages other than primary production

Annex II of the regulation lays down additional general hygiene requirements that must be met by food business operators carrying out production, processing and distribution of food at all stages following primary production. A summary of Chapters I to IV of Annex II is provided as follows:

8.3.2.1 Chapter I

Chapter I applies to all food premises, except premises to which Chapter III applies.

- Food premises must be kept clean, and maintained in good repair and condition, so as to permit hygienic performance of operations. Their layout should allow for this.
- The environment should allow GHPs and provide temperature-controlled handling and storage conditions where necessary, and enable foods to be kept at correct temperatures and be monitored.
- Additionally, there are requirements for provision of adequate lavatories, basins, ventilation, lighting and drainage.

8.3.2.2 Chapter II

Chapter II applies to all rooms where food is prepared, treated or processed, except dining areas and premises to which Chapter III applies.

- The design and layout of rooms should allow for GHPs between and during operations. Therefore floor and wall surfaces, ceilings and windows should be constructed to prevent dirt accumulating.
- Surfaces where food is handled must be well-maintained and allow easy cleaning and disinfection, preferably using smooth, washable corrosion-resistant and non-toxic materials.
- There should be facilities for cleaning or disinfecting, and for storing working utensils or equipment. Clean potable water and adequate provision for washing food is needed.

8.3.2.3 Chapter III

Chapter III applies to temporary premises (e.g. marquees, market stalls, and mobile sales vehicles), premises used primarily as a private dwelling-house, but where foods are regularly prepared for placing on the market, and vending machines.

- Premises and vending machines should be practically sited, designed, constructed and kept clean and maintained in good repair and condition so as to avoid the risk of contamination, in particular by animals and pests.
- Facilities should allow adequate personal hygiene, and surfaces in contact with food should be easy to clean. Enough potable water and storage arrangements for hazardous or inedible substances are required, as well as adherence to food safety requirements.

8.3.2.4 Chapter IV:

Chapter IV applies to all transportation.

- Conveyances and/or containers used for transporting foodstuffs are to be kept clean and maintained in good repair and condition to protect foodstuffs from contamination and are, where necessary, to be designed and constructed to permit adequate cleaning and/or disinfection.
- Food should be maintained at appropriate temperatures.

8.3.2.5 Chapter V

Chapter V refers to equipment requirements.

- Adequate cleaning and disinfection is to be carried out frequently for articles, fittings and equipment in contact with food where contamination needs to be avoided.
- Equipment should be installed to allow adequate cleaning, and be fitted with the required control device.

8.3.2.6 Chapter VI

Chapter VI refers to food waste.

- Food waste, non-edible by-products and other refuse is to be removed from rooms where food is present as quickly as possible to avoid accumulation. Such waste is to be deposited in closable containers, to allow easy cleaning.
- Refuse stores should allow easy cleaning and be free of pests.
- Waste must be eliminated hygienically in accordance with EC legislation.

8.3.2.7 Chapter VII

Chapter VII refers to the water supply.

- There must be an adequate supply of potable water, which should be used whenever necessary to ensure foodstuffs are not contaminated. There are also requirements for recycled water, ice in contact with food, use of steam, and for the water used in the cooling process for heat-treated foods in hermetically sealed containers.
- Recycled water used in processing or as an ingredient must not present a contamination risk. It must be of the same standard as potable water, unless the competent authority is satisfied that the quality of water cannot affect the wholesomeness of food in the finished form.

Additionally, ice that comes into contact with food or that could possibly contaminate it needs to be made from potable water.

8.3.2.8 Chapter VIII

Chapter VIII sets out personal hygiene requirements for those working in a food handling area, including clean protective clothing and the requirement that those carrying or suffering from a disease are not permitted to handle food.

8.3.2.9 Chapter IX

Chapter IX covers provisions applicable to foodstuffs.

- A food business operator should not accept raw materials or ingredients, other than live animals, or any other material used in processing products, if they are known to be contaminated with parasites, pathogenic microorganisms or foreign substances, to be toxic or decomposed to such an extent that, even after the business operator applied normal hygienic processing, the product would be inedible.
- Raw materials must be kept under appropriate conditions throughout production, processing and distribution. In particular, temperature control (i.e. cold chain and food thawing) requirements are laid down.

8.3.2.10 Chapter X

Chapter X lays down provisions applicable to the wrapping and packaging of foodstuffs to avoid contamination of any form.

8.3.2.11 Chapter XI

Chapter XI lays down heat treatment requirements for food that is placed on the market in hermetically sealed containers. The main relevant process parameters (particularly temperature, pressure, sealing and microbiology) including use of automatic devices, must be checked by the food business operator to ensure that the required heat treatment has been achieved.

Also, the process used should comply with an internationally recognised standard (e.g. pasteurisation, ultra high temperature or sterilisation).

8.3.2.12 Chapter XII

Chapter XII states training requirements for food business operators to ensure that food handlers are trained in food hygiene matters and in the application of HACCP principles.

8.3.3 Registration

The Regulation requires that food business operators must notify the relevant competent authority of each establishment under their control that carries out any of the stages of production, processing and distribution of food, with a view to the registration of each establishment.

Food business operators must also ensure that the competent authority always has up-to-date information on establishments, including the notification of significant changes in activity, and closure of an existing establishment.

Food business operators must ensure that establishments are approved by the competent authority, following at least one on-site visit, when approval is required by the national law of the Member State in which the establishment is located, or under Regulation (EC) No. 853/2004 for products of animal origin, or by a separate decision adopted.

Additional rules apply for businesses producing products of animal origin.

8.3.4 HACCP

Food business operators, other than at the level of primary production, and associated operations must put in place, implement and maintain a permanent procedure or procedures based on principles of the HACCP system. Emphasis is placed on risk-related control, with responsibility placed on the proprietor of the food business to ensure that potential hazards are identified, and systems are developed to control them. Under HACCP, food business operators must amongst other considerations, identify hazards to be prevented or eliminated, or reduced to acceptable levels, identify critical control points (CCP) at which control is essential to prevent, eliminate or reduce hazards and establish critical limits at these points, implement effective monitoring procedures, and establish corrective actions in the case where a CCP is out of control. Procedures must be followed to confirm the above is in place and up-to-date, as well as providing the competent authority with documents and records as evidence, when required.

8.4 Regulation (EC) No. 853/2004 Laying Down Specific Hygiene Rules for Food of Animal Origin

Regulation (EC) No. 853/2004 lays down hygiene rules for products of animal origin that apply in addition to the general hygiene rules of Regulation (EC) No. 852/2004. Regulation (EC) No. 853/2004 applies to unprocessed and processed products of animal origin and lays down the hygiene requirements to be respected by food businesses handling food of animal origin at all stages of the food chain.

8.4.1 Definitions

8.4.1.1 The following definitions apply for meat and meat products:

Meat means edible parts of the animals referring to domestic ungulates, poultry, lagomorphs, wild game, farmed game, small wild game, and large wild game, including blood.

Meat products means processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat.

Domestic ungulates means domestic bovine (including *Bubalus* and Bison species), porcine, ovine and caprine animals, and domestic solipeds.

Poultry means farmed birds, including birds that are not considered as domestic but which are farmed as domestic animals, with the exception of ratites.

Lagomorphs mean rabbits, hares and rodents.

Wild game means:
- wild ungulates and lagomorphs, as well as other land mammals that are hunted for human consumption and are considered to be wild game under the applicable law in the Member State concerned, including mammals living in enclosed territory under conditions of freedom similar to those of wild game; and
- wild birds that are hunted for human consumption.

Farmed game means farmed ratites and farmed land mammals other than those referred to in the definition of ‘domestic ungulates’.

Small wild game means wild game birds and lagomorphs living freely in the wild.

Large wild game means wild land mammals living freely in the wild that do not fall within the definition of small wild game.

Carcase means the body of an animal after slaughter and dressing.

Fresh meat means meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.

Offal means fresh meat other than that of the carcase, including viscera and blood.

Viscera mean the organs of the thoracic, abdominal and pelvic cavities, as well as the trachea and oesophagus and, in birds, the crop.

Minced meat means boned meat that has been minced into fragments and contains less than 1% salt.

Mechanically separated meat (MSM) means the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure.

Meat preparations means fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.

Slaughterhouse means an establishment used for slaughtering and dressing animals, the meat of which is intended for human consumption.

Cutting plant means an establishment used for boning and/or cutting up meat.

Game-handling establishment means any establishment in which game and game meat obtained after hunting are prepared for placing on the market.

Products of animal origin means:

- food of animal origin, including honey and blood;
- live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods intended for human consumption; and
- other animals destined to be prepared with a view to being supplied live to the final consumer.

8.4.1.2 The following definitions apply for eggs

Eggs means eggs in shell - other than broken, incubated or cooked eggs - that are produced by farmed birds and are fit for direct human consumption or for the preparation of egg products.
Liquid egg means unprocessed egg contents after removal of the shell.

Cracked eggs means eggs with damaged shell and intact membranes.

Packing centre means an establishment where eggs are graded by quality and weight.

Egg products mean processed products resulting from the processing of eggs, or of various components or mixtures of eggs, or from the further processing of such processed products.

8.4.2 Meat and meat product requirements

8.4.2.1 Identification marking

Identification marking is applicable to both products of animal origin that have been processed and those that are unprocessed. In determining whether a product of animal origin is processed or unprocessed it is important to take into account all the relevant definitions contained in the hygiene regulations, in particular, the definitions of 'processing', 'unprocessed products' and 'processed products' in Article 2 of Regulation (EC) No. 852/2004.

Food containing both products of plant origin, and processed products of animal origin are outside the scope of Regulation (EC) No. 853/2004. However, processed products of animal origin used to prepare such foods must be obtained and handled in accordance with the requirements of this Regulation.

Health marking is separate to identification marking even though the presentation is similar, as health marks are applicable only to red meat animals (domestic ungulates, farmed game mammals other than lagomorphs, and large wild game) having undergone ante- and post-mortem inspections in accordance with Regulation (EC) No. 854/2004 (further information in section ***8.5.2***).

An identification mark needs to be applied following the requirements of Annex II, Section I of Regulation (EC) No. 853/2004. An identification mark can only be applied provided the relevant requirements as stated above in relation to Regulation (EC) No. 852/2004 have been met in particular for the registration and approval of establishments.

Extracts of the requirements of Annex II, Section I of Regulation (EC) No. 853/2004 are given as follows:

8.4.2.1.1 Application of the identification mark

(i) The identification mark must be applied before the product leaves the establishment of production.

(ii) However, when a product's packaging and/or wrapping is removed or is further processed in another establishment, a new mark must be applied to the product. In this case, the new mark must show the approval number of the establishment where this occurred.

(iii) Food business operators must have in place systems and procedures to identify food business operators from whom they have received, and to whom they have delivered products of animal origin.

(iv) An identification mark is not necessary for eggs in respect of which Regulation (EC) No. 1907/90 (Council Regulation (EEC) No. 1907/90 of 26 June 1990 on certain marketing standards for eggs (OJ L 173, 6.7.1990, p. 5). Regulation as last amended by Regulation (EC) No. 2052/2003 (OJ L 305, 22.11.2003, p. 1)) lays down requirements concerning labelling or marking.

8.4.2.1.2 Form of the identification mark

(i) The mark must be legible and indelible, and the characters easily decipherable and be clearly displayed.

(ii) The mark must indicate the name of the country in which the establishment is located and written out in full or shown as a two-letter code in accordance with the relevant International Organisation of Standards (ISO) standard. For the UK, the code would be UK; for other codes, please refer to Regulation (EC) No. 853/2004.

(iii) Additionally, the mark must show the approval number of the establishment. If an establishment manufactures both food to which Regulation (EC) No. 853/2004 applies, and food to which it does not, the food business operator may apply the same identification mark to both types of food.

(iv) If the identification mark is applied in an establishment within the Community, the mark must be oval shaped and include the abbreviation CE, EC, EF, EG, EK, EO, EY, ES, EÜ, EK, EB or WE.

8.4.2.1.3 Method of marking

(i) The mark is to be applied directly to the product, the wrapping or the packaging, or be printed on a label affixed to the product, its wrapping or the

packaging. It may also be a tag made of a resistant material affixed in such a way that it cannot be removed.

(ii) For products of animal origin that are placed in transport containers or large packages, and are intended for further handling, processing, wrapping or packaging in another establishment, the mark may be applied to the outer surface of the container or packaging.

(iii) In the case of liquid, granulate and powdered products of animal origin carried in bulk, and fishery products carried in bulk, an identification mark is not necessary if accompanying documentation contains the name of the country or its two-letter code, the approval number of the establishment and the abbreviation for the community where appropriate.

(iv) If products of animal origin are placed in a package destined for direct supply to the final consumer, it is sufficient to apply the mark to the exterior of that package only.

(v) When the mark is applied directly to products of animal origin, the colourings used must be EC authorised colours.

8.4.2.2 Hygiene requirements for meat of domestic ungulates

Extracts of the requirements of Annex III, Section I of EC Regulation. 853/2004, as amended, specifically relating to meat of domestic ungulates are given below; for full requirements, reference should be made to the actual Regulation.

8.4.2.2.1 Chapter I: Transport of live animals to the slaughterhouse

Food business operators transporting live animals to slaughterhouses must ensure compliance with the following requirements.

1. During collection and transport, animals must be handled carefully without causing unnecessary distress.

2. Animals showing symptoms of disease or originating in herds known to be contaminated with agents of public health importance may only be transported to the slaughterhouse when the competent authority so permits.

8.4.2.2.2 Chapter II: Requirements for slaughterhouses

Food business operators must ensure that the construction, layout and equipment of slaughterhouses in which domestic ungulates are slaughtered meet the following requirements.

1. (a) Slaughterhouses must have adequate and hygienic lairage facilities or, climate permitting, waiting pens that are easy to clean and disinfect.

   These facilities must be equipped for watering the animals and, if necessary, feeding them. The drainage of the waste water must not compromise food safety.

   (b) They must also have separate lockable facilities or, climate permitting, pens for sick or suspect animals with separate draining and sited in such a way as to avoid contamination of other animals, unless the competent authority considers that such facilities are unnecessary.

   (c) The size of the lairage facilities must ensure that the welfare of the animals is respected. Their layout must facilitate ante-mortem inspections, including the identification of the animals or groups of animals.

2. To avoid contaminating meat, they must:

   (a) have a sufficient number of rooms, appropriate to the operations being carried out;

   (b) have a separate room for the emptying and cleaning of stomachs and intestines, unless the competent authority authorises the separation in time of these operations within a specific slaughterhouse on a case-by-case basis;

   (c) ensure separation in space or time of the following operations:

      (i) stunning and bleeding;

      (ii) in the case of porcine animals, scalding, depilation, scraping and singeing;

      (iii) evisceration and further dressing;

      (iv) handling clean guts and tripe;

      (v) preparation and cleaning of other offal, particularly the handling of skinned heads if it does not take place at the slaughter line;

      (vi) packaging offal; and

      (vii) dispatching meat;

   (d) have installations that prevent contact between the meat and the floors, walls and fixtures; and

(e) have slaughter lines (where operated) that are designed to allow constant progress of the slaughter process and to avoid cross contamination between the different parts of the slaughter line. Where more than one slaughter line is operated in the same premises, there must be adequate separation of the lines to prevent cross contamination.

3. They must have facilities for disinfecting tools with hot water supplied at not less than 82 °C, or an alternative system having an equivalent effect.

4. The equipment for washing hands used by the staff engaged in handling exposed meat must have taps designed to prevent the spread of contamination.

5. There must be lockable facilities for the refrigerated storage of detained meat and separate lockable facilities for the storage of meat declared unfit for human consumption.

6. There must be a separate place with appropriate facilities for the cleaning, washing and disinfection of means of transport for livestock. However, slaughterhouses need not have these places and facilities if the competent authority so permits and official authorised places and facilities exist nearby.

7. They must have lockable facilities reserved for the slaughter of sick and suspect animals. This is not essential if this slaughter takes place in other establishments authorised by the competent authority for this purpose, or at the end of the normal slaughter period.

8. If manure or digestive tract content is stored in the slaughterhouse, there must be a special area or place for that purpose.

9. They must have an adequately equipped lockable facility or, where needed, room for the exclusive use of the veterinary service.

8.4.2.2.3 Chapter III: Requirements for cutting plants

Food business operators must ensure that cutting plants handling meat of domestic ungulates:

1. are constructed so as to avoid contamination of meat, in particular by:

   (a) allowing constant progress of the operations; or

   (b) ensuring separation between the different production batches;

2. have rooms for the separate storage of packaged and exposed meat, unless stored at different times or in such a way that the packaging material and the manner of storage cannot be a source of contamination for the meat;

3. have cutting rooms equipped to ensure compliance with the requirements laid down in Chapter V;

4. have equipment for washing hands with taps designed to prevent the spread of contamination, for use by staff engaged in handling exposed meat; and

5. have facilities for disinfecting tools with hot water supplied at not less than 82 °C, or an alternative system having an equivalent effect.

8.4.2.2.4 Chapter IV: Slaughter Hygiene

Food business operators operating slaughterhouses in which domestic ungulates are slaughtered must ensure compliance with the following requirements.

1. After arrival in the slaughterhouse, the slaughter of the animals must not be unduly delayed. However, where required for welfare reasons, animals must be given a resting period before slaughter.

2. (a) Meat from animals other than those referred to in subparagraphs (b) and (c) must not be used for human consumption if they die otherwise than by being slaughtered in the slaughterhouse.

   (b) Only live animals intended for slaughter may be brought into the slaughter premises, with the exception of:

      (i) animals that have undergone emergency slaughter outside the slaughterhouse in accordance with Chapter VI;

      (ii) animals slaughtered at the place of production in accordance with Section III; and

      (iii) wild game, in compliance with Section IV, Chapter II.

   (c) Meat from animals that undergo slaughter following an accident in a slaughterhouse may be used for human consumption if, on inspection, no serious lesions other than those due to the accident are found.

3. The animals or, where appropriate, each batch of animals sent for slaughter must be identified so that their origin can be traced.

4. Animals must be clean.

5. Slaughterhouse operators must follow the instructions of the veterinarian appointed by the competent authority in accordance with Regulation (EC) No. 854/2004 to ensure that ante-mortem inspection of every animal to be slaughtered is carried out under suitable conditions.

6. Animals brought into the slaughter hall must be slaughtered without undue delay.

7. Stunning, bleeding, skinning, evisceration and other dressing must be carried out without undue delay and in a manner that avoids contaminating the meat.

   In particular:

   (a) the trachea and oesophagus must remain intact during bleeding, except in the case of slaughter according to a religious custom;

   (b) during the removal of hides and fleece:

      (i) contact between the outside of the skin and the carcase must be prevented; and

      (ii) operators and equipment coming into contact with the outer surface of hides and fleece must not touch the meat;

   (c) measures must be taken to prevent the spillage of digestive tract content during and after evisceration and to ensure that evisceration is completed as soon as possible after stunning; and

   (d) removal of the udder must not result in contamination of the carcase with milk or colostrum.

8. Carcases and other parts of the body intended for human consumption must be completely skinned, except in the case of porcine animals, the heads of ovine and caprine animals and calves, the muzzle and lips of bovine animals and the feet of bovine, ovine and caprine animals. Heads, including muzzle and lips, and feet must be handled in such a way as to avoid contamination.

9. When not skinned, porcine animals must have their bristles removed immediately.

   The risk of contamination of the meat with scalding water must be minimised. Only approved additives may be used for this operation. Porcine animals must be thoroughly rinsed afterwards with potable water.

10. The carcases must not contain visible faecal contamination. Any visible contamination must be removed without delay by trimming or alternative means having an equivalent effect.

11. Carcases and offal must not come into contact with floors, walls or work stands.

12. Slaughterhouse operators must follow the instructions of the competent authority to ensure that post-mortem inspection of all slaughtered animals is carried out under suitable conditions in accordance with Regulation (EC) No. 854/2004.

13. Until post-mortem inspection is completed, parts of a slaughtered animal subject to such inspection must:

    (a) remain identifiable as belonging to a given carcase; and

    (b) come into contact with no other carcase, offal or viscera, including those that have already undergone post-mortem inspection.

    However, provided that it shows no pathological lesion, the penis may be discarded immediately.

14. Both kidneys must be removed from their fatty covering. In the case of bovine and porcine animals, and solipeds, the peri-renal capsule must also be removed.

15. If the blood or other offal of several animals is collected in the same container before completion of post-mortem inspection, the entire contents must be declared unfit for human consumption if the carcase of one or more of the animals concerned has been declared unfit for human consumption.

16. After post-mortem inspection:

    (a) the tonsils of bovine animals, porcine animals and solipeds must be removed hygienically;

    (b) parts unfit for human consumption must be removed as soon as possible from the clean sector of the establishment;

    (c) meat detained or declared unfit for human consumption and inedible by-products must not come into contact with meat declared fit for human consumption; and

(d) viscera or parts of viscera remaining in the carcase, except for the kidneys, must be removed entirely and as soon as possible, unless the competent authority authorises otherwise.

17. After completion of slaughter and post-mortem inspection, the meat must be stored in accordance with the requirements laid down in Chapter VII.

18. When destined for further handling:

(a) stomachs must be scalded or cleaned;

(b) intestines must be emptied and cleaned; and

(c) heads and feet must be skinned or scalded and depilated.

19. Where establishments are approved for the slaughter of different animal species or for the handling of carcases of farmed game and wild game, precautions must be taken to prevent cross contamination by separation either in time or in space of operations carried out on the different species. Separate facilities for the reception and storage of unskinned carcases of farmed game slaughtered at the farm and for wild game must be available.

20. If the slaughterhouse does not have lockable facilities reserved for the slaughter of sick or suspect animals, the facilities used to slaughter such animals must be cleaned, washed and disinfected under official supervision before the slaughter of other animals is resumed.

8.4.2.2.5 Chapter V: Hygiene during cutting and boning

Food business operators must ensure that cutting and boning of meat of domestic ungulates takes place in accordance with the following requirements.

1. Carcases of domestic ungulates may be cut into half-carcases or quarters, and half-carcases into no more than three wholesale cuts, in slaughterhouses. Further cutting and boning must be carried out in a cutting plant.

2. The work on meat must be organised in such a way as to prevent or minimise contamination. To this end, food business operators must ensure in particular that:

(a) meat intended for cutting is brought into the workrooms progressively as needed;

(b) during cutting, boning, trimming, slicing, dicing, wrapping and packaging, the meat is maintained at not more than 3 °C for offal and 7 °C for

other meat, by means of an ambient temperature of not more than 12 °C or an alternative system having an equivalent effect; and

(c) where the premises are approved for the cutting of meat of different animal species, precautions are taken to avoid cross contamination, where necessary by separation of the operations on the different species in either space or time.

3. However, meat may be boned and cut before it reaches the temperature referred to in point 2(b) in accordance with Chapter VII, point 3.

4. Meat may also be boned and cut prior to reaching the temperature referred to in point 2(b) when the cutting room is on the same site as the slaughter premises. In this case, the meat must be transferred to the cutting room either directly from the slaughter premises, or after a waiting period in a chilling or refrigerating room. As soon as it is cut and, where appropriate, packaged, the meat must be chilled to the temperature referred to in point 2(b).

8.4.2.2.6 Chapter VI: Emergency slaughter outside the slaughterhouse

Food business operators must ensure that meat from domestic ungulates that have undergone emergency slaughter outside the slaughterhouse may be used for human consumption only if it complies with all the following requirements.

1. An otherwise healthy animal must have suffered an accident that prevented its transport to the slaughterhouse for welfare reasons.

2. A veterinarian must carry out an ante-mortem inspection of the animal.

3. The slaughtered and bled animal must be transported to the slaughterhouse hygienically and without undue delay. Removal of the stomach and intestines, but no other dressing, may take place on the spot, under the supervision of the veterinarian. Any viscera removed must accompany the slaughtered animal to the slaughterhouse and be identified as belonging to that animal.

4. If more than two hours elapse between slaughter and arrival at the slaughterhouse, the animal must be refrigerated. Where climatic conditions so permit, active chilling is not necessary.

5. A declaration by the food business operator who reared the animal, stating the identity of the animal and indicating any veterinary products or other treatments administered to the animal, dates of administration and withdrawal periods, must accompany the slaughtered animal to the slaughterhouse.

6. A declaration issued by the veterinarian recording the favourable outcome of the ante-mortem inspection, the date and time of, and reason for, emergency slaughter, and the nature of any treatment administered by the veterinarian to the animal, must accompany the slaughtered animal to the slaughterhouse.

7. The slaughtered animal must be fit for human consumption following post-mortem inspection carried out in the slaughterhouse in accordance with Regulation (EC) No 854/2004, including any additional tests required in the case of emergency slaughter.

8. Food business operators must follow any instructions that the official veterinarian may give after post-mortem inspection concerning the use of the meat.

9. Food business operators may not place meat from animals having undergone emergency slaughter on the market unless it bears a special health mark which cannot be confused either with the health mark provided for in Regulation (EC) No. 854/2004 or with the identification mark provided for in Annex II, Section I to this Regulation. Such meat may be placed on the market only in the Member State where slaughter takes place and in accordance with national law.

8.4.2.2.7 Chapter VII: Storage and transport

Food business operators must ensure that the storage and transport of meat of domestic ungulates takes place in accordance with the following requirements.

1. (a) Unless other specific provisions provide otherwise, post-mortem inspection must be followed immediately by chilling in the slaughterhouse to ensure a temperature throughout the meat of not more than 3 °C for offal and 7 °C for other meat along a chilling curve that ensures a continuous decrease of the temperature. However, meat may be cut and boned during chilling in accordance with Chapter V, point 4.

   (b) During the chilling operations, there must be adequate ventilation to prevent condensation on the surface of the meat.

2. Meat must attain the temperature specified in point 1 and remain at that temperature during storage.

3. Meat must attain the temperature specified in point 1 before transport, and remain at that temperature during transport. However, transport may also take place if the competent authority so authorises to enable the production of specific products, provided that:

(a) such transport takes place in accordance with the requirements that the competent authority specifies in respect of transport from one given establishment to another; and

(b) the meat leaves the slaughterhouse, or a cutting room on the same site as the slaughter premises, immediately and transport takes no more than two hours.

4. Meat intended for freezing must be frozen without undue delay, taking into account where necessary a stabilisation period before freezing.

5. Exposed meat must be stored and transported separately from packaged meat, unless stored or transported at different times or in such a way that the packaging material and the manner of storage or transport cannot be a source of contamination for the meat.

8.4.2.3 Hygiene requirements for meat from poultry and lagomorphs

Extracts of the requirements of Annex III, Section II of Regulation (EC) No. 853/2004, as amended, specifically relating to meat from poultry and lagomorphs are given below; for full requirements, reference should be made to the actual Regulation.

8.4.2.3.1 Chapter I: Transport of live animals to the slaughterhouse

Food business operators transporting live animals to slaughterhouses must ensure compliance with the following requirements.

1. During collection and transport, animals must be handled carefully without causing unnecessary distress.

2. Animals showing symptoms of disease or originating in flocks known to be contaminated with agents of public health importance may only be transported to the slaughterhouse when permitted by the competent authority.

3. Crates for delivering animals to the slaughterhouse and modules, where used, must be made of non-corrodible material and be easy to clean and disinfect.

   Immediately after emptying and, if necessary, before re-use, all equipment used for collecting and delivering live animals must be cleaned, washed and disinfected.

8.4.2.3.2 Chapter II: Requirements for slaughterhouses

Food business operators must ensure that the construction, layout and equipment of slaughterhouses in which poultry or lagomorphs are slaughtered meet the following requirements.

1. They must have a room or covered space for the reception of the animals and for their inspection before slaughter.

2. To avoid contaminating meat, they must:

   (a) have a sufficient number of rooms, appropriate to the operations being carried out;

   (b) have a separate room for evisceration and further dressing, including the addition of seasonings to whole poultry carcases, unless the competent authority authorises separation in time of these operations within a specific slaughterhouse on a case-by-case basis;

   (c) ensure separation in space or time of the following operations:

      (i) stunning and bleeding;

      (ii) plucking or skinning, and any scalding; and

      (iii) dispatching meat;

   (d) have installations that prevent contact between the meat and the floors, walls and fixtures; and

   (e) have slaughter lines (where operated) that are designed to allow a constant progress of the slaughter process and to avoid cross contamination between the different parts of the slaughter line. Where more than one slaughter line is operated in the same premises, there must be adequate separation of the lines to prevent cross contamination.

3. They must have facilities for disinfecting tools with hot water supplied at not less than 82 °C, or an alternative system having an equivalent effect.

4. The equipment for washing hands used by the staff engaged in handling exposed meat must have taps designed to prevent the spread of contamination.

5. There must be lockable facilities for the refrigerated storage of detained meat and separate lockable facilities for the storage of meat declared unfit for human consumption.

6. There must be a separate place with appropriate facilities for the cleaning, washing and disinfection of:

   (a) transport equipment such as crates; and

   (b) means of transport.

   These places and facilities are not compulsory for (b) if officially authorised places and facilities exist nearby.

7. They must have an adequately equipped lockable facility or, where needed, room for the exclusive use of the veterinary service.

8.4.2.3.3 Chapter III: Requirements for cutting plants

1. Food business operators must ensure that cutting plants handling meat from poultry or lagomorphs:

   (a) are constructed so as to avoid contamination of meat, in particular by:

      (i) allowing constant progress of the operations; or

      (ii) ensuring separation between the different production batches;

   (b) have rooms for the separate storage of packaged and exposed meat, unless stored at different times or in such a way that the packaging material and the manner of storage cannot be a source of contamination for the meat;

   (c) have cutting rooms equipped to ensure compliance with the requirements laid down in Chapter V;

   (d) have equipment for washing hands used by staff handling exposed meat with taps designed to prevent the spread of contamination; and

   (e) have facilities for disinfecting tools with hot water supplied at not less than 82 °C, or an alternative system having an equivalent effect.

2. If the following operations are undertaken in a cutting plant:

   (a) the evisceration of geese and ducks reared for the production of *foie gras*, which have been stunned, bled and plucked on the fattening farm; or

(b) the evisceration of delayed eviscerated poultry, food business operators must ensure that separate rooms are available for that purpose.

8.4.2.3.4 Chapter IV: Slaughter hygiene

Food business operators operating slaughterhouses in which poultry or lagomorphs are slaughtered must ensure compliance with the following requirements.

1. (a) Meat from animals other than those referred to in (b) must not be used for human consumption if they die otherwise than by being slaughtered in the slaughterhouse.

   (b) Only live animals intended for slaughter may be brought into the slaughter premises, with the exception of:

   (i) delayed eviscerated poultry, geese and ducks reared for the production of *foie gras* and birds that are not considered as domestic but which are farmed as domestic animals, if slaughtered at the farm in accordance with Chapter VI;

   (ii) farmed game slaughtered at the place of production in accordance with Section III; and

   (iii) small wild game in accordance with Section IV, Chapter III.

2. Slaughterhouse operators must follow the instructions of the competent authority to ensure that ante-mortem inspection is carried out under suitable conditions.

3. Where establishments are approved for the slaughter of different animal species or for the handling of farmed ratites and small wild game, precautions must be taken to prevent cross contamination by separation either in time or in space of the operations carried out on the different species. Separate facilities for the reception and storage of carcases of farmed ratites slaughtered at the farm and for small wild game must be available.

4. Animals brought into the slaughter room must be slaughtered without undue delay.

5. Stunning, bleeding, skinning or plucking, evisceration and other dressing must be carried out without undue delay in such a way that contamination of the meat is avoided. In particular, measures must be taken to prevent the spillage of digestive tract contents during evisceration.

6. Slaughterhouse operators must follow the instructions of the competent authority to ensure that the post-mortem inspection is carried out under suitable conditions, and in particular that slaughtered animals can be inspected properly.

7. After post-mortem inspection:

   (a) parts unfit for human consumption must be removed as soon as possible from the clean sector of the establishment;

   (b) meat detained or declared unfit for human consumption and inedible by-products must not come into contact with meat declared fit for human consumption; and

   (c) viscera or parts of viscera remaining in the carcase, except for the kidneys, must be removed entirely, if possible, and as soon as possible, unless otherwise authorised by the competent authority.

8. After inspection and evisceration, slaughtered animals must be cleaned and chilled to not more than 4 °C as soon as possible, unless the meat is cut while warm.

9. When carcases are subjected to an immersion chilling process, account must be taken of the following.

   (a) Every precaution must be taken to avoid contamination of carcases, taking into account parameters such as carcase weight, water temperature, volume and direction of water flow and chilling time.

   (b) Equipment must be entirely emptied, cleaned and disinfected whenever this is necessary and at least once a day.

10. Sick or suspect animals, and animals slaughtered in application of disease eradication or control programmes, must not be slaughtered in the establishment except when permitted by the competent authority. In that event, slaughter must be performed under official supervision and steps taken to prevent contamination; the premises must be cleaned and disinfected before being used again.

8.4.2.3.5 Chapter V: Hygiene during and after cutting and boning

Food business operators must ensure that cutting and boning of meat of poultry and lagomorphs takes place in accordance with the following requirements.

1. The work on meat must be organised in such a way as to prevent or minimise contamination. To this end, food business operators must ensure in particular that:

   (a) meat intended for cutting is brought into the workrooms progressively as needed;

   (b) during cutting, boning, trimming, slicing, dicing, wrapping and packaging, the temperature of the meat is maintained at not more than 4 °C by means of an ambient temperature of 12 °C or an alternative system having an equivalent effect; and

   (c) where the premises are approved for the cutting of meat of different animal species, precautions are taken to avoid cross contamination, where necessary by separation of the operations on the different species in either space or time.

2. However, meat may be boned and cut prior to reaching the temperature referred to in point 1(b) when the cutting room is on the same site as the slaughter premises, provided that it is transferred to the cutting room either:

   (a) directly from the slaughter premises; or

   (b) after a waiting period in a chilling or refrigerating room.

3. As soon as it is cut and, where appropriate, packaged, the meat must be chilled to the temperature referred to in point 1(b).

4. Exposed meat must be stored and transported separately from packaged meat, unless stored or transported at different times or in such a way that the packaging material and the manner of storage or transport cannot be a source of contamination for the meat.

8.4.2.3.6 Chapter VI: Slaughter on the farm

Food business operators may slaughter poultry referred to in Chapter IV, point 1 (b)(i), on the farm only with the authorisation of the competent authority and in compliance with the following requirements.

1. The farm must undergo regular veterinary inspection.

2. The food business operator must inform the competent authority in advance of the date and time of slaughter.

3. The holding must have facilities for concentrating the birds to allow an ante-mortem inspection of the group to be made.

4. The holding must have premises suitable for the hygienic slaughter and further handling of the birds.

5. Animal welfare requirements must be complied with.

6. The slaughtered birds must be accompanied to the slaughterhouse by a declaration by the food business operator who reared the animal indicating any veterinary products or other treatments administered to the animal, dates of administration and withdrawal periods, and the date and time of slaughter.

7. The slaughtered animal must be accompanied to the slaughterhouse by a certificate issued by the official veterinarian or approved veterinarian in accordance with Regulation (EC) No. 854/2004.

8. In the case of poultry reared for the production of *foie gras*, the uneviscerated birds must be transported immediately and, if necessary, refrigerated to a slaughterhouse or cutting plant. They must be eviscerated within 24 hours of slaughter under the supervision of the competent authority.

9. Delayed eviscerated poultry obtained at the farm of production may be kept for up to 15 days at a temperature of not more than 4 °C. It must then be eviscerated in a slaughterhouse or in a cutting plant located in the same member state as the farm of production.

8.4.2.3.7 Chapter VII: Water retention agents

Food business operators shall ensure that poultry meat that has been treated specifically to promote water retention is not placed on the market as fresh meat but as meat preparations or used for the production of processed products.

8.4.2.4 Hygiene requirements for meat of farmed game

Extracts of the requirements of Annex III, Section III of Regulation (EC) No. 853/2004, as amended, specifically relating to meat of farmed game are given below; for full requirements, reference should be made to the actual Regulation.

8.4.2.4.1 Meat of farmed game

1. The provisions of Section I apply to the production and placing on the market of meat from even-toed farmed game mammals (Cervidae and Suidae), unless the competent authority considers them inappropriate.

2. The provisions of Section II apply to the production and placing on the market of meat from ratites. However, those of Section I apply where the competent authority considers them appropriate. Appropriate facilities must be provided, adapted to the size of the animals.

3. Notwithstanding points 1 and 2, food business operators may slaughter farmed ratites and farmed ungulates referred to in point 1 at the place of origin with the authorisation of the competent authority if:

   (a) the animals cannot be transported, to avoid any risk for the handler or to protect the welfare of the animals;

   (b) the herd undergoes regular veterinary inspection;

   (c) the owner of the animals submits a request;

   (d) the competent authority is informed in advance of the date and time of slaughter of the animals;

   (e) the holding has procedures for concentrating the animals to allow an ante-mortem inspection of the group to be made;

   (f) the holding has facilities suitable for the slaughter, bleeding and, where ratites are to be plucked, plucking of the animals;

   (g) animal welfare requirements are complied with;

   (h) slaughtered and bled animals are transported to the slaughterhouse hygienically and without undue delay. If transport takes more than two hours, the animals are, if necessary, refrigerated. Evisceration may take place on the spot, under the supervision of the veterinarian;

   (i) a declaration by the food business operator who reared the animals, stating their identity and indicating any veterinary products or other treatments administered, dates of administration and withdrawal periods, accompanies the slaughtered animals to the slaughterhouse; and

   (j) during transport to the approved establishment, a certificate issued and signed by the official veterinarian or approved veterinarian, attesting to a favourable result of the ante-mortem inspection, correct slaughter and bleeding and the date and time of slaughter, accompanies the slaughtered animals.

4. Food business operators may also slaughter bison on the farm in accordance with point 3 in exceptional circumstances.

8.4.2.5 Hygiene requirements for wild game meat

Extracts of the requirements of Annex III, Section IV of Regulation (EC) No. 853/2004, as amended, specifically relating to wild game meat are given below; for full requirements, reference should be made to the actual Regulation.

8.4.2.5.1 Chapter I: Training of hunters in health and hygiene

1. Persons who hunt wild game with a view to placing it on the market for human consumption must have sufficient knowledge of the pathology of wild game, and of the production and handling of wild game and wild game meat after hunting, to undertake an initial examination of wild game on the spot.

2. It is however enough if at least one person of a hunting team has the knowledge referred to in point 1. References in this section to a 'trained person' are references to that person.

3. The trained person could also be the gamekeeper or the game manager if he or she is part of the hunting team or located in the immediate vicinity of where hunting is taking place. In the latter case, the hunter must present the wild game to the gamekeeper or game manager and inform them of any abnormal behaviour observed before killing.

4. Training must be provided to the satisfaction of the competent authority to enable hunters to become trained persons. It should cover at least the following subjects:

   (a) the normal anatomy, physiology and behaviour of wild game;

   (b) abnormal behaviour and pathological changes in wild game due to diseases, environmental contamination or other factors which may affect human health after consumption;

   (c) the hygiene rules and proper techniques for the handling, transportation, evisceration, etc. of wild game animals after killing; and

   (d) legislation and administrative provisions on the animal and public health and hygiene conditions governing the placing on the market of wild game.

5. The competent authority should encourage hunters' organisations to provide such training.

8.4.2.5.2 Chapter II: Handling of large wild game

1. After killing, large wild game must have their stomachs and intestines removed as soon as possible and, if necessary, be bled.

2. The trained person must carry out an examination of the body, and of any viscera removed, to identify any characteristics that may indicate that the meat presents a health risk. The examination must take place as soon as possible after killing.

3. Meat of large wild game may be placed on the market only if the body is transported to a game-handling establishment as soon as possible after the examination referred to in point 2. The viscera must accompany the body as specified in point 4. The viscera must be identifiable as belonging to a given animal.

4. (a) If no abnormal characteristics are found during the examination referred to in point 2, no abnormal behaviour was observed before killing, and there is no suspicion of environmental contamination, the trained person must attach to the animal body a numbered declaration stating this. This declaration must also indicate the date, time and place of killing. In this case, the head and the viscera need not accompany the body, except in the case of species susceptible to Trichinosis (porcine animals, solipeds and others), whose head (except for tusks) and diaphragm must accompany the body. However, hunters must comply with any additional requirements imposed in the member state where hunting takes place, in particular to permit the monitoring of certain residues and substances in accordance with Directive 96/23/EC;

   (b) In other circumstances, the head (except for tusks, antlers and horns) and all the viscera except for the stomach and intestines must accompany the body. The trained person who carried out the examination must inform the competent authority of the abnormal characteristics, abnormal behaviour or suspicion of environmental contamination that prevented him or her from making a declaration in accordance with (a);

   (c) If no trained person is available to carry out the examination referred to in point 2 in a particular case, the head (except for tusks, antlers and horns) and all the viscera except for the stomach and the intestines must accompany the body.

5. Chilling must begin within a reasonable period of time after killing and achieve a temperature throughout the meat of not more than 7 °C. Where climatic conditions so permit, active chilling is not necessary.

6. During transport to the game-handling establishment, heaping must be avoided.

7. Large wild game delivered to a game-handling establishment must be presented to the competent authority for inspection.

8. In addition, unskinned large wild game may be skinned and placed on the market only if:

   (a) before skinning, it is stored and handled separately from other food and not frozen; and

   (b) after skinning, it undergoes a final inspection in accordance with Regulation (EC) No. 854/2004.

9. The rules laid down in Section I, Chapter V, apply to the cutting and boning of large wild game.

8.4.2.5.3 Chapter III: Handling of small wild game

1. The trained person must carry out an examination to identify any characteristics that may indicate that the meat presents a health risk. The examination must take place as soon as possible after killing.

2. If abnormal characteristics are found during the examination, abnormal behaviour was observed before killing, or environmental contamination is suspected, the trained person must inform the competent authority.

3. Meat of small wild game may be placed on the market only if the body is transported to a game-handling establishment as soon as possible after the examination referred to in point 1.

4. Chilling must begin within a reasonable period of time of killing and achieve a temperature throughout the meat of not more than 4 °C. Where climatic conditions so permit, active chilling is not necessary.

5. Evisceration must be carried out, or completed, without undue delay upon arrival at the game-handling establishment, unless the competent authority permits otherwise.

6. Small wild game delivered to a game-handling establishment must be presented to the competent authority for inspection.

7. The rules laid down in Section II, Chapter V apply to the cutting and boning of small wild game.

8.4.2.6 Hygiene requirements for minced meat, meat preparations and mechanically separated meat (MSM)

Extracts of the requirements of Annex III, Section V of Regulation (EC) No. 853/2004, as amended, specifically relating to minced meat, meat preparations and MSM are given below; for full requirements, reference should be made to the actual Regulation.

8.4.2.6.1 Chapter I: Requirements for production establishments

Food business operators operating establishments producing minced meat, meat preparations or MSM must ensure that they:

1. are constructed so as to avoid contamination of meat and products, in particular by:

   (a) allowing constant progress of the operations; or

   (b) ensuring separation between the different production batches;

2. have rooms for the separate storage of packaged and exposed meat and products, unless stored at different times or in such a way that the packaging material and the manner of storage cannot be a source of contamination for the meat or products;

3. have rooms equipped to ensure compliance with the temperature requirements laid down in Chapter III;

4. have equipment for washing hands used by staff handling exposed meat and products with taps designed to prevent the spread of contamination; and

5. have facilities for disinfecting tools with hot water supplied at not less than 82 °C, or an alternative system having an equivalent effect.

8.4.2.6.2 Chapter II: Requirements for raw materials

Food business operators producing minced meat, meat preparations or MSM must ensure that the raw materials used satisfy the following requirements.

1. The raw material used to prepare minced meat must meet the following requirements.

   (a) It must comply with the requirements for fresh meat;

(b) It must derive from skeletal muscle, including adherent fatty tissues;

(c) It must not derive from:

(i) scrap cuttings and scrap trimmings (other than whole muscle cuttings);

(ii) MSM;

(iii) meat containing bone fragments or skin; or

(iv) meat of the head with the exception of the masseters, the non-muscular part of the *linea alba*, the region of the carpus and the tarsus, bone scrapings and the muscles of the diaphragm (unless the serosa has been removed).

2. The following raw material may be used to prepare meat preparations:

(a) fresh meat;

(b) meat meeting the requirements of point 1; and

(c) if the meat preparation is clearly not intended to be consumed without first undergoing heat treatment:

(i) meat derived from the mincing or fragmentation of meat meeting the requirements of point 1 other than point 1(c)(i); and

(ii) MSM meeting the requirements of Chapter III, point 3(d).

3. The raw material used to produce MSM must meet the following requirements.

(a) It must comply with the requirements for fresh meat;

(b) The following material must not be used to produce MSM:

(i) for poultry, the feet, neck skin, and head; and

(ii) for other animals, the bones of the head, feet, tails, femur, tibia, fibula, humerus, radius and ulna.

8.4.2.6.3 Chapter III: Hygiene during and after production

Food business operators producing minced meat, meat preparations, or MSM must ensure compliance with the following requirements.

1. The work on meat must be organised in such a way as to prevent or minimise contamination. To this end, food business operators must ensure in particular that the meat used is:

   (a) at a temperature of not more than 4 °C for poultry, 3 °C for offal and 7 °C for other meat; and

   (b) brought into the preparation room progressively, as needed.

2. The following requirements apply to the production of minced meat and meat preparations.

   (a) Unless the competent authority authorises boning immediately before mincing, frozen or deep-frozen meat used for the preparation of minced meat or meat preparations must be boned before freezing. It may be stored only for a limited period.

   (b) When prepared from chilled meat, minced meat must be prepared:

      (i) in the case of poultry, within no more than three days of their slaughter;

      (ii) in the case of animal other than poultry, within no more than six days of their slaughter; or

      (iii) within no more than 15 days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.

   (c) Immediately after production, minced meat and meat preparations must be wrapped or packaged and be:

      (i) chilled to an internal temperature of not more than 2 °C for minced meat and 4 °C for meat preparations; or

      (ii) frozen to an internal temperature of not more than -18 °C.

      These temperature conditions must be maintained during storage and transport.

3. The following requirements apply to the production and use of MSM produced using techniques that do not alter the structure of the bones used in the production of MSM and the calcium content of which is not significantly higher than that of minced meat.

   (a) Raw material for deboning from an on-site slaughterhouse must be no more than seven days old; otherwise, raw material for deboning must be no more than five days old. However, poultry carcases must be no more than three days old.

   (b) Mechanical separation must take place immediately after deboning.

   (c) If not used immediately after being obtained, MSM must be wrapped or packaged and then chilled to a temperature of not more than 2 °C or frozen to an internal temperature of not more than -18 °C. These temperature requirements must be maintained during storage and transport.

   (d) If the food business operator has carried out analyses demonstrating that MSM complies with the microbiological criteria for minced meat adopted in accordance with Regulation (EC) No. 852/2004, it may be used in meat preparations that are clearly not intended to be consumed without first undergoing heat treatment and in meat products.

   (e) MSM not shown to comply with the criteria referred to in (d) may be used only to manufacture heat-treated meat products in establishments approved in accordance with this Regulation.

4. The following requirements apply to the production and use of MSM produced using techniques other than those mentioned in point 3.

   (a) Raw material for deboning from an on-site slaughterhouse must be no more than seven days old; otherwise, raw material for deboning must be no more than five days old. However, poultry carcases must be no more than three days old.

   (b) If mechanical separation does not take place immediately after deboning, the flesh-bearing bones must be stored and transported at a temperature of not more than 2 °C or, if frozen, at a temperature of not more than -18 °C.

   (c) Flesh-bearing bones obtained from frozen carcases must not be refrozen.

   (d) If not used within one hour of being obtained, MSM must be chilled immediately to a temperature of not more than 2 °C.

(e) If, after chilling, MSM is not processed within 24 hours, it must be frozen within 12 hours of production and reach an internal temperature of not more than -18 °C within six hours.

(f) Frozen MSM must be wrapped or packaged before storage or transport, must not be stored for more than three months and must be maintained at a temperature of not more than -18 °C during storage and transport.

(g) MSM may be used only to manufacture heat-treated meat products in establishments approved in accordance with this Regulation.

5. Minced meat, meat preparations and MSM must not be re-frozen after thawing.

8.4.2.6.4 Chapter IV: Labelling

1. In addition to the requirements of Directive 2000/13/EC (Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs (OJ L 109, 6.5.2000, p. 29). Directive as last amended by Directive 2003/89/EC (OJ L 308, 25.11.2003, p. 15)), food business operators must ensure compliance with the requirement of point 2 if, and to the extent that, national rules in the Member State in the territory of which the product is placed on the market so require.

2. Packages intended for supply to the final consumer containing minced meat from poultry or solipeds or meat preparations containing MSM must bear a notice indicating that such products should be cooked before consumption.

8.4.2.7 Hygiene requirements for meat products

Extracts of the requirements of Annex III, Section VI of Regulation (EC) No. 853/2004, as amended, specifically relating to meat products are given below; for full requirements, reference should be made to the actual Regulation.

1. Food business operators must ensure that the following items are not used in the preparation of meat products:

   (a) genital organs of either female or male animals, except testicles;

   (b) urinary organs, except the kidneys and the bladder;

   (c) the cartilage of the larynx, the trachea and the extra-lobular bronchi;

(d) eyes and eyelids;

(e) the external auditory meatus;

(f) horn tissue; and

(g) in poultry, the head — except the comb and the ears, the wattles and caruncles — the oesophagus, the crop, the intestines and the genital organs.

2. All meat, including minced meat and meat preparations, used to produce meat products must meet the requirements for fresh meat. However, minced meat and meat preparations used to produce meat products need not satisfy other specific requirements of Section V.

8.4.2.8 Hygiene requirements for egg and egg products

8.4.2.8.1 Chapter I: Eggs

1. At the producer's premises, and until sale to the consumer, eggs must be kept clean, dry, free of extraneous odour, effectively protected from shocks and out of direct sunshine.

2. Eggs must be stored and transported until sale to the final consumer at a temperature, preferably constant, that is best suited to assure optimal conservation of their hygiene properties, unless the competent authority imposes national temperature requirements for egg storage facilities and for vehicles transporting eggs between such storage facilities.

3. Eggs must be delivered to the consumer within a maximum time limit of 21 days of laying.

8.4.2.8.2 Chapter II: Egg products

I. Requirements for establishments

Food business operators must ensure that establishments for the manufacture of egg products are constructed, laid out and equipped so as to ensure separation of the following operations:

1. washing, drying and disinfecting dirty eggs, where carried out;

2. breaking eggs, collecting their contents and removing parts of shells and membranes; and

3. operations other than those referred to in points 1 and 2.

II. Raw materials for the manufacturers of egg products

Food business operators must ensure that raw materials used to manufacture egg products comply with the following requirements.

1. The shells of eggs used in the manufacture of egg products must be fully developed and contain no breaks. However, cracked eggs may be used for the manufacture of liquid egg or egg products if the establishment of production or a packing centre delivers them directly to an establishment approved for the manufacture of liquid egg or a processing establishment, where they must be broken as soon as possible.

2. Liquid egg obtained in an establishment approved for that purpose may be used as raw material. Liquid egg must be obtained in accordance with the requirements of points 1, 2, 3, 4 and 7 of Part III.

III. Special hygiene requirements for the manufacturers of egg products

Food business operators must ensure that all operations are carried out in such a way as to avoid any contamination during production, handling and storage of egg products, in particular by ensuring compliance with the following requirements.

1. Eggs must not be broken unless they are clean and dry.

2. Eggs must be broken in a manner that minimises contamination, in particular by ensuring adequate separation from other operations.

   Cracked eggs must be processed as soon as possible.

3. Eggs other than those of hens, turkeys or guinea fowl must be handled and processed separately. All equipment must be cleaned and disinfected before processing of hens', turkeys' and guinea fowls' eggs is resumed.

4. Egg contents may not be obtained by the centrifuging or crushing of eggs, nor may centrifuging be used to obtain the remains of egg whites from empty shells for human consumption.

5. After breaking, each particle of the liquid egg must undergo processing as quickly as possible to eliminate microbiological hazards or to reduce them to an acceptable level. A batch that has been insufficiently processed may immediately undergo processing again in the same establishment if this processing renders it fit for human consumption. Where a batch is found to

be unfit for human consumption, it must be denatured to ensure that it is not used for human consumption.

6. Processing is not required for egg white intended for the manufacture of dried or crystallised albumin destined subsequently to undergo heat treatment.

7. If processing is not carried out immediately after breaking, liquid egg must be stored either frozen or at a temperature of not more than 4 °C.

   The storage period before processing at 4 °C must not exceed 48 hours.

   However, these requirements do not apply to products to be de-sugared, if de-sugaring process is performed as soon as possible.

8. Products that have not been stabilised so as to be kept at room temperature must be cooled to not more than 4 °C. Products for freezing must be frozen immediately after processing.

IV. Analytical specifications

1. The concentration of 3-OH-butyric acid must not exceed 10 mg/kg in the dry matter of the unmodified egg product.

2. The lactic acid content of raw material used to manufacture egg products must not exceed 1 g/kg of dry matter. However, for fermented products, this value must be the one recorded before the fermentation process.

3. The quantity of eggshell remains, egg membranes and any other particles in the processed egg product must not exceed 100 mg/kg of egg product.

V. Labelling and identification marking

1. In addition to the general requirements for identification marking laid down in Annex II, Section I, consignments of egg products, destined not for retail but for use as an ingredient in the manufacture of another product, must have a label giving the temperature at which the egg products must be maintained and the period during which conservation may thus be assured.

2. In the case of liquid egg, the label referred to in point 1 must also bear the words: ‘non-pasteurised liquid egg - to be treated at place of destination’ and indicate the date and hour of breaking.

8.5 Regulation (EC) No. 854/2004 of the European Parliament and of the Council Laying Down Specific Rules for the Organisation of Official Controls on Products of Animal Origin Intended for Human Consumption

Regulation (EC) No. 854/2004 gives requirements for official controls on products of animal origin and states requirements for those enforcing the provisions.

In this regulation, general principles for official controls in respect of all products of animal origin falling within the scope of the regulation are given. It is a requirement that food business operators give assistance to ensure that official controls carried out by the competent authority can be done properly. The official controls include audits of GHPs and HACCP-based procedures.

Meat products in particular must comply with the requirements of Annex I of the regulation. This refers to official controls of production and the placing on the market of meat products along with the use of an official veterinarian appointed by the competent authorities. The requirements refer to hygienic conditions and regular inspections both ante- and post-mortem to ensure compliance. Specific requirements regarding inspections are laid down for the following: bovine animals; sheep and goats; swine; poultry; lagomorphs and wild game.

8.5.1 Regulation (EC) No. 854/2004 Annex I: Decisions concerning meat

Chapter V of Section II states that meat products are to be declared unfit for human consumption if it:

(a) derives from animals that have not undergone ante-mortem inspection, except for hunted wild game;

(b) derives from animals the offal of which has not undergone post-mortem inspection, unless otherwise provided for under this Regulation or Regulation (EC) No 853/2004;

(c) derives from animals which are dead before slaughter, stillborn, unborn or slaughtered under the age of seven days;

(d) results from the trimming of sticking points;

(e) derives from animals affected by an (OIE) List A or, where appropriate, OIE List B disease, unless otherwise provided for in Section IV;

(f) derives from animals affected by a generalised disease, such as generalised septicaemia, pyaemia, toxaemia or viraemia;

(g) is not in conformity with microbiological criteria laid down under Community legislation to determine whether food may be placed on the market;

(h) exhibits parasitic infestation, unless otherwise provided for in Section IV;

(i) contains residues or contaminants in excess of the levels laid down in Community legislation. Any overshooting of the relevant level should lead to additional analyses whenever appropriate;

(j) without prejudice to more specific Community legislation, derives from animals or carcases containing residues of forbidden substances or from animals that have been treated with forbidden substances;

(k) consists of the liver and kidneys of animals more than two years old from regions where implementation of plans approved in accordance with Article 5 of Directive 96/23/EC has revealed the generalised presence of heavy metals in the environment;

(l) has been treated illegally with decontaminating substances;

(m) has been treated illegally with ionising or UV-rays;

(n) contains foreign bodies (except, in the case of wild game, material used to hunt the animal);

(o) exceeds the maximum permitted radioactivity levels laid down under Community legislation;

(p) indicates patho-physiological changes, anomalies in consistency, insufficient bleeding (except for wild game) or organoleptic anomalies, in particular a pronounced sexual odour;

(q) derives from emaciated animals;

(r) contains specified risk material, except as provided for under Community legislation;

(s) shows soiling, faecal or other contamination;

(t) consists of blood that may constitute a risk to public or animal health owing to the health status of any animal from which it derives or contamination arising during the slaughter process;

(u) in the opinion of the official veterinarian, after examination of all the relevant information, it may constitute a risk to public or animal health or is for any other reason not suitable for human consumption.

The official veterinarian may impose requirements concerning the use of meat derived from animals having undergone emergency slaughter outside the slaughterhouse.

8.5.2 Regulation (EC) No. 854/2004, Annex I: Health marking

1. The official veterinarian is to supervise health marking and the marks used.

2. The official veterinarian is to ensure, in particular, that:

   (a) the health mark is applied only to animals (domestic ungulates, farmed game mammals other than lagomorphs, and large wild game) having undergone ante-mortem and post-mortem inspection in accordance with this Regulation and when there are no grounds for declaring the meat unfit for human consumption. However, the health mark may be applied before the results of any examination for trichinosis is available, if the official veterinarian is satisfied that meat from the animal concerned will be placed on the market only if the results are satisfactory; and

   (b) health-marking takes place on the external surface of the carcase, by stamping the mark in ink or hot branding, and in such a manner that if carcases are cut into half carcases or quarters, or half carcases are cut into three pieces, each piece bears a health mark.

3. The health mark must be an oval mark at least 6.5 cm wide by 4.5 cm high bearing the following information in perfectly legible characters:

   (a) the mark must indicate name of the country in which the establishment is located, which may be written out in full in capitals or shown as a two-letter code in accordance with the relevant ISO standard.

   In the case of Member States, however, these codes are BE, BG, CZ, DK, DE, EE, GR, ES, FR, IE, IT, CY, LV, LT, LU, HU, MT, NL, AT, PL, PT, RO, SI, SK, FI, SE and UK;

   (b) the mark must indicate the approval number of the slaughterhouse; and

   (c) when applied in a slaughterhouse within the Community, the mark must include the abbreviation CE, EC, EF, EG, EK, EO, EY, ES, EÜ, EK, EB or WE.

4. Letters must be at least 0,8 cm high and figures at least 1 cm high. The dimensions and characters of the mark may be reduced for health marking of lamb, kids and piglets.

5. The colours used for health marking must be authorised in accordance with Community rules on the use of colouring substances in foodstuffs.

6. The health mark may also include an indication of the official veterinarian who carried out the health inspection of the meat.

7. Meat from animals having undergone emergency slaughter outside the slaughterhouse must bear a special health mark, which cannot be confused either with the health mark provided for in this Chapter or with the identification mark provided for in Annex II, Section I, to Regulation (EC) No. 853/2004.

8. Meat from unskinned wild game cannot bear a health mark unless, after skinning in a game handling establishment, it has undergone post-mortem inspection and been declared fit for human consumption.

9. This Chapter is to apply without prejudice to animal health rules on health marking.

8.5.3 Imports

Chapter III of Regulation (EC) No. 854/2004 lays down procedures concerning imports including provisions for lists of third countries and parts of third countries from which imports of specified products of animal origin are permitted, and list of establishments from which imports of specified products of animal origin are permitted.

Fresh meat, minced meat, meat preparations, meat products and MSM may be imported into the Community only if they have been manufactured from meat obtained in slaughterhouses and cutting plants appearing on lists drawn up and updated in accordance with Article 12 of this regulation or in approved Community establishments.

8.6 Regulation (EC) No. 2073/2005 on Microbiological Criteria for Foodstuffs

Regulation (EC) No. 2073/2005, which has applied since 1 January 2006, establishes microbiological criteria for a range of foods.

The aim of this legislation is to complement food hygiene requirements, ensuring that foods being placed on the market do not pose a risk to human health, and the legislation applies to all businesses involved in food production and handling.

The definition of *microbiological criterion* means a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of *microorganisms*, and/or on the quantity of their toxins or metabolites, per unit(s) of mass, volume, area or batch;

> *microorganisms* means bacteria, viruses, yeasts, moulds, algae, parasitic protozoa, microscopic parasitic helminths, and their toxins and metabolites;

Two kinds of criteria have been established: *food safety criteria*, applying to products placed on the market, and *process hygiene criteria* that are applied during the manufacturing process.

> *food safety criterion* means a criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market;

> *process hygiene criterion* means a criterion indicating the acceptable functioning of the production process. Such a criterion is not applicable to products placed on the market. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law;

8.6.1 Food safety criteria

Chapter 1 of Regulation (EC) No. 2073/2005 focuses on food safety criteria that cover foods such as ready-to-eat foods (RTE) and meat products. The relevant criteria are outlined in Table 8.I:

Table 8.I
Food safety criteria

Food category	Microorganisms	Sampling plan(1)		Limit(2)		Analytical reference method(3)	Stage where the criterion apply
		n	c	m	M		
1.1 RTE foods intended for infants and RTE foods for special medical purposes (4)	*Listeria monocytogenes*	10	5	Absence in 25 g		EN/ISO 11290-1	Products placed on the market during their shelf life
1.2 RTE foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	*L. monocytogenes*	5	0	100 cfu/g (5)		EN/ISO 11290-2 (6)	Products placed on the market during their shelf life
		5	0	Absence in 25 g (7)		EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3 RTE foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes(4) (8)	*L. monocytogenes*	5	0	100 cfu/g		EN/ISO 11290-2 (6)	Products placed on the market during their shelf life
1.4 Minced meat and meat preparations intended to be eaten raw	*Salmonella*	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf life
1.5 Minced meat and meat preparations made from poultry meat intended to be eaten cooked	*Salmonella*	5	0	From 1.1.2006 Absence in 10 g From 1.1.2010 Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf life

Food category	Microorganisms	Sampling plan[1]		Limit[2]		Analytical reference method[3]	Stage where the criterion apply
		n	c	m	M		
1.6 Minced meat and meat preparations made from other species than poultry intended to be eaten cooked	*Salmonella*	5	0	Absence in 10 g		EN/ISO 6579	Products placed on the market during their shelf life
1.7 MSM [9]	*Salmonella*	5	0	Absence in 10 g		EN/ISO 6579	Products placed on the market during their shelf life
1.8 Meat products intended to be eaten raw, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk	*Salmonella*	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf life
1.9 Meat products made from poultry meat intended to be eaten cooked	*Salmonella*	5	0	From 1.1.2006 Absence in 10 g From 1.1.2010 Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf life
1.10 Gelatine and collagen	*Salmonella*	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf life
1.14 Egg products, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk	*Salmonella*	5	0	Absence in 25 g		EN/ISO 6579	Products placed on the market during their shelf life
1.15 RTE foods containing raw eggs, excluding products where the manufacturing process or the composition of the product will eliminate the *Salmonella* risk	*Salmonella*	5	0	Absence in 25 g or ml		EN/ISO 6579	Products placed on the market during their shelf life

1 n = number of units comprising the sample; c = number of sample units giving values between m and M

2 For point 1.1 - 1.25, m = M.

3 The most recent edition of the standard shall be used.

4 Regular testing against the criterion is not required in normal circumstances for the following RTE foods:
- those which have received heat treatment or other processing effective to eliminate *L. monocytogenes*, when recontamination is not possible after this treatment (for example, products heat treated in their final package),
- fresh, uncut and unprocessed vegetables and fruits, excluding sprouted seeds,
- bread, biscuits and similar products,
- bottled or packed waters, soft drinks, beer, cider, wine, spirits and similar products,
- sugar, honey and confectionery, including cocoa and chocolate products,
- live bivalve molluscs.

5 This criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf life. The operator may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf life.

6 1 ml of inoculum is plated on a petri dish of 140 mm diameter or on three petri dishes of 90 mm diameter.

7 This criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf life.

8 Products with pH $\leq$ 4,4 or $a_w \leq$ 0,92, products with pH $\leq$ 5,0 and $a_w \leq$ 0,94, products with a shelf life of less than five days shall be automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.

9 This criterion shall apply to MSM produced with the techniques referred to in paragraph 3 of Chapter III of Section V of Annex III to Regulation (EC) No. 853/2004 of the European Parliament and of the Council.

Interpretation of the test results relating to Table 8.I

The limits given refer to each sample unit tested.

The test results demonstrate the microbiological quality of the batch tested(1).

L. monocytogenes in RTE foods intended for infants and for special medical purposes:
- satisfactory, if all the values observed indicate the absence of the bacterium,
- unsatisfactory, if the presence of the bacterium is detected in any of the sample units.

L. monocytogenes in RTE foods able to support the growth of *L. monocytogenes* before the food has left the immediate control of the producing food business operator when he is not able to demonstrate that the product will not exceed the limit of 100 cfu/g throughout the shelf life:
- satisfactory, if all the values observed indicate the absence of the bacterium,
- unsatisfactory, if the presence of the bacterium is detected in any of the sample units.

L. monocytogenes in other RTE foods:
- satisfactory, if all the values observed are ≤ the limit,
- unsatisfactory, if any of the values are > the limit.

Salmonella in different food categories:
- satisfactory, if all the values observed indicate the absence of the bacterium,
- unsatisfactory, if the presence of the bacterium is detected in any of the sample units.

(1) The test results may be used also for demonstrating the effectiveness of the HACCP principles or GHP of the process.

8.6.2 Process hygiene criteria

Chapter 2 focuses on process hygiene criteria, with chapter 2.1 referring to meat and products thereof. The relevant criteria are outlined in Table 8.II A and 8.II B:

Table 8.II A
Process hygiene criteria: Meat and products thereof

Food category	Micro-organisms	Sampling plan[1]		Limit[2]		Analytical reference method[3]	Stage where the criterion apply	Action in case of unsatisfactory results
		n	c	m	M			
2.1.1 Carcases of cattle, sheep, goats, horses [4]	Aerobic colony count			3,5 log cfu/ cm^2 daily mean log	5,0 log cfu/ cm^2 daily mean log	ISO 4833	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
	Enterobacteriaceae			1,5 log cfu/ cm^2 daily mean log	2,5 log cfu/ cm^2 daily mean log	ISO 21528-2	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
2.1.2 Carcases of pigs [4]	Aerobic colony count			4,0 log cfu/ cm^2 daily mean log	5,0 log cfu/ cm^2 daily mean log	ISO 4833	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
	Enterobacteriaceae			2,0 log cfu/ cm^2 daily mean log	3,0 log cfu/ cm^2 daily mean log	ISO 21528-2	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls
2.1.3 Carcases of cattle, sheep, goats and horses	*Salmonella*	50 [5]	2 [6]	Absence in the area tested per carcase		EN/ISO 6579	Carcases after dressing but before chilling	Improvements in slaughter hygiene, review of process controls and of origin of animals
2.1.4 Carcases of pigs	*Salmonella*	50 [5]	5 [6]	Absence in the area tested per carcase		EN/ISO 6579	Carcases after dressing but before chilling	Improvements in slaughter hygiene and review of process controls, origin of animals and of the biosecurity measures in the farms of origin

Food category	Micro-organisms	Sampling plan[1]		Limit[2]		Analytical reference method[3]	Stage where the criterion apply	Action in case of unsatisfactory results
		n	c	m	M			
2.1.5 Poultry carcases of broilers and turkeys	*Salmonella*	50 [5]	7 [6]	Absence in 25 g of a pooled sample of neck skin		EN/ISO 6579	Carcases after chilling	Improvements in slaughter hygiene and review of process controls, origin of animals and biosecurity measures in the farms of origin
2.1.6 Minced meat	Aerobic colony count [7]	5	2	5×10^5 cfu/g	5×10^6 cfu/g	ISO 4833	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials
	E. coli [8]	5	2	50 cfu/g	500 cfu/g	ISO 16649-1 or 2	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials
2.1.7 MSM [9]	Aerobic colony count	5	2	5×10^5 cfu/g	5×10^6 cfu/g	ISO 4833	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials
	E. coli [8]	5	2	50 cfu/g	500 cfu/g	ISO 16649-1 or 2	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials
2.1.8 Meat preparations	*E. coli* [8]	5	2	500 cfu/g or cm^2	5000 cfu/g or cm^2	ISO 16649-1 or 2	End of the manufacturing process	Improvements in production hygiene and improvements in selection and/or origin of raw materials

1 n = number of units comprising the sample; c = number of sample units giving values between m and M

2 For points 2.1.3 - 2.1.5 m = M.

3 The most recent edition of the standard shall be used.

4 The limits (m and M) shall apply only to samples taken by the destructive method. The daily mean log shall be calculated by first taking a log value of each individual test result and then calculating the mean of these log values.

5 The 50 samples shall be derived from 10 consecutive sampling sessions in accordance with the sampling rules and frequencies laid down in this Regulation.

6 The number of samples where the presence of *Salmonella* is detected. The c value is subject to review in order to take into account the progress made in reducing the *Salmonella* prevalence. Member States or regions having low *Salmonella* prevalence may use lower c values even before the review.

7 This criterion shall not apply to minced meat produced at retail level when the shelf life of the product is less then 24 hours.

8 *E. coli* is used here as an indicator of faecal contamination.

9 These criteria apply to MSM produced with the techniques referred to in paragraph 3 of Chapter III of Section V of Annex III to Regulation (EC) No. 853/2004 of the European Parliament and of the Council.

Interpretation of the test results relating to Table 8.II A

The limits given refer to each sample unit tested, excluding testing of carcases where the limits refer to pooled samples.

The test results demonstrate the microbiological quality of the process tested.

Enterobacteriaceae and aerobic colony count in carcases of cattle, sheep, goats, horses and pigs:
- satisfactory, if the daily mean log is ≤ m,
- acceptable, if the daily mean log is between m and M,
- unsatisfactory, if the daily mean log is > M.

Salmonella in carcases:
- satisfactory, if the presence of *Salmonella* is detected in a maximum of c/n samples,
- unsatisfactory, if the presence of *Salmonella* is detected in more than c/n samples.

After each sampling session, the results of the last ten sampling sessions shall be assessed in order to obtain the n number of samples.

E. coli and aerobic colony count in minced meat, meat preparations and MSM:
- satisfactory, if all the values observed are ≤ m,
- acceptable, if a maximum of c/n values are between m and M, and the rest of the values observed are ≤ m,
- unsatisfactory, if one or more of the values observed are > M or more than c/n values are between m and M.

Table 8.II B
Process hygiene criteria: Egg products

Food category	Micro-organisms	Sampling plan[1]		Limit		Analytical reference method[2]	Stage where the criterion apply	Action in case of unsatisfactory results
		n	c	m	M			
2.3.1 Egg products	Enterobacteriaceae	5	2	10 cfu/g or ml	100 cu/g or ml	ISO 21528-2	End of the manufacturing process	Checks on the efficiency of the heat treatment and prevention of recontamination

1 n = number of units comprising the sample; c = number of sample units giving values between m and M

2 The most recent edition of the standard shall be used

Interpretation of the test results relating to Table 8.II B

The limits given refer to each sample unit tested

The test results demonstrate the microbiological quality of the process tested.

Enterobacteriaceae in egg products:
- satisfactory, if all the values observed are ≤ m,
- acceptable, if a maximum of c/n values are between m and M, and the rest of the values observed are ≤ m,
- unsatisfactory, if one or more of the values observed are > M or more than c/n values are between m and M

8.7 Food Hygiene (England) Regulations 2006, S.I. 2006 No. 14 (Hygiene Requirements Specific to the U.K)

8.7.1 Temperature control requirements

In the UK, Schedule 4 of the Food Hygiene (England) Regulations 2006, S.I. 2006 No. 14, details temperature control requirements for foods in general.

The regulations prescribe a chilled food holding temperature of 8 °C or less, but there is also a general requirement that foods must not be kept at temperatures that would result in a risk to health, and particularly that perishable foodstuffs must not be kept at above the maximum recommended storage temperature, which overrides the 8 °C requirement. Hot-held foods (food having been cooked or reheated and is for service or on display for sale) must not be kept below 63 °C.

The regulations provide for defences in relation to upward variations of the 8 °C temperature, tolerance periods for chill-holding of foods and hot-holding variations. The defendant may be required to produce well-founded scientific proof to support his claims. For example, with chill holding tolerance periods, the defendant will need to prove that the food was on service or display, had not been previously put on display at more than 8 °C and had been kept there for less than four hours. Alternatively, it would need to be proven that the food was being transferred to or from a vehicle used for the activities of a food business, to or from premises (including vehicles) at which the food was to be kept at or below 8 °C or the recommended temperature, or, was kept at above 8 °C or the recommended temperature for an unavoidable reason, such as that below, and was kept at above 8 °C or the recommended temperature, for a limited period consistent with food safety. The permitted reasons are given below:

- to facilitate handling during and after processing or preparation
- the defrosting of equipment, or
- temporary breakdown of equipment

For Scotland there are separate provisions to include requirements to hold food under refrigeration or in a cool ventilated place, or at a temperature above 63 °C and to reheat food to a temperature of at least 82 °C (The Food Hygiene (Scotland) Regulations 2006, S.S.I. 2006 No. 3).

Schedule 4 of the Food Hygiene (England) Regulations 2006 contains several definitions, including:

Shelf life: where the minimum durability or 'use by' indication is required according to Regulation 20 or 21 of the Food Labelling Regulations 1996 (form of indication of minimum durability and form of indication of ‘use-by date’) the

period up to and including that date. For other food, the period for which it can be expected to remain fit for sale when kept in a manner consistent with food safety.

Recommended temperature: a specified temperature that has been recommended in accordance with a food business responsible for manufacturing, preparing or processing the food recommending that it be kept at or below a specified temperature between 8 ºC and ambient temperatures.

It should be noted that the temperature control requirements as detailed in Schedule 4 of the Food Hygiene (England) Regulations 2006 (S.I. 2006 No. 14) do not apply to any food covered by Regulation (EC) No. 853/2004 on hygiene of products of animal origin or any food business operation carried out on a ship or aircraft.

8.8 Guidance

In the U.K, the Food Standards Agency (FSA) has published guidance notes on the requirements of the EU hygiene and microbiological criteria regulations. These guidance notes should be read in conjunction with the regulations to aid interpretation. The FSA guidance notes can be accessed at the following link: http://www.food.gov.uk/foodindustry/guidancenotes/hygguid/fhlguidance/

8.9 Other Relevant Legislation

The following regulations about meat designations need to be considered:

- Council Regulation (EC) No. 1234/2007 of 22 October 2007 establishing a common organisation of agricultural markets and on specific provisions for certain agricultural products (Single CMO Regulation) (OJ *European Communities* L 299, 16.11.2007, p. 1-149). In addition to general meat provisions, this Regulation contains information regarding marketing standards for poultry, a definition for fresh poultry meat and specific provisions on carcase grading.

- Commission Regulation (EC) No. 566/2008 of 18 June 2008 laying down detailed rules for the application of Council Regulation (EC) No. 1234/2007 as regards the marketing of the meat of bovine animals aged 12 months or less (OJ *European Communities* L 160, 19.6.2008, p. 22-5).

- Commission Regulation (EC) No. 1665/2006 amending Regulation (EC) No. 2075/2005 laying down specific rules on official controls for *Trichinella* in meat (OJ *European Communities* L 320, 18.11.2006, p. 46).

8.10 References

1. Regulation (EC) No. 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs (OJ *European Communities* L139, 30.4.2004, p. 1). The revised text of Regulation (EC) No. 852/2004 is now set out in a Corrigendum (OJ *European Communities* L226, 25.6.2004, p. 3) as amended by Regulation (EC) No. 1019/2008 and as read with Regulation 2073/2005.

2. Regulation (EC) No. 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin (OJ *European Communities* L139, 30.4.2004, p. 55). The revised text of Regulation (EC) No. 853/2004 is now set out in a Corrigendum (OJ *European Communities* L226, 25.6.2004, p. 22) as amended by Regulation (EC) Nos. 2074/2005, 2076/2005, 1662/2006, 1791/2006 and 1020/2008 and as read with EC Directive 2004/41, Regulation (EC) No. 1688/2005, Regulation (EC) No. 2074/2005 and Regulation (EC) No. 2076/2005.

3. Regulation (EC) No. 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption (OJ *European Communities* L139, 30.4.2004, p. 206). The revised text of Regulation (EC) No. 854/2004 is now set out in a Corrigendum (OJ No. L226, 25.6.2004, p. 83) as amended by Regulation Nos. 882/2004, 2074/2005, 2076/2005, 1663/2006, 1791/2006 and 1021/2008 and as read with EC Directive 2004/41, Regulation (EC) No. 2074/2005, Regulation (EC) No. 2075/2005 and Regulation (EC) No. 2076/2005.

4. Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs (OJ *European Communities* L338, 22.12.2005, p. 1, as read with the corrigenda at OJ No. L283, 14.10.2006, p. 62) as amended by Regulation (EC) No. 1441/2007.

5. Commission Regulation (EC) No. 2074/2005 laying down implementing measures for certain products under Regulation (EC) No. 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No. 854/2004 of the European Parliament and of the Council and Regulation (EC) No. 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No. 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No. 853/2004 and (EC) No. 854/2004, as amended by Commission Regulation (EC) No. 1022/2008 (OJ *European Communities* L 338, 22.12.2005, p. 27)

6. Commission Regulation (EC) No. 2076/2005 laying down transitional arrangements for the implementation of Regulations (EC) No. 853/2004, (EC) No. 854/2004 and (EC) No. 882/2004 of the European Parliament and of the Council and amending Regulations (EC) No. 853/2004 and (EC) No. 854/2004 (OJ *European Communities* L 338, 22.12.2005, p. 83).

9. PATHOGEN PROFILES

9.1 *Campylobacter* spp.

9.1.1 Morphology

Gram-negative spirally curved rods; 0.2 - 0.8 x 0.5 - 5.0 μm.

9.1.2 Oxygen requirements

Campylobacter is both microaerophilic and 'capnophilic' (liking carbon dioxide); its growth is favoured by an atmosphere containing 10% carbon dioxide and 5 - 6% oxygen. Growth is also enhanced by hydrogen. The organism will normally die rapidly in the presence of air; it is particularly sensitive to oxygen breakdown products. Because of this and other growth characteristics (see below), these organisms are not normally capable of growing in foods.

Vacuum- or gas-packing appears to have little major impact on the survival of *Campylobacters* on chilled meat or poultry (1, 2).

9.1.3 Temperature

Campylobacter jejuni and *Campylobacter coli* only grow at temperatures above about 30 °C; they (and *Campylobacter lari*) are consequently referred to as the thermophilic group of *Campylobacters*. Their optimum temperature for growth is between 42 and 43 °C, with a maximum of 45 °C (3).

Campylobacter survives poorly at room temperatures (around 20 - 23 °C); it dies much more quickly than at refrigeration temperatures. It can survive well for short periods at chill temperatures. On the other hand, it is generally more sensitive to freezing, although there may be some survival for long periods (1, 4, 5).

9.1.4 Heat resistance

C. jejuni is very heat-sensitive. Heat injury can occur at 46 °C or higher. z-values range from 4.5 to 8 minutes for temperatures between 48 - 60 °C depending on pH (3) . With meat and poultry, heat treatments sufficient to kill *Salmonella* will also readily kill *Campylobacter*. D-values ranging from a few seconds to less than 1 minute at temperatures between 57 and 60 °C have been reported for *Campylobacter* in meat. Low z-values (*ca* 3 - 5 °C) have also been demonstrated for *Campylobacter* (6).

9.1.5 pH

Campylobacter has an optimum pH for growth in the range 6.5 - 7.5, and no growth is observed below pH 4.9 (3). The response of *C. jejuni* to pH is influenced by temperature and the type of acid used to adjust the pH. At similar pH values, the organism was most rapidly inactivated at 42 °C, at an intermediate rate at 25 °C, and slowly at 4 °C (6). Lactic acid is more inhibitory than hydrochloric acid at the same pH level (1).

9.1.6 A_w/Sodium chloride

Campylobacter is particularly sensitive to drying; it does not survive well in dry environments. The minimum water activity for growth is 0.98. *Campylobacter* is also quite sensitive to sodium chloride (NaCl); levels of 2% or more can be bactericidal to the organism. The effect is temperature-dependent; the presence of even 1% NaCI can be inhibitory or bactericidal, depending on temperature. The bactericidal effect falls with decreasing temperature (2).

9.2 *Clostridium botulinum*

Seven different types of *C. botulinum* are known, forming at least seven different toxins, A to G. Types A, B, E and, to a lesser extent, F are the types that are responsible for most cases of human botulism (7, 8). All type A strains are proteolytic and type E strains are usually non-proteolytic; types B and F can be either. There are four main groupings of the organism, and groups I and II are those responsible for the botulism cases.

9.2.1 Morphology

Gram-positive spore-forming rods; 0.5 - 2.4 x 1.7 - 22.0 μm.

9.2.2 Oxygen requirements

Although *C. botulinum* is an obligate anaerobe, many foods that are not obviously 'anaerobic' can provide adequate conditions for growth. Thus an aerobically packed product may not support the growth of the organism on the surface, but the interior of the food may do so. It is also important to note that the inclusion of oxygen as a packaging gas cannot ensure that growth of *C. botulinum* is prevented.

9.2.3 Temperature

All strains of *C. botulinum* grow reasonably well in the temperature range of 20 - 45 ºC, but the minimum temperatures required to inhibit Groups I (proteolytic group) and II (non-proteolytic group) are different. Group I will not grow at temperatures of 10 ºC or less, but Group II strains are psychrotrophic, being capable of slow growth and toxin production at low temperatures - even as low as 3 ºC (9).

9.2.4 Heat resistance

The vegetative cells of *C. botulinum* are not particularly heat-resistant, but the spores of this organism are more so. All *C. botulinum* types produce heat labile toxins, which may be inactivated by heating at 80 ºC for 20 - 30 minutes, at 85 ºC for 5 minutes, or at 90 ºC for a few seconds.

9.2.5 pH

The minimum pH for the growth of proteolytic and non-proteolytic strains is pH 4.6 and pH 5.0, respectively (10, 11).

9.2.6 A_w/ Sodium chloride

The minimum a_w for growth of *C. botulinum* depends on solute, pH and temperature, but under optimum growth conditions 10% (w/w) NaCl is required to prevent growth of Group I and 5% (w/w) NaCl is necessary to prevent growth of Group II organisms. These concentrations correspond to limiting a_w of 0.94 for Group I and 0.97 for Group II (7). These values have been established under carefully controlled laboratory conditions. In commercial situations, safety margins must be introduced to allow for process variability. Note also that if humectants other than salt are used, the minimum aw for growth may be lower.

9.2.7 Characteristics of C. botulinum spores

The most heat-resistant spores of Group I *C. botulinum* are produced by type A and proteolytic B strains ($D_{121\,°C}$=*ca* 0.21 minutes) (11).

The spores of Group II (non-proteolytic/psychotrophic) strains are less heat-resistant than Group I strains. However, they may survive mild heat treatments (70 - 85 °C) and their ability to grow at refrigeration temperatures necessitates their control in foods capable of supporting their growth (e.g. vacuum-packed, part-cooked meals with pH value >5.0 and a_w >0.97) (12, 13).

9.3 *Clostridium perfringens*

9.3.1 Morphology

Gram-positive spore-forming rods; 0.3 - 1.9 x 2.0 - 10.0 μm.

9.3.2 Oxygen requirements

C. perfringens - like other clostridia - is an obligate anaerobe. It will not, therefore, grow on the surface of foods unless they are vacuum- or gas-packed. The organism will grow well in the centre of meat or poultry dishes, where oxygen levels are reduced, particularly by cooking.

9.3.3 Temperature

The most significant characteristic of *C. perfringens* in relation to food safety is the organism's ability to grow extremely rapidly at high temperatures. Its optimum temperature for growth is 43 - 45 ºC, although *C. perfringens* has the potential ability to grow within the temperature range 15 - 50 ºC, depending on strain and other conditions. While some growth can occur at 50 ºC, death of the vegetative cells of this organism usually occurs rapidly above this temperature (14, 15). At cold temperatures of 0 - 10 ºC, vegetative cells die rapidly (14).

9.3.4 Heat resistance

Exposure to a temperature of 60 ºC or more will result in the death of vegetative cells of *C. perfringens*, although prior growth at high temperatures or the presence of fat in a food will result in increased heat resistance (16). In addition, the enterotoxin is not heat-resistant - it is destroyed by heating at 60 ºC for 10 minutes (16, 17, 18). The D-value for *C. perfringens* in roasted beef at 60 ºC is 14.5 minutes (19).

9.3.5 pH

C. pe*rfringens* is not tolerant of very low or very high pH. It grows best at pH values between 6 and 7 (the same pH as most meats). Under otherwise ideal conditions, very limited growth may occur at pH values over the range pH ≤5 and ≥8.3. Spores, however, will survive greater extremes of pH (and a_w) (14, 15).

9.3.6 A_w/Sodium chloride

C. perfringens is not tolerant of low water activities. As in the case of other factors limiting the growth or survival of this organism, the limits for a_w are affected by temperature, pH, type of solute, etc. The lowest a_w recorded to support the growth of *C. perfringens* appears to be 0.93 and 0.97 using glycerol and sucrose respectively (15, 20). Salt concentrations of 6 - 8% inhibit growth of most *C. perfringens* strains; lower concentrations may be effective in combination with other factors. Some studies indicate that the presence of 3% NaCl delays growth of *C. perfringens* in vacuum-packed beef (20).

9.3.7 Characteristics of C. perfringens spores

The spores of *C. perfringens* can vary quite considerably in their heat resistance, which also affected by the heating substrate. Recorded heat-resistance values at 95 ºC (D-values) range from 17.6 - 64.0 minutes for heat-resistant spores to 1.3 - 2.8 minutes for heat-sensitive spores (14).

9.4 *Escherichia coli* O157

9.4.1 Morphology

Gram-negative short rods; 1.1 - 1.5 x 2.0 - 6.0 µm.

9.4.2 Oxygen requirements

E. coli O157 is a facultative anaerobe; it grows well under aerobic or anaerobic conditions. High levels of carbon dioxide may inhibit its growth.

9.4.3 Temperature

The growth range for *E. coli* O157 is thought to be between 7 and 45 °C, with an optimum of approximately 37 °C (21). (Note: *E. coli* O157:H7 grows poorly at 44 - 45 °C and does not grow within 48 hours at 45.5 °C. Therefore, traditional

detection methods for *E. coli* in foods cannot be relied upon to detect *E. coli* O157:H7).

The organism appears to survive well at low temperatures and to resist freezing.

9.4.4 Heat resistance

E. coli O157 is not a heat-resistant organism. D-values at 57 and 63 °C in meat have been reported as approximately 5 and 0.5 minutes, respectively (22). Anaerobic growth, reduced a_w, high fat content and exposure to prior heat shock may result in higher D-values. The organism is more sensitive to heat than typical isolates from salmonellae.

9.4.5 pH

The minimum pH for growth, under optimal conditions, is 4.0 - 4.4 (using hydrochloric acid as an acidulant) (23, 24). The minimum value is affected by the acidulant used, with both lactic and acetic acids being more inhibitory than hydrochloric acid (21). *E. coli* O157 is unusually acid-tolerant and survives well in foods with low pH values (3.6 - 4.0), especially at chill temperatures (25).

9.4.6 A_w/Sodium chloride

Current published data suggest that *E. coli* O157 grows well at NaCl concentrations up to 2.5% and may grow at concentrations of at least 6.5% (w/v) (a_w less than 0.97) under otherwise optimal conditions (26). The organism appears to be able to tolerate certain drying processes (25).

9.5 *Listeria* spp.

9.5.1 Morphology

Gram-positive short rods; 0.4 - 0.5 x 0.5 - 2.0 μm.

9.5.2 Oxygen requirements

Aerobe or microaerophilic.

9.5.3 Temperature

Listeria monocytogenes is unusual amongst foodborne pathogens in that it is psychrotrophic, being potentially capable of growing - albeit slowly - at refrigeration temperatures down to, or even below 0 ºC. However, -0.4 °C is probably a more likely minimum in foods (27). Its optimum growth temperature, however, is between 30 and 37 ºC; growth at low temperatures can be very slow, requiring days or weeks to reach maximum numbers. The upper temperature limit for the growth of *L. monocytogenes* is reported to be 45 ºC (28).

9.5.4 Heat resistance

L. monocytogenes is not a particularly heat-resistant organism; it is not a spore-former, so can be destroyed by pasteurisation. It has been reported to have slightly greater heat resistance than certain other foodborne pathogens.

D-values at 65 °C for *L. monocytogenes* in beef, chicken leg, and chicken breast were reported as 0.93, 0.53 and 0.52 minutes respectively (29).

9.5.5 pH

The ability of *Listeria* to grow at different pH values (as with other bacteria) is markedly affected by the type of acid used, and the temperature. Under ideal conditions, the organism is able to grow at pH values well below pH 5 (pH 4.3 is the lowest value where growth has been recorded, using hydrochloric acid as acidulant). In foods however, the lowest limit for growth is likely to be considerably higher - especially at refrigeration temperatures and, where acetic acid is used as an acidulant; pH <5.2 has been suggested as the lowest working limit (30).

9.5.6 A_w/Sodium chloride

L. monocytogenes is quite tolerant of high sodium chloride/low a_w. It is likely to survive, or even grow, at salt levels found in foods (10 - 12% NaCl or more). It grows best at a_w of ≥0.97, but has been shown to be able to grow at a a_w level of 0.90. The bacterium may survive for long periods at a_w values as low as 0.83 (28).

9.6 *Salmonella* spp.

9.6.1 Morphology

Gram-negative short rods; peritrichous flagella; 0.5 - 0.7 x 1.0 - 3.0 µm.

9.6.2 Oxygen requirements

Facultative anaerobe.

9.6.3 Temperature

Salmonellae can grow in the temperature range of 7 - 48 ºC. However, some strains are able to grow at temperatures as low as 4 °C (31). Growth is slow at temperatures below about 10 ºC, the optimum being 35 - 37 ºC.

Salmonellae are quite resistant to freezing.

9.6.4 Heat resistance

Salmonella is not a spore-forming organism. It is not, therefore, a heat-resistant organism; pasteurisation and equivalent treatments will destroy the organism under normal circumstances. $D_{60\ °C}$ values normally range from about 1 to 10 minutes, with a z-value of 4 - 5 ºC. However, high fat or low moisture (low a_w) will reduce the effectiveness of heat treatments, and appropriate heat treatments must be determined experimentally for low-a_w foods. Furthermore, strains vary in their ability to withstand heating; *Salmonella senftenberg* 775W is about 10 - 20 times more heat-resistant than the average strain of *Salmonella* at high a_w (32). The D-value for *S. senftenberg* in beef bouillon at 65.5 ºC is 0.66 minutes, and the D-value for *Salmonella typhimurium* in ground beef at 63 ºC is 0.36 minutes (32).

9.6.5 pH

Salmonella has a pH range for growth of pH 3.8 - 9.5, under otherwise ideal conditions, and with an appropriate acid. Some death will occur at pH values of less than about 4.0, depending on the type of acid and temperature. The optimal pH for *Salmonella* growth is between 6.5 - 7.5.

9.6.6 A_w/Sodium chloride

Where all other conditions are favourable, *Salmonella* has the potential to grow at a_w levels as low as 0.945, or possibly 0.93 (as reported in dried meat and dehydrated soup), depending on serotype, substrate, temperature and pH. Salmonellae are quite resistant to drying.

The growth of *Salmonella* is generally inhibited by the presence of 3 - 4% NaCl, although salt tolerance increases with increasing temperature (33).

9.7 *Staphylococcus aureus*

9.7.1 Morphology

Gram-positive cocci; 0.7 - 0.9 μm diameter.

9.7.2 Oxygen requirements

Facultative anaerobe.

NB: The growth of *Staph. aureus* is more limited under anaerobic than aerobic conditions. The limits for toxin production are also narrower than for growth. The following relate to limits for growth only.

9.7.3 Temperature

Under otherwise ideal conditions, *Staph. aureus* can grow within the temperature range 7 - 48.5 ºC, with an optimum of 35 - 37 ºC (34). It can survive well at low temperatures.

Freezing and thawing have little effect on *Staph. aureus* viability, but may cause some cell damage (35).

9.7.4 Heat resistance

Heat resistance depends very much on the food type in which the organism is being heated (conditions relating to pH, fat content, a_w, etc.). As is the case with other bacteria, stressed cells can also be less tolerant of heating.

Under most circumstances, however, the organism is heat-sensitive and will be destroyed by pasteurisation. In meat macerate, the $D_{60\,^{o}C}$ value is 2 - 20 minutes, depending on a_w.

9.7.5 pH

The pH at which a staphylococcal strain will grow is dependent on the type of acid (acetic acid is more effective at destroying *Staph. aureus* than citric acid), a_w and temperature (sensitivity to acid increases with temperature). Most strains of staphylococci can grow within the pH range 4.2 to 9.3 (optimum 7.0 - 7.5), under otherwise ideal conditions (34, 36).

9.7.6 A_w/Sodium chloride

Staph. aureus is unusual amongst food-poisoning organisms in its ability to tolerate low a_w. It can grow from a_w 0.83 - >0.99 aerobically under otherwise optimal conditions. However, a_w of 0.86 is the generally recognised minimum in foods (37).

Staphylococci are more resistant to salt present in foods than other organisms. Generally, *Staph. aureus* can grow in 7 - 10% NaCl, but certain strains can grow in 20% NaCl. An effect of increasing salt concentration is to raise the minimum pH of growth.

9.7.7 Limits permitting toxin production

Temperature	10 - 46 ºC (optimum between 35 and 40 ºC) (very little toxin is produced at the upper and lower extremes) (36)
pH	5.2 - 9.0 (optimum 7.0 - 7.5) (34, 36)
*A_w	between 0.87 and >0.99
Atmosphere	little or no toxin production in anaerobically packed foods, especially vacuum-packed foods (38)
Heat Resistance	Enterotoxins are quite heat-resistant. In general, heating at 100 °C for at least 30 minutes may be required to destroy unpurified toxin (36, 39).

*dependent on temperature, pH, atmosphere, strain, and solute.

9.8 *Yersinia* spp.

9.8.1 Morphology

Gram-negative short rods (occasionally coccoid); 0.5 - 1.0 x 1.0 - 2.0 µm.

9.8.2 Oxygen requirements

Facultative anaerobe. Carbon dioxide has some inhibitory effect on the growth of *Yersinia enterocolitica*. Vacuum packaging can retard growth to a lesser extent.

9.8.3 Temperature

Yersinias are psychrotrophic organisms, being capable of growth at refrigeration temperatures. Extremely slow growth has been recorded at temperatures as low as 0 to -1.3 °C. However, the optimum temperature for growth of *Y. enterocolitica* is 28 - 29 °C with the reported growth range of -2 - 42 °C (40, 41, 42). The maximum temperature at which growth has been recorded is 44 °C (42, 43).

The organism is quite resistant to freezing and has been reported to survive in frozen foods for long periods (40, 41).

9.8.4 Heat resistance

The organism is sensitive to heat, being easily killed at temperatures above about 60 °C. Internal temperatures of 60 °C in beef roasts inactivate up to a million cells of *Yersinia* per gram, whereas cooking at 51 °C leaves some survivors (44).

9.8.5 pH

Yersinia is sensitive to pH values of less than 4.6 (more typically 5.0) in the presence of organic acids, e.g. acetic acid. *Y. enterocolitica* are not able to grow at pH <4.2 or >9.0. A lower pH minimum for growth (pH 4.1 - 4.4) has been observed with inorganic acids, under otherwise optimal conditions. Its optimum is pH 7.0 - 8.0; they tolerate alkaline conditions extremely well (45).

9.8.6 A_w/Sodium chloride

Yersinia may grow at salt concentrations up to about 5% (a_w 0.96), but no growth occurs at 7% (a_w 0.945). Growth is retarded in foods containing 5% salt (42, 45).

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Useful Web Sites

Gateway to Government food safety information (US)
http://www.FoodSafety.gov/

World Health Organisation (WHO): Food safety programmes and projects
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European Commission: Activities of the European Union food safety
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