RODRIGO TARTÉ
Editor

Ingredients in Meat Products

Properties, Functionality and Applications

Springer

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Preface

There is little doubt that today's food industry is faced with a rapidly changing market landscape. The obvious need to continue to provide consumers with nutritious, delectable, safe, and affordable food products which are also profitable for food manufacturers, as well as the ongoing challenge of ensuring the delivery of adequate nutrition to hundreds of millions of disadvantaged people around the world, appears – at least as much as, if not more than, ever – to be at odds with the challenges posed by soaring energy and food commodity prices; fast-paced changes in consumer demographics, habits, and preferences; and the continual need to stay ahead of current and emerging food safety issues. In addition to this, the present ubiquity in the industry of terms such as *functional foods, nutraceuticals, low sodium, low fat, clean label, minimal processing*, and *natural* – to name a few – underscores yet a different dimension of the challenges faced by food processors today.

On the other hand, however, the solutions of many of these challenges may, concurrently, present the food industry with unique and exciting opportunities. The processed meat industry, despite its long history and tradition, is certainly not exempt from having to face these modern challenges, nor excluded from realizing the promises of the opportunities that may lie ahead. Fortunately, at the same time as the market landscape has been changing, the technological landscape has continued to evolve and advance as a result of continuous scientific breakthroughs and innovations in the areas of process and ingredient technology. If the meat processing industry is to successfully address the issues it faces now and in the future, and if its product offering is to remain relevant to twenty-first century consumers, it must take advantage of these latest technological advances by adequately understanding their fundamental underlying scientific principles and translating them into real, cost-effective solutions to consumers' needs and wants.

With this in mind, *Ingredients in Meat Products: Properties, Functionality and Applications* aims to present the most up-to-date information regarding nonmeat ingredients and their utilization in the manufacturing of processed meat products, and to do so in a way that is both comprehensive and practical. As the subtitle indicates, emphasis has been placed on helping the reader attain a fundamental understanding of: (1) the properties of each of these groups of ingredients, as we understand them today; (2) how these properties affect their functionality in meat

systems; and (3) how to take advantage of the ingredients' functional properties to maximize their application in real-life situations.

The volume discusses all the major types of ingredients used in processed meat products today, and does so both in the context of the ingredients' traditional uses, as well as, when applicable, in the context of their more novel - and in many instances commercially unexploited – applications. It draws on the individual knowledge and expertise of 20 contributors who, collectively, bring with them a diversity of backgrounds and experiences – half of them being from academia and half from industry, and representing five different countries of North America and Europe.

This book is intended as a primary reference on the subject for university, industry, and government meat science researchers; graduate and undergraduate students with an interest in the topic; and meat and food industry product development, quality, production and marketing personnel. It is my hope that it will make a significant contribution to the science and practice of meat processing by becoming a useful tool that the industry can use to successfully undertake and overcome the challenges of the twenty-first century.

This book, like all others, did not just *happen*. Many people were involved in helping me transform this project from a rough idea into the book you are reading right now. First and foremost, I express my most sincere appreciation to the 19 other authors and co-authors who partnered with me in this effort. They all saw the project's potential from the beginning and I am grateful to them for agreeing to enrich it with their knowledge and perspectives. Gratitude is also expressed to all those who served as reviewers of the individual chapters.

Very special thanks go to the management of the Research & Development organization of Kraft Foods Inc. for their continuous support of this endeavor. Many of my other colleagues at Kraft were also most helpful, encouraging, and supportive, especially my co-workers in the Kraft Meat Science Research Group.

My wife, Mercedes, and kids, Daniel and David, have sacrificed the most during this project. For much too long, the one constant of most of their weekends (and many a weeknight) was the sight of their husband and father sitting in front of a laptop computer for hours at a time working on the "meat ingredient" book. And, despite that, they *still* eat hot dogs! You guys are the best and I love you!

Finally, I thank the publishers for their encouragement, advice, and patience, and in particular my editor, Susan Safren, who was the first to encourage me to embark on this adventure.

Rodrigo Tarté

Contents

1 Basic Curing Ingredients 1
Joseph G. Sebranek

2 Starches 25
Ghislaine Joly and Björn Anderstein

3 Nonstarch Hydrocolloids 57
James W. Lamkey

4 Fiber 83
Jon M. Bodner and Jürgen Sieg

5 Plant Proteins 111
William Russell Egbert and C. Tony Payne

6 Dairy Proteins 131
Youling L. Xiong

7 Meat-Derived Protein Ingredients 145
Rodrigo Tarté

8 Enzymes 173
C. Tony Payne

9 Spices, Seasonings, and Flavors 199
Peter M. Brown

10 Smoke Flavor 211
Jeffrey J. Rozum

11 Fermentation and Acidification Ingredients 227
Frédéric Leroy and Luc De Vuyst

12 Coating Ingredients 253
Susana M. Fiszman

13 Antioxidants 291
Ingolf U. Grün

14 Antimicrobial Ingredients 301
Catherine A. Simpson and John N. Sofos

15 Alternative Curing Systems 379
Jeffrey J. Sindelar and Terry A. Houser

Index 406

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Chapter 1
Basic Curing Ingredients

Joseph G. Sebranek

Introduction

Background

Meat curing is an ancient process that was developed from the necessity to preserve a highly perishable food product. It is generally believed the cured meat processes were derived from preservation treatments first developed with salt. There is evidence of meat preservation with salt as early as 3,000 B.C. (Romans, Costello, Carlson, Greaser, & Jones, 2001), and it is clear that the Romans utilized rock salt for a variety of meat preservation treatments (Pegg & Shahidi, 2000). Several reviews of meat curing (Binkerd & Kolari, 1975; Cassens, 1990; Pegg & Shahidi, 2000; Pierson & Smoot, 1982; Sebranek, 1979) have suggested that the use of salt from a variety of sources probably resulted in observations that certain types of salt created a very attractive reddish pink meat color. Over time, it was recognized that salt contaminated with salt peter (potassium nitrate) provided a superior salted meat color and thus the multifunctional contributions of curing ingredients to cured meats began to become more obvious. Once saltpeter was recognized as a source of cured meat color, direct addition of nitrate to meat curing mixtures became common.

In the mid-to-late 1800s, the meat industry began to evolve from what was primarily an animal slaughter industry to a slaughter-processing-preservation industry as population demographics began to shift and a greater need for commercial food preservation developed. The turn of the century brought applications of chemistry to the meat industry and a major discovery by German chemists showed that an essential step in the meat curing process was conversion of nitrate to nitrite. Subsequent research established the concentrations of nitrite required and in 1926, the United States Department of Agriculture (USDA) published regulations describing permitted uses and concentrations of nitrite as a cured meat ingredient (Pegg & Shahidi, 2000). The last half of the twentieth century brought rapid developments in meat curing systems including cure accelerators (reductants and acidulants), new equipment (multiple-needle injectors, tumblers, and massagers), water-binding ingredients (phosphates and others) and USDA regulations governing water addition and retention in processed meats. Today meat curing processes have become a highly sophisticated science. It has become

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critical for meat processors to have a good fundamental understanding of the functional properties of all of the ingredients used in order to utilize those ingredients to best advantage in a highly competitive marketplace.

Definitions

"Cure" is commonly understood in the meat industry as both a verb and a noun. "To cure" is to add nitrite and/or nitrate with salt to a meat product to achieve improved preservation. "A cure" is often used to describe the chemical entities of nitrite and/or nitrate. While "cure" and "curing" are sometimes used to describe meat products preserved with salt alone, the typical use of these terms means that nitrite and/or nitrate are included.

Dissolving nitrite and/or nitrate in water with salt and other ingredients prior to addition to meat comprises a "curing brine" or a "pickle," while applying a dry mixture of curing ingredients to the outside of meat pieces is a "dry cure." "Immersion curing" means that meat pieces are immersed in a curing brine for a period of time to allow "pickup" of the water and curing ingredients. "Injection curing" utilizes single or multiple-needle injectors to inject the curing brine into the interior of the meat pieces being cured. In "massaging" and "tumbling,"injected meat pieces are slowly mixed or agitated to help equilibrate the brine and improve the pickup. Additional curing brine may be added to the massager or tumbler if the injection procedure has not achieved the targeted amount. "Reductants" and "acidulants" are compounds used to accelerate curing reactions by reducing (with reductants such as ascorbate or erythorbate) nitrite to nitric oxide or by lowering product pH (with acidulants such as sodium acid pyrophosphate or glucono-delta-lactone).

Water

Water is often overlooked as a functional ingredient in processed meats. While water is a major component of raw meat, when additional water is added as part of curing processes, the water becomes a nonmeat ingredient as well as a meat component. Because water is typically lost during cooking due to evaporation, there is a perception that water is added to cured meats during processing simply to compensate for expected weight loss. While improved yields is a very practical reason for adding water to cured meats, water has several important functions in meat products that result from the unique properties of water molecules.

Properties

Water is the singly most abundant component of meat, making up some 60–70% of the lean meat that is used for processed products. Water is both simple, in terms of chemical

structure, and unusual, in terms of properties, compared to other chemical compounds (Ruan & Chen, 1998). The two hydrogen atoms in water form a V-shaped molecule with the single oxygen atom. The molecule is characterized by an angle of 104.5° between the two O–H bonds. The two covalent bonds result in two positively charged poles (hydrogens) and two equally spaced negatively charged poles on the opposite sides of the oxygen atom from the two hydrogen poles. The four charged poles arrange themselves into a tetrahedron shape around the central oxygen atom, with each pole equidistant from the others. A very significant result of this arrangement is the ability of each water molecule to form hydrogen bonds with four other water molecules, utilizing the hydrogen–oxygen attractive forces on each molecule. Because each water molecule has two hydrogen poles to bind to two oxygen poles of adjacent molecules and two oxygen poles to bind to two hydrogen poles on two more adjacent molecules, the intermolecular hydrogen bonding is equilateral, and each molecule can bind to four others. This equally distributed attraction between water molecules is the reason for the unusual properties of water including high melting and boiling points, high latent heat of vaporization, high dielectric constant and reduced density in crystalline (ice) form (Le Meste, Roudaut, Champion, Blond, & Simatos, 2006). The practical effects of these properties include attractive forces between water molecules that help to "hold" them together in meat and the interaction of water with other polar molecules, particularly the polar structures on meat proteins. These interactions are the basis for the functional contributions of water to cured meats.

Functions

Water is often called a "universal solvent" because it will dissolve a large number of substances, including those used as ingredients in cured meats. Water serves as a solvent, carrier, and dispersing agent for salt, nitrate, nitrite, sugar, phosphates, and other ingredients typically included in cured meat. This is especially critical for uniform dispersion of sodium nitrite, which for comminuted meat products is restricted to 156 ppm (7 g/45.4 kg). Without water to dissolve and disperse such a small quantity, it would be very difficult to achieve a uniform distribution of nitrite during mixing. The solvent property of water is also essential for meat protein extraction, a critical step for meat emulsion stability and for cooked product textural properties (Tarté & Amundson, 2006). Salt (sodium chloride) is necessary for protein solubilization because it is the salt-soluble (myofibrillar) proteins that are most important; however, added water (often 10–20% of the meat weight) also plays an important role in protein solubility. Solubilizing meat proteins with salt and added water facilitates the formation of an interfacial protein film around fat globules in finely chopped meat products such as frankfurters and bologna. If properly formed, these protein films stabilize the fat globules during cooking and prevent fat separation from the meat mixture. Solubilized meat proteins are also critical to the three-dimensional crosslinking and gelation that occurs during heating of meat mixtures. Heat-set gelation of meat proteins is responsible for trapping and holding water and fat, and for the textural properties of the semisolid,

three-dimensional structure that results. A great deal of finished product tenderness, juiciness, and mouthfeel are dictated by the gel structure that is formed. The gelation properties also determine the extent of water and fat retention during cooking and, consequently, affect product yields.

Water is also an important consideration for two other properties of cured meat products, namely, brine strength and available water. These two properties of cured meats are closely related. The first of these, brine strength, is defined as

$$\frac{\%\ \text{salt}}{\%\ \text{salt} + \%\ \text{water}} = \%\ \text{brine strength}$$

For example, frankfurters that have 2.5% salt and 60% water would have a brine strength of

$$\frac{2.5}{2.5+60} = \frac{2.5}{62.5} = 0.04 = 4.0\%$$

Brine strength is important to bacterial inhibition and product shelf life, as well as for salt-soluble protein extraction. While the salt concentration is obviously critical, added water will clearly alter brine strength if salt is kept constant. Where this becomes important is when formulations are changed, as in the case of reduced fat products. Typically, added water is increased in reduced fat products to keep the product texture and mouthfeel acceptable, and to essentially replace some of the fat that has been removed. Salt, however, is usually not increased so that excessively salty flavor does not become a problem. As a result, brine strength is reduced. If the frankfurters used in the example earlier were reduced in fat content from 29+% to less than 10%, water were increased to 80%, and salt kept the same, the brine strength would decline from 4% to 3%.

$$\frac{2.5}{2.5+80} = \frac{2.5}{82.5} = 0.03 = 3.0\%$$

This would have a significant impact on bacterial growth, product shelf life, and protein solubility, if no other changes are made. Reduced-fat processed meats also usually include additional water-binding ingredients such as starches, hydrocolloids, or nonmeat proteins to help retain the additional added water during cooking and storage of the products.

The second consideration for water that is important to cured meat properties is the water activity or available water (A_w). Water activity is the vapor pressure of water in a product at equilibrium and at a constant temperature relative to pure water at the same temperature, and is indicative of the biological and chemical availability of the water in the product. Available water is very valuable for predicting bacterial growth because it takes into account water binding by all components in food including salt, sugar, proteins, and any other molecules that offer bonding sites for water molecules. This makes available water a major factor in control of

spoilage and pathogenic bacteria, and a significant contributor to the hurdle concept for food preservation (Leistner, 1994). Most spoilage bacteria will not grow below A_w of 0.91 and pathogens such as *Staphylococcus aureus* are limited by an A_w of 0.86 (Jay, Loessner, & Golden, 2005).

Despite the positive contributions of water to cured meats, added water can also be a source of problems. Hard water, for example, may contain contaminants that cause color losses, flavor changes, and shorter shelf life. Water sources within a processing plant can become contaminated with bacteria as well. Consequently, water quality is important to cured meats and must be monitored for both mineral content (hardness) and chemical and microbiological contamination.

Regulations

Most of the relevant regulations governing addition of water as well as the use of various other ingredients in processed meats can be found in Title 9 of the Code of Federal Regulations, Chapter III, Parts 317–319 (Code of Federal Regulations [CFR], 2007a). Other useful sources for how the regulations are applied include the *USDA Processing Inspectors' Calculations Handbook* (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 1995) and the *Food Standards and Labeling Policy Book* (USDA-FSIS, 2005).

In the United States, regulations governing water content in cured meats vary widely depending on the product category considered. In any case where water is added as an ingredient, it must be included in the ingredient statement. The only exceptions are when water is added to starter cultures for rehydration or to products that are freeze-dried or spray-dried (USDA-FSIS, 2005). Some examples of limits for added water include cooked sausage such as frankfurters, where added water plus fat content cannot exceed 40% (CFR, 2007d). Because added water cannot be differentiated from water inherently present in meat, it is assumed that the meat block will have a water content equal to four times the protein content. This is close to the actual water:protein ratio that is typical of meat without any added water. The water content above the level of four times the protein content is then considered added water. It is important to note that "added water" has a regulatory meaning in this context. The USDA limits the amount of water that may be added above what is considered to be a reasonably typical amount for the meat ingredients in order to retain expected product properties and to prevent excessive addition of water to meat product formulations. In the case of cured pork products like hams, water content is limited on a protein-fat-free (PFF) basis. A minimum protein content of 20.5%, on a fat-free basis, for example, is required for a product labeled "Ham," while products with lower protein content (higher moisture content) must be labeled as "Ham with natural juices" (18.5–20.5% PFF), "Ham-water added" (17.0–18.5% PFF), or "Ham and water product" (<17.0% PFF) (CFR, 2007b, 2007c). The protein content of the product is dependent on the amount of added ingredients, most of which are water and, consequently, the PFF labeling requirement serves to restrict the amount of water that can be added to these products.

Water content is also used to define dried cured meat products, where moisture:protein (M:P) ratios are used. For example, pepperoni must be dried to an M:P ratio of 1.6:1 or less, while jerky is required to have an M:P ratio of 0.75:1 or less. Because fresh raw meat has an M:P ratio of 3.6–3.8:1, it is clear that a considerable amount of water must be removed during the drying process to qualify these products for the intended product label. There are also a number of products that have specific regulatory limits on the amount of water that can be picked up (absorbed) from a curing brine. Beef briskets, for example, cannot exceed 20% over fresh-brisket weight after an application of a curing solution. Application of curing solutions to beef cuts other than briskets or tongues is limited to 10% weight gain (USDA-FSIS, 1995). Because of many different regulations on added water, it is best to consult with the regulatory agencies on questions concerning use of added water for processed meats.

Salt

Salt (sodium chloride) is one of the oldest ingredients used for meat preservation and one that is fundamental to all cured meat products. Salt, in terms of quantity and frequency of use, is the most common ingredient in cured meats. Despite concerns about excessive sodium in human diets and efforts to reduce sodium consumption, salt is so critical to meat processing that this ingredient cannot be eliminated.

Properties

Salt (sodium chloride) is a white crystalline solid that is available as evaporated salt, rock salt, or solar salt. Most food grade salt is produced by vacuum evaporation of salt brines which produces the highest purity and cleanest salt. Because salt is a Generally Recognized As Safe (GRAS) substance, food grade salt must comply with the chemical tolerances of the *Food Chemicals Codex* (Institute of Medicine [IOM], 2003). For vacuum evaporated salt, the minimum purity is 99.0% NaCl (IOM, 2003) and most commercial evaporated salt is 99.8–99.9% pure (Strietelmeier, 1988). Rock salt is mined from mineral deposits and solar salt or sea salt is produced by natural evaporation of sea water. Rock salt and solar (sea) salt are required to contain at least 97.5% sodium chloride for food applications (IOM, 2003).

Functions

Salt is widely recognized as a multifunctional ingredient in cured meats. Because salt is highly water soluble and forms sodium (Na^+) and chloride (Cl^-) ions in solution, the functions that salt provides in meat mixtures are largely determined by the dissociated

ions. The ionic strength, for example, is critical to solubilization and extraction of the salt-soluble proteins that are necessary for stabilizing fat in emulsion products and determining the product texture that results from heat-set protein gelation. An ionic strength of 0.5 or more will cause muscle myofibrils to swell and disintegrate, depolymerize myosin filaments, and solubilize the myofibrillar proteins (Hamm, 1986). A salt concentration of 2% or more in most meat formulations will achieve the necessary ionic strength. However, even at lower concentrations such as 0.5–1.0% as used for many moisture-enhanced fresh meats, the Cl^- ion from salt will interact with meat proteins to increase the negative electrical charges on the proteins and increase the water-binding properties of the meat mixture. This is an essential role of chloride ions in meat systems because the interaction with meat proteins that swells the protein structure is responsible for allowing the proteins to hold more of the weakly bound water within and between their structure. The increased retention of water by the protein structure in the presence of chloride ions has a major impact on cooking yields, juiciness, tenderness, and mouthfeel when the product is consumed. The chloride ion is much more important than the sodium ion for achieving increased water binding by meat proteins. The chloride ions from salt may also play a role in cured color because Cl^- has been reported to accelerate cured color formation in cured meats by increasing the rate of nitric oxide formation from nitrite (Sebranek & Fox, 1991).

Salt concentration plays an important role in the control of microbial growth as described earlier. The available water (A_w) in a meat system is significantly reduced with addition of salt and this reduced A_w is believed to be one of the primary antimicrobial effects of salt in meat products. With all else equal, reducing the salt content of heat-pasteurized cured meats will reduce product shelf life and may have implications for increased risk of growth of pathogenic microorganisms.

While chloride ions play a major role in water binding by meat proteins, it is the sodium ion that is responsible for the flavor that is derived from salt. An important function of sodium in flavor perception is not only the saltiness contributed by sodium but also the increased intensity of other flavors that result in the presence of sodium (Ruusunen & Puolanne, 2005). Thus, salt is not only an important flavor contributor but also serves as a flavor enhancer for other flavor components in food. Despite extensive research efforts, no suitable substitutes for the sodium flavor have been discovered. For cured meats, the sodium concentration can be reduced by using a mixture of potassium chloride with sodium chloride. Blends of up to 50:50 potassium chloride:sodium chloride have been reported to provide acceptable flavor while maintaining sufficient chloride concentration for adequate water binding (Romans et al., 2001). It should be noted that, because the molecular weight of potassium is greater than that of sodium, a 1:1 substitution of sodium chloride with potassium chloride will result in a somewhat lower chloride ion concentration. This could have implications for the chloride ion-dependent functionality of the meat proteins for water binding and fat emulsification. More than 50% substitution of sodium chloride with potassium chloride usually results in undesirable flavors from the potassium ion. When considering sodium effects in meat systems and sodium/potassium ratios, it is important to consider all ingredients being used that may contribute sodium and potassium ions. Several ingredients contribute sodium (sodium erythorbate, sodium phosphates, sodium lactate, and others) and some can be

used as potassium salts as well (potassium lactate, potassium nitrate, potassium nitrite). Thus, it is important to consider all sources of sodium and potassium in a product formulation when considering substitution of sodium with potassium. Sodium chloride concentration can also be reduced to less than 2.0% of the product if alkaline phosphates are included to supplement the protein-based binding of water molecules. However, these effects are very dependent upon specific product formulations and product type; consequently sodium reduction efforts in cured meats must be done on a case-by-case basis. It appears that less than about 1.5% sodium chloride, however, is likely to result in significant changes in cured meat properties. Clearly, salt is an essential ingredient in cured meats.

Regulations

Sodium chloride in processed meats is a GRAS substance, and because products will become unpalatable with high concentrations of salt, this ingredient is considered self-limiting. Consequently, there are no regulations that restrict the amount of sodium chloride added to cured meats.

Curing Agents

Curing agents (nitrate and nitrite) are essential ingredients for cured meats because these compounds are responsible for the unique, distinctive properties that characterize cured meat products. While either nitrate or nitrite may be used, nitrate is effective as a curing agent only if it is reduced to nitrite. Because nitrate reduction in meat is typically achieved by microorganisms, adequate time and temperature for microbial conversion are necessary. Consequently, for high-volume cured meat products such as frankfurters, bologna and most hams, that are cooked within hours of blending with curing agents, nitrate is considered “useless and superfluous” (Honikel, 2004). Because the true curing agent is nitrite, most cured meats are formulated with nitrite as the only curing agent. However, for some products such as dry sausage and dry-cured hams that are slowly cured over an extended period of time, nitrate is used to provide a long-term reservoir of nitrite during the extended curing time. More recently, a number of natural and organic meat products have been developed that utilize natural sources of nitrate from vegetables which, when combined with a bacterial starter culture, result in properties characteristic of nitrite-cured products. These natural and organic products represent a new category of cured meats.

Nitrate

It is not clear when nitrate was first recognized as a curing agent but historical records show that nitrate, either as a contaminant of salt, or as saltpeter (potassium

nitrate), was used for centuries before research chemists determined, in the late 1800s, that nitrite was the active form of the compound (Pegg & Shahidi, 2000). As a result, nitrate is rarely used in current cured meat products except for a few specialty products where long curing processes are used.

Properties

Nitrate (NO_3^-) salts are highly soluble in water and are often found in ground water sources due to the use of nitrate fertilizers. Nitrate in cured meat is a relatively inert ingredient and does not contribute to meat curing until converted to nitrite. Nitrate conversion is not easily achieved except by bacterial reduction. In traditional meat curing processes, naturally contaminating bacterial cultures can be encouraged to grow by holding meat mixtures for extended periods of time after addition of salt and curing agents. The salt will inhibit many spoilage bacteria while allowing salt-tolerant cocci such as *Kocuria* (formerly *Micrococcus*) and coagulase-negative *Staphylococcus* strains to grow and reduce nitrate to nitrite (Leroy, Verluyten, & DeVuyst, 2006). While these bacteria will reduce nitrate over a relatively wide temperature range (Casaburi, Blaiotta, Mauriello, Pepe, & Villani, 2005), significant time is necessary, and direct addition of nitrite permits much faster production of cured meats in modern commercial processing facilities.

Functions

While nitrate functions only by serving as a source for nitrite, this can be an important role for nitrate in products that are dry cured or that are dried over an extended period of time. Because nitrite is very reactive as a curing agent, it is depleted from cured meats relatively quickly, and nitrate serves as an important reservoir in these products to maintain an effective nitrite concentration during drying or storage for long periods of time.

The recent rapid market growth of natural and organic foods has brought a new function for nitrate in processed meats (Sebranek & Bacus, 2007). The regulatory and labeling requirements for natural and organic processed meats do not permit the addition of nitrate or nitrite. However, processors have developed methods by which natural sources of nitrate such as vegetable juices and concentrates are added as ingredients. Vegetables are recognized as a very significant source of nitrate with concentrations of 1,500–2,800 ppm commonly found in celery, lettuce, and beets (National Academy of Sciences, 1981). Dried vegetable juice powders have been found to contain over 2.5% nitrate or more than 25,000 ppm (Sindelar, 2006). Addition of vegetable juice powder at 0.2–0.4% of a meat product formulation provides sufficient nitrate concentration to achieve typical cured meat properties. When a nitrate-reducing starter culture is also included with appropriate incubation conditions, reduction of nitrate to nitrite can be achieved within a relatively short period of time. The result is very typical cured meat characteristics for hams, bacon, frankfurters, and a host of other products for which consumers expect cured meat

color and flavor. It is interesting to note that many of these products are labeled "Uncured" and "No nitrite or nitrate added." The labeling of these products may change in the near future because the USDA is currently reviewing the regulatory and labeling requirements for meat products categorized as "natural," and is expected to issue new regulations for these products.

Regulations

Concentrations of nitrate that are permitted in cured meats vary around the world and it is important to determine the regulatory requirements for the locations in which products are to be marketed. In the United States, for example, either sodium or potassium nitrate are limited to an ingoing concentration of 1,718 ppm in comminuted products, 700 ppm in immersion cured or injected products and 2,187 ppm in dry cured products. Nitrate is not permitted, however, in bacon so that actual concentrations of nitrite can be more precisely controlled. The limits for sodium and potassium nitrate added to cured meats are the same despite the greater molecular weight of the potassium salt. This means that there is less nitrate ion present from potassium nitrate than from sodium nitrate when equal quantities of each are considered. In cases where nitrate and nitrite are used together, the ingoing limits for nitrate are the same as if nitrate were used alone, but the combination must not result in more than 200 ppm of analytically measured nitrite, calculated as sodium nitrite, in the finished product (USDA-FSIS, 1995).

The European Union restricts sodium nitrate to 300 ppm ingoing for cured meats with 250 ppm residual nitrate. Nitrate may be used for bacon but residual levels are limited to 175 ppm, expressed as sodium nitrite (Honikel, 2004). Russia, on the other hand, does not permit use of nitrate in meat products.

Nitrite

The chemistry of nitrite in meat curing is a fascinating, highly complex mixture of reactions involving a wide array of different reactants and producing many different end products. Nitrite reactions are the source of the unique and distinct properties that characterize cured meat products and, for that reason, nitrite is the true meat curing agent.

Properties

Sodium or potassium nitrite is a pale yellow, nearly white, crystalline compound that is highly soluble in water. The appearance of pure crystalline sodium nitrite is very similar to salt, sugar, and a variety of other white, crystalline food ingredients, and can be easily mistaken for other commonly used food ingredients. Because nitrite is a toxic substance, however, mistaken use has potentially serious conse-

quences. There have been cases where salt shakers were mistakenly filled with sodium nitrite and where nitrite was mistakenly used in a punch mixture instead of citric acid. High doses of nitrite will produce methemoglobinemia in humans, which is not unlike carbon monoxide poisoning. A lethal dose of sodium nitrite for humans has been estimated to be about 1 g (Ellenhorn & Barceloux, 1988).

Nitrite toxicity is recognized by the USDA. Secure storage and written records of use of nitrite are required of processors who utilize this compound for meat curing. It is also common in the meat industry to use a salt/nitrite mixture rather than pure nitrite. "Curing salts" or "curing blends" are most often 6.25% nitrite with the balance of the mixture made up of salt (sodium chloride). In many cases, a red or pink coloring agent is also included. The use of curing salts with a coloring agent makes the mixture visually obvious and largely prevents mistaken overuse because of the high salt content. Some of the European Union countries have mandated that only curing blends or curing salts can be used in meat processing applications.

Nitrite is a highly reactive compound that can function as an oxidizing, reducing, or nitrosating agent, and is converted to a variety of related compounds when added to meat. The reaction products of nitrite are the source of the functional contributions of nitrite to cured meat.

Functions

Nitrite, like salt, is a multifunctional ingredient in cured meat but, in the case of nitrite, these functions are accomplished with extremely small concentrations. Addition of 50–100 ppm of nitrite is adequate to achieve the multiple functions of nitrite in cured meat. Consequently, nitrite is sometimes called a "magic" ingredient for the unique and potent effects it creates when added to meat.

Because the effects of nitrite are unique, the functions of nitrite in cured meat have been studied extensively and it is clear that nitrite is responsible for cured meat color, cured flavor, flavor protection (as an antioxidant), and bacterial inhibition. However, the means by which nitrite achieves these functions is not completely understood in all cases.

The effect of nitrite on meat color is the best understood and most obvious result of adding nitrite to meat. It is also a good example of the complexity of nitrite reactions in meat because nitrite does not act directly as a nitrosylating (transfer of nitric oxide) agent but rather forms nitric oxide by several different mechanisms, depending on conditions. The production of nitric oxide from nitrite is a necessary step for cured meat color because it is nitric oxide that subsequently reacts with myoglobin to produce the red/pink meat pigment that is typical of cured meat color. Because nitrite in meat is dissolved in the water phase, nitrite ions (NO_2^-) are available to react with H^+ ions in the weakly acid (pH 5.5–6.0) conditions of a meat mixture and, in doing so, form nitrous acid (HNO_2) (Honikel, 2004; Pegg & Shahidi, 2000).

$$NaNO_2 \rightarrow Na^+ + NO_2^-$$
$$NO_2^- + H^+ \leftrightarrow HNO_2$$

Nitrous acid is in equilibrium with N_2O_3, which dissociates to form NO (nitric oxide) and NO_2 (nitrogen dioxide). The nitric oxide can then react with the raw meat pigment to produce red nitric oxide myoglobin, which converts to pink nitrosylhemochrome upon cooking.

$$4HNO_2 \leftrightarrow 2N_2O_3 + H_2O$$
$$2N_2O_3 \leftrightarrow 2NO + 2NO_2$$

$$NO + \text{myoglobin} \rightarrow \text{NO-myoglobin(red)} \xrightarrow{\text{cooking}} \text{nitrosylhemochrome(pink)}$$

At the same time, the NO_2 formed can react with water to reform nitrous acid (HNO_2) which re-enters the nitric oxide sequence. This reaction also produces HNO_3 (nitric acid) which dissociates to form nitrate (NO_3^-).

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$$

$$HNO_3 \rightarrow H^+ + NO_3^-$$

Thus, one result of this sequence of reactions, besides color development, is formation of nitrate (Honikel, 2004). This explains why nitrate is commonly found in cured meats even when it has not been added.

In the presence of reductants such as sodium ascorbate or erythorbate, the N_2O_3 formed from nitrous acid can be reduced to nitric oxide by an oxidation–reduction reaction (Møller & Skibsted, 2002)

$$N_2O_3 + 2H^+ - \text{ASC} \rightarrow 2NO + 2\text{dehydro} - \text{ASC} + H_2O$$

The above reactions describe two of the most important factors governing nitrite reactions during meat curing, namely pH and reductants. A small decrease in pH of 0.2–0.3 pH units will double the rate of nitric oxide production (Fox, 1974; Fox, Townsend, Ackerman, & Swift, 1967) and, when coupled with reductants, meat curing can be rapidly accelerated.

While pH and reductants are major controlling factors for nitrite curing reactions, several other nitrite reactions contribute to nitric oxide production and further exemplify the complexity of nitrite-meat chemistry. For example, when nitrite is added to meat in the presence of oxygen, the meat quickly turns brown because nitrite acts as a strong heme pigment (myoglobin) oxidant, and is, in turn, reduced to nitric oxide. This reaction provides a quick visual check for processors to confirm the addition of nitrite to a meat mixture because the brown color will not develop if nitrite has not been added. Addition of nitrate, for example, will not oxidize the pigment to brown metmyoglobin and in this case, a raw meat mixture will retain its red color.

$$NO_2^- + Myo - Fe^{+2}\,(\text{red myoglobin}) \rightarrow Myo - Fe^{+3}$$
$$(\text{brown metmyoglobin}) + NO + OH^-$$

The nitric oxide generated in this reaction then combines with the oxidized heme, which is then reduced by reductants or is converted to nitrosylhemochrome during subsequent cooking. Heat processing denatures and separates the protein portion (globin) of myoglobin from the nonprotein heme and contributes to the visual color change to the final pink cooked cured color of the nitrosylhemochrome pigment. To further complicate the system, all cured meats include sodium chloride in varying amounts, and nitrous acid reacts with the chloride ion to produce nitrosyl chloride (NOCl), which is a more reactive nitrosylating agent than N_2O_3 (Møller & Skibsted, 2002; Sebranek & Fox, 1991).

$$HNO_2 + H^+ + Cl^- \rightarrow NOCl + H_2O$$

Consequently, chloride ions accelerate cured color development in cured meat. Further, nitrous acid can react with sulfhydryl groups on meat proteins to release nitric oxide in an oxidation–reduction reaction that results in a disulfide (Pegg & Shahidi, 2000).

$$2HNO_2 + 2R\text{-}SH \rightarrow 2R\text{-}S\text{-}S\text{-}R + 2NO + 2H_2O$$

In addition to generating nitric oxide, the above reaction has been suggested as a partial explanation for textural changes in cured meats. Crosslinking between proteins, if significant, could contribute to firmer product texture.

These reaction sequences probably also play a role in the formation of cured meat flavor by nitrite. Cured meat flavor is the least understood aspect of nitrite chemistry in cured meat and has eluded numerous researchers. While it is relatively easy to distinguish the flavor of cooked, cured ham from that of fresh roast pork, the chemical identity of cured flavor remains unknown. Numerous volatile compounds have been identified as components of cured meats and several include nitrogen- or nitrogen/oxygen-containing components that could be derived from nitrite, but no suitable mechanisms for flavor development have been proposed (Pegg & Shahidi, 2000). It has been suggested that the flavor difference observed in cured meat may be due to suppression of lipid oxidation by nitrite. While nitrite is a highly effective antioxidant, other antioxidants do not produce typical cured meat flavor. It seems likely that cured flavor is the result of nitrite reactions in meat, but the specific compounds that are involved are unknown.

The nitrite reaction sequences that generate nitric oxide for color formation probably also play an important role in the strong antioxidant function of nitrite in cured meat that serves to protect flavor from becoming rancid. This

relationship between color reactions and antioxidant function is likely because the proposed mechanisms for the antioxidant effect of nitrite include reactions with heme proteins and metal ions, radical chelation activity by nitric oxide, and formation of nitroso- and nitrosyl compounds that have antioxidant properties (Pegg & Shahidi, 2000). All of these reactions are also closely related to cured color development. Whether nitrite, nitric oxide, or some reaction product of these compounds is responsible for the antioxidant function of nitrite, it is clear that nitrite is a very potent antioxidant in cured meat. As little as 50 ppm has been shown to reduce rancidity in beef, pork, and chicken meat by 50–64% (Morrissey & Techivangana, 1985), an effect that was even greater at higher concentrations of nitrite. For example, in the same study, 200 ppm of nitrite reduced rancidity measures by 87–91% in similar meat samples.

In addition to color, flavor, and antioxidant functions, nitrite in cured meat is an important antimicrobial agent. Nitrite is strongly inhibitory to anaerobic bacteria, most importantly *Clostridium botulinum*, and contributes to control of other pathogenic microorganisms such as *Listeria monocytogenes*. Nitrite is not considered to be effective for control of Gram-negative enteric pathogens such as *Escherichia coli*, though recent research has reported reduced growth of *E. coli* in salami with nitrite compared with a similar uncured product (Pichner, Hechelmann, Steinrueck, & Gareis, 2006). The effects of nitrite and its likely inhibitory mechanism probably differ in different bacterial species (Tompkin, 2005). Because the effectiveness of nitrite as an antimicrobial agent is strongly pH dependent (Tompkin, 2005), it seems likely that nitrite reaction sequences that generate nitric oxide and other reaction products are important to its antimicrobial effects. Once again, however, it is not clear how nitrite achieves the antimicrobial and antibotulinal impact. It has been suggested that both the amounts of added and residual nitrite in meat are important to antibotulinal protection. USDA regulations, for example, specify a minimum ingoing nitrite concentration of 120 ppm for cured products that must be refrigerated (USDA-FSIS, 1995). However, the ingoing nitrite may be important because it affects the subsequent residual concentration. Tompkin (2005) concluded that the residual nitrite present at the time of temperature abuse is critical to its antibotulinal effect, and that depletion of residual nitrite during product storage will reach some point at which inhibitory effects are also depleted. The antibotulinal effect of nitrite may well be the most important role of this ingredient in vacuum-packaged, refrigerated meat products because its use provides insurance for the safety of these products in the event that product temperature is not well controlled.

While nitrite reactions are highly beneficial to cured meats, the highly reactive nature of nitrite has also been grounds for concern. Toxicity of nitrite is one issue, as previously discussed, but under its normally controlled use in meat curing, this represents no risk. However, the reactivity of nitrite is also a concern relative to potential formation of carcinogenic nitrosamines in cured meat or in the stomach after ingestion of nitrite. This was a major issue in the 1970s and, fortunately, changes in manufacturing practices and reductions in levels used for meat curing solved the problem (National Academy of Sciences, 1982). Despite clear evidence

that the use of nitrite for meat curing was not a safety issue, a background concern about nitrite has lingered. A series of epidemiological studies in the 1990s reported relationships between consumption of cured meats and childhood leukemia and brain cancer (Peters et al., 1994; Preston-Martin et al., 1996; Sarasua & Savitz, 1994). These reports received extensive coverage in the popular media and re-ignited concerns about nitrite in cured meat. Once again, however, subsequent studies and careful scientific review largely resolved the issue (Milkowski, 2006). Most recently, however, the issue of ingested nitrite was again raised by the International Agency for Research on Cancer (IARC) with the conclusion that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans" (Coughlin, 2006). While it is clear that less than 5% of ingested nitrite is derived from cured meat (Archer, 2002; Cassens, 1997; Milkowski, 2006) and that cured meat does not pose a risk, it is likely that the IARC conclusions will again stimulate considerable debate about the safety of nitrite as a food ingredient.

Regulations

As for nitrate, regulations on the use of nitrite in the United States vary with the method of curing used and the product that is cured. For comminuted products, sodium or potassium nitrite are restricted to 156 ppm (7 g/45.4 kg) based on the green weight of the meat block (USDA-FSIS, 1995). For immersion cured or injected products, the maximum ingoing sodium or potassium nitrite is 200 ppm, again based on the green weight of the meat block. Dry cured products are limited to 625 ppm ingoing sodium or potassium nitrite. The USDA requires a minimum of 120 ppm ingoing sodium nitrite for all cured "Keep refrigerated" products unless "… safety is assured by some other preservation process …" (USDA-FSIS, 1995).

Bacon is an exception to the general limits on curing agents. For injected skinless bacon, 120 ppm of sodium nitrite or 148 ppm of potassium nitrite is required along with 550 ppm of sodium ascorbate or sodium erythorbate, which is also required. An important distinction to the bacon regulation is that these are required amounts (of both nitrite and reductant) rather than upper limits. The objective of this regulation is to provide sufficient nitrite to achieve bacterial inhibition while limiting the residual nitrite to levels that will not result in nitrosamine formation when the bacon is fried. To allow for variation in injection procedures and drainage of brine from injected product, the regulations allow ±20% deviation from the target nitrite concentrations at the time of injection. Two exceptions to these regulations for specific amounts of curing agents in injected bacon are permitted: first, 100 ppm of sodium nitrite (or 123 ppm of potassium nitrite) may be used with "an appropriate partial quality control program" and, second, 40–80 ppm of sodium nitrite (or 49–99 ppm of potassium nitrite) is permitted if sugar and a lactic acid starter culture are included (USDA-FSIS, 1995). In this case, reduced concentrations of nitrite are used to achieve cured color and flavor while the lactic acid culture is included to provide for microbial inhibition in the event of temperature

abuse. Immersion cured bacon is similar to injected bacon in that the ingoing limit is 120 ppm sodium nitrite or 148 ppm of potassium nitrite while dry cured bacon is limited to 200 or 246 ppm, respectively.

As for nitrate, other countries differ somewhat in regulatory requirements regarding the use of nitrite. The European Union limits ingoing nitrite to 150 ppm for most cured meats (Honikel, 2004), while Canada allows up to 200 ppm (Pegg, 2004). Canadian regulations, however, are based on the total formulation weight as opposed to the weight of the meat block.

Cure Accelerators

Because the reduction of nitrite to nitric oxide is a critical and necessary step for cured meat color development and probably for several other cured meat properties as well, the use of reducing compounds and acidulants has become an important part of meat curing. Ascorbic acid, sodium ascorbate, erythorbic acid, and sodium erythorbate are reducing compounds that are widely used for cured meat processing. These reductants are particularly advantageous for high volume, rapid processes, and have enabled the development of high-speed, continuous processing lines because less time is required for nitric oxide production before cooking and color fixation. They are sometimes coupled with acidulants in comminuted products, a combination that can dramatically accelerate production of nitric oxide from nitrite. Fumaric acid, sodium acid pyrophosphate, and glucono-delta-lactone are compounds that may be utilized as acidulants if very rapid reduction of nitrite is desired.

Reductants

Properties

Ascorbic acid and erythorbic acid are water soluble organic acids with significant antioxidant properties. Both exist as white to pale yellow crystals that are relatively stable. L-Ascorbic acid has vitamin C activity and, consequently, provides this biological property in addition to reductant and antioxidant properties. It may be identified on ingredient statements as vitamin C. Erythorbic acid is the molecular stereoisomer (mirror image) of ascorbic acid, and while its chemical properties are the same as for ascorbic acid, it does not provide significant biological activity. A 1% solution of these compounds has a pH of 2.8–3.1 and, consequently, direct contact of the acid forms of these compounds with nitrite can result in extremely fast reduction and excessive loss of nitric oxide from the curing mixture.

Sodium ascorbate and sodium erythorbate are sodium salts of the acids and are the most common forms of the reductants used in commercial meat curing. The

chemical properties and reductant activity of the salts are very similar to those of the acids. However, an aqueous solution of sodium ascorbate or erythorbate will be pH 5.5–8.0 and, while contact with nitrite will result in nitric oxide production, the reaction will be slower than if the acid forms of the reductants were used.

Functions

The reductants provide for accelerated production of nitric oxide from nitrite, probably by serving as one-half of the oxidation–reduction pairing with N_2O_3 formed from nitrite, as discussed earlier. Because acidic conditions control the amount of the N_2O_3 intermediate formed, it is easy to understand the major interactive role of acidulants and reductants in meat curing. The reductants are also very effective for reducing oxidized metmyoglobin to reduced myoglobin (Shahidi & Samaranayaka, 2004), a function that facilitates cured color development, particularly in cases where addition of nitrite oxidizes the meat pigment. Ascorbate and erythorbate are extremely effective for maintaining fresh meat color as bright red oxymyoglobin (Manu-Tawiah, Amman, Sebranek, & Molins, 1991), but are not permitted to be used for this purpose in the United States. However, the reductants are allowed to be used in a 10% solution to spray the surfaces of cured meat products prior to packaging to improve color stability, providing that no significant additional moisture is added to the product (USDA-FSIS, 1995).

Regulations

Ascorbic acid and erythorbic acid, alone and in combination, may be added to cured meat or poultry (injected, immersed, comminuted, or dried) at a maximum concentration of 469 ppm, based on the green weight of the meat block. Sodium ascorbate and sodium erythorbate are limited to 547 ppm. Citric acid or sodium citrate may be used to replace up to half of any one of the above reductants. While the above regulations apply to all cured meats, bacon is an exception. For injected bacon, sodium ascorbate or sodium erythorbate is required (not optional) at 550 ppm (USDA-FSIS, 1995).

Acidulants

Functions

The acidulants (fumaric acid, sodium acid pyrophosphate, and glucono-delta-lactone) are utilized to provide a more acid environment to encourage faster nitrite-to-nitric oxide conversion. While reducing the pH will accelerate curing reactions, reduced pH may also reduce the water binding of the meat mixture and may reduce

product yields. Consequently, pH changes must be considered with care. The acidulants will generally reduce meat pH by 0.2–0.3 pH units when added at the maximum concentrations (0.5%) permitted.

Regulations

The acidulants are permitted as cure accelerators only in comminuted cured products. Fumaric acid may be used at a maximum concentration of 650 ppm in cured, comminuted meat and poultry products. Glucono-delta-lactone and sodium acid pyrophosphate may be used only in comminuted meat products (not poultry) at a maximum level of 5,000 ppm (0.5%), based on finished product weight. While sodium acid pyrophosphate is limited to 5,000 ppm alone or in combination with other cure accelerators, glucono-delta-lactone may also be used at up to 1% (10,000 ppm) in Genoa salami (USDA-FSIS, 1995). In the case of Genoa salami, glucono-delta-lactone helps to provide the low pH and acid tang desired for a fermented sausage.

Phosphates

Phosphates are included in many curing solutions and cured meat formulations because of numerous beneficial effects that they bring to cured meat products. However, phosphates are not distinctive ingredients that characterize cured meats, as is the case for nitrite and salt. Cured meats can be manufactured successfully without phosphates and will demonstrate all the typical properties of cured meats expected by consumers. At the same time, the advantages of including phosphates are such that these compounds have become a common ingredient in most cured meats, except for dry and semidry products.

Properties

Phosphates differ from many other food ingredients in that several different forms may be used. Further, the different forms of phosphates vary greatly in properties and, consequently, in applications. For example, there are at least ten specific phosphates that can be used in processed meat applications. These phosphates vary widely in two critical properties: pH and solubility in water (Molins, 1991). A 1% solution of the different phosphates in water, for example, can range from less than pH 5.0 to over pH 10.0. Solubility of the phosphates can range from less than 10 g/100 g of water to over 100 g/100 g of water. Generally speaking, the commonly used phosphates in processed meats are alkaline because one objective of their use is to increase meat pH and, in doing so, increase water retention. The commonly used phosphates are also somewhat limited in solubility, such that when added to injection brines where concentration of other compounds (sodium chlo-

ride, sugar, etc.) is expected to be high, it is imperative that the phosphates be dissolved first or they may not dissolve completely.

The phosphates can be categorized into four groups based on their structure. The *orthophosphates* are the simplest and consist of a single phosphate (PO_4) unit. Both sodium and potassium salts may be used in processed meats. Most of the orthophosphates are alkaline except for the monosodium and monopotassium phosphates, which are acidic. Single phosphate units may be condensed into longer chain phosphates with combinations of two phosphate units (di-phosphate) normally termed *pyrophosphates*. Pyrophosphates can also be used in processed meats as either sodium or potassium salts and, while most are alkaline, this group includes sodium acid pyrophosphate, which can be used as an acidulant for accelerating curing reactions. Longer chain phosphates include *tripolyphosphates*, which are condensed, three-phosphate chains. Both sodium and potassium salts of tripolyphosphate are basic but are significantly different in their water solubility. Finally, longer chain condensed phosphates are called simply *polyphosphates* because the number of phosphate units may vary from about 10 to about 25 (Molins, 1991).

Metaphosphates are multiple phosphate units arranged in a ring structure and are not used in foods. A traditional name used for one of the polyphosphates has been sodium hexametaphosphate, which is technically inaccurate and has caused some confusion. Sodium hexametaphosphate is not a true metaphosphate, but rather a straight chain polyphosphate of 10–15 phosphate units (Molins, 1991).

Functions

Phosphates, like many of the other cured meat ingredients, perform multiple functions when added to meat mixtures. One of the most important functions of phosphates is the increased capacity of meat proteins to bind and retain water. The net result of improved water retention is not only improved cooking yields, but also improved product texture, tenderness, and juiciness (Xiong, 2005). The effect of phosphates on meat proteins is twofold. First, the use of alkaline phosphates significantly increases the pH and ionic strength of meat mixtures, an effect that is well recognized as a means of improving water retention by increasing protein charge repulsion. However, since the pioneer work of Offer and Trinick (1983), it has become clear that phosphates also facilitate removal of transverse myofibrillar proteins that, in the presence of salt alone, serve to constrain swelling of myofibrils and extraction of myosin. Sodium pyrophosphate and tripolyphosphate appear to be the most effective in this regard (Xiong, 2005). One of the major advantages of the protein-specific effects of phosphates is the opportunity to reduce sodium chloride content in cured meats while retaining the water-binding capacity of the higher salt concentration. Sofos (1985) reported that salt content could be reduced by about 50% to 1.1%, in cooked comminuted meat by addition of phosphate. Ruusunen and Poulanne (2005) suggested that salt content in cooked sausage could be reduced to about 1.4% with addition of phosphate.

The effect of phosphates on meat protein solubility is also an important contributor to the binding properties of meat pieces in restructured products, heat-set protein gel properties, emulsion stability, and many other functional properties of processed meats that are affected by soluble myofibrillar proteins. Improved solubility of myofibrillar proteins provides greater protein concentration for the functional roles of these proteins in processed meat products.

Phosphates have an important function as contributors to antioxidant activity in processed meat products. There have been extensive studies with a wide variety of products that document phosphates as very effective for suppressing oxidation in meat products (Molins, 1991). Because sodium chloride is recognized as a prooxidant in meat, the addition of phosphates is particularly advantageous in those products where rancidity could quickly develop (Vasavada, Dwivedi, & Cornforth, 2006). Cured meats are well protected by the antioxidant activity of nitrite, but in uncured, moisture-enhanced fresh meats injected with water, salt, and phosphates, or in uncured, cooked meat products, the phosphate provides a critical protective role (Detienne & Wicker, 1999). Phosphates are not typically classified as antioxidants, despite their positive contribution to lipid stability, because it is generally believed that phosphates exert their antioxidant role indirectly. The most common explanation of how phosphates protect lipids from oxidation is metal chelation and removal of catalysts that serve to initiate lipid oxidation (Molins, 1991). While other mechanisms may be involved, phosphates are recognized as effective synergists for antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT), an effect that supports the suggested role of phosphates as chelators.

Although not considered to be primary antimicrobial agents, there are several studies that have reported antimicrobial effects of phosphates in meat products. Most of these have found phosphates to contribute to bacterial control and shelf life of fresh meat (Molins, 1991; Molins, Kraft, & Marcy, 1987) but these effects are less obvious in cured meats. The presence of other significant microbial inhibitors in cured meat has made it difficult to separate any potential antimicrobial effects of phosphates from those of other food additives.

While color is not considered to be a major function of alkaline phosphates in meat, these compounds are likely to have an effect on product color. In the case of cured meats, the elevated pH resulting from phosphates may slow the nitrite curing reaction and extend the time needed to generate sufficient nitric oxide for good cured color development. Without adequate time, cured color will be less intense. On the positive side, a higher pH is conducive to greater cured color stability during extended storage of the finished product. An important factor in maintaining cured color during storage is a small amount of residual nitrite, which is likely to be better maintained with high product pH.

Because the phosphates vary widely in pH and solubility characteristics, it is important to consider the different phosphate properties for specific product applications. Solubility, for example, will be more critical for a phosphate to be dissolved in an injection brine than for a phosphate added to a comminuted product. Further, phosphate suppliers have developed blends of phosphates to provide the

best combinations of properties for specific applications. Consequently, the best course of action for meat and poultry processors when considering how to best use phosphates is to work closely with suppliers to determine recommended uses for specific phosphates and phosphate blends.

Regulations

Although there are many forms of the phosphates that may be used in cured meat and poultry, the regulations that apply do not differentiate between the various phosphates or phosphate blends. All are restricted to 0.5% (5,000 ppm) based on finished product weight when used to reduce moisture losses, to protect flavor, or as a cure accelerator. Most processors utilize 0.3–0.4% to compensate for the small amount of phosphate naturally present in meat. While all of the phosphates that are generally approved for use can be used in both cured meat and cured poultry, there is one exception; when sodium acid pyrophosphate is used as a cure accelerator, it may be used in cured meat but not in cured poultry.

When phosphates are used to increase product pH, the USDA will also allow the addition of sodium hydroxide in a ratio of 1 part sodium hydroxide to 4 part phosphate. The function of the sodium hydroxide is solely for achieving greater increase in product pH over that of phosphates alone.

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Chapter 2
Starches

Ghislaine Joly and Björn Anderstein

Introduction

Starch is a well-known ingredient in the food industry for its water-binding capabilities. It has been mass-produced in the United States since the end of World War II and has become very available worldwide as a commodity ingredient. Beyond its famous use in gravy and cherry pie filling, increased use of starch by the U.S. meat industry has been driven by higher levels of poultry usage and a demand for affordable, quality meat products. Starch can also be combined with other hydrocolloids and proteins in meat formulation to achieve the preferred yields, texture, and mouthfeel.

In this chapter, starch sources and modifications are presented as they pertain to providing economical, innovative, and desirable meat products. Guidelines and understanding of starch technology in meat will be given. Practical information is provided to make starch selection easier in regard to formulation in the kitchen, pilot plant, or full-scale manufacturing in combination with other ingredients.

History of the Use of Starch in Meat

Food historians confirm that starches have been used as meat fillers from ancient times forward. Recipes were developed according to custom and cuisine. Examples are ancient Roman Minces, Swedish Meatballs, or American Meatloaves, which all have the same historical culinary background. Earliest starch-filled meat minces were generally made with cooked/preserved starch-containing vegetables. It is also confirmed that prehistoric cooks used starches to thicken soups and stews, by acting as a binding agent. More importantly, it stretched the food in order to feed more people. The high carbohydrate content of most starches satisfies hunger better than liquid-thinned recipes. It is also possible that meat minces evolved from quick-cooking techniques widely practiced in the Middle Eastern/Asian region, where cooking fuel was scarce. Stir-fry and kebabs are two examples of this (Olver, 2007).

It took many more centuries, however, for starch to become industrially available for usage in food and meat. As Bergsma (2000) describes, the first “industrial

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processes" for the manufacture of wheat or potato starches date back to 1772. Further advances were made in the nineteenth century, but the most significant industrial development occurred between 1940 and 1960 with the finding that a variety of reagents could be reacted with ungelatinized starch granules in water systems to achieve degrees of derivatization sufficient to markedly change the properties of the treated starches.

The type of starches used in meat products varies from region to region. In European meat products where starch is part of the recipe, potato starches are traditionally used, for availability and functionality. As a part of the recipe for meat products and certain dishes, especially in Scandinavia, boiled and coarse-cut or mashed potatoes can be found, which are now commonly replaced by industrial food starches. In other regions such as Asia, the traditionally used native tapioca or wheat starches are being replaced by modified starches of any type.

Starch Structure and Function

Source

Starch is a form of energy reserve for vegetables. It is usually stored in the grain/seed to be used as an energy source during germination and initial growth of the plant. This family is represented by corn (maize), wheat, rice, oats, etc. It is also present in roots, tubers, and tree trunks (stems) for the same purpose. This starch family is found in potatoes, tapioca (cassava), sago trees, etc. In North America, the most commonly used starch in meat applications is corn-based, while in Europe potato starch is the most predominant. These particular preferences for starch sources are primarily determined by the predominant crop of each continent. As shown in Table 2.1, each starch source presents unique characteristics important to its selection for use in a meat formulation. Each starch source can be identified microscopically by the shape, diameter range, and coloration of its granules when stained by potassium iodine. The microscope is the tool most commonly used to identify starch (and its cook status) when its source is unknown in a meat product.

Structure

Native Structure

Refined starch is a fine white powder made of starch granules. Each granule is organized in a "ring" pattern of glucose chains: amylose and amylopectin polymers, the two pillars of starch functionality. Refined starch also has residual proteins, lipids, minerals, and moisture at levels that are dependent on the starch source and on the "washing" step during the starch manufacture.

Table 2.1 Starch sources and unique characteristics

Source	Type	Diameter range (μm)	Granule shape	Amylose content (%)	Iodine stain	Gelatinization temperature (°C)
Corn (dent)	Cereal	5–25	Round polygonal	25	Blue	62–72
Waxy corn (maize)	Cereal	5–25	Round polygonal	<1	Red violet	63–72
Tapioca	Tuber	5–25	Truncated round, oval	17	Blue	62–73
Potato	Tuber	15–100	Round, oval	20	Blue	59–68
Rice	Cereal	3–8	Round polygonal	19	Blue	68–78
Wheat	Cereal	3–35[a]	Round lenticular	20	Blue	58–64
High amylose corn	Cereal	3–24	Round elongated	63–92[b]	Blue	50–90

[a]Wheat starch has two populations: large granules (20–35 μm) and small granules (3–10 μm).
[b]High amylose corn is not completely gelatinized in boiling water.

The starch source is the first thing to select when choosing to formulate with a starch. With chemical and/or physical modifications, it is the key to providing the desired attributes to the meat.

As explained before, there are many options, and we will only present the most common here. Table 2.1 describes the characteristics of common starches and their ratio of the glucose polymers amylose and amylopectin found in their native states. In this table, it is assumed that amylose and amylopectin combined make up 100% of the starch. For example, potato starch is made up of 20% amylose and 80% amylopectin, and this ratio will give it unique functional properties when it is applied to a meat product.

Amylose and amylopectin are solely differentiated by their glucose chain linkages and provide different water-binding "types." As Fig. 2.1 illustrates, amylose is a linear glucose polymer chain with α-(1,4)-glucosidic linkages. Amylopectin is a branched glucose polymer with α-(1,4) and α-(1,6) linkages. These linkages will impart different water-binding characteristics, enabling the structure to stay bound over a long period of time in refrigerated conditions, for example. This structure can undergo different chemical and physical modifications to manage water binding during the manufacturing process and the product's shelf life.

Modified Structure

Starch modifications are made to improve native starch performance. Native starches have limitation in terms of heat-, shear-, and acid-resistance and in stability in refrigerated or freeze/thaw conditions. Modified starches are less limiting in extreme processes and formulations. Some modifications also allow for the addition of some functional attributes to the starch, such as emulsification properties.

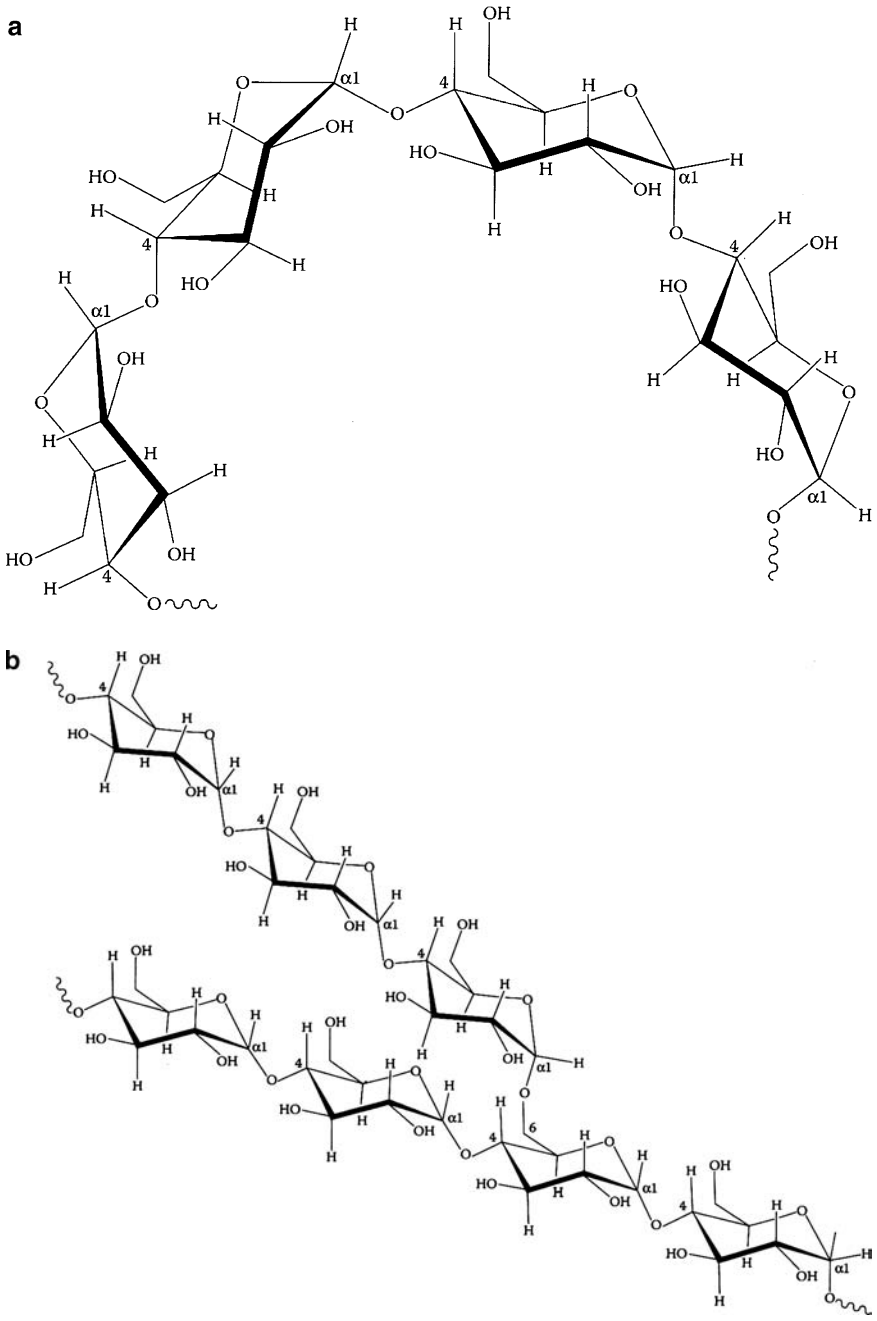

Fig. 2.1 Starch glucose polymer structures (From Mitolo, 2006. Reproduced with permission).

Regulations. In the United States, the Food and Drug Administration has regulated the starch modifications that are permitted for food use (Code of Federal Regulations [CFR], 2007c). This regulation also limits the types and quantities of chemicals that can be applied to raw starch during its manufacture, as well as the levels of residual chemicals present in the finished starch. The use of modified food starches must be declared on the ingredient line of meat product labels either by the generic term "modified food starch" or by identifying the starch source, such as, for example, "modified *tapioca* starch," "modified *potato* starch," or "modified *corn* starch."

In Europe, chemically modified starches are classified as food additives. They are assigned *E* numbers and are regulated in accordance with Directive 95/2/EC on "food additives other than colors and sweeteners," as amended (European Parliament and Council, 2006). They have to be manufactured in accordance of specified purity criteria. An overview of the *E* numbers is given in Table 2.2.

Chemical Modifications. The array of chemistry available gives meat processors much latitude when choosing a starch that will fit their requirements (Table 2.3). The modification for better freeze/thaw stability, often required in processed meats, is called stabilization (or substitution) as illustrated in Fig. 2.2. This modification preserves the starch's functionality even after freezing. This freeze/thaw stability can be provided to the starch by a propylene oxide reaction. A native starch containing amylose will retrograde over time, upon aging in refrigerated conditions or after freezing/thawing. This is due to the collapse of the amylose chains, which get closer together and release the water in the medium the starch is in. This is called syneresis. It is also referred to in meat products as purge or drip loss. Amylopectin can also retrograde, but at a slower rate and less strongly, thus creating softer "gels."

Another well-known modification is cross-linking which makes the granule resistant to heat, acid, and shear abuses during the process and preserves its integrity. Cross-linking (Fig. 2.3) can be achieved, for instance, by forming a diester with phosphoric acid ($POCl_3$ is the most common reagent) or by forming an ether bond by reacting with epichlorhydrin. A cross-linked starch may be preferred for retorted meat or meat undergoing several heating processes or a very long cooking procedure.

Some other modifications can be desirable. For instance, the emulsifying properties conferred by an OSA (octenyl succinic anhydride) modification (comprising dual functional hydrophilic and hydrophobic groups) can help in a fatty processed meat to stabilize the fat and keep it in during cooking.

Physical Modifications

1. Pregelatinization. Although most of the starches used in meat are cook-up starches because they will be cooked in a smokehouse, industrial oven or consumer grill, pregelatinized starches can be used to help in forming a ground meat product

Table 2.2 *E* numbers for modified starches

Starch	Legislation	Classified as	*E* number	EU product description
Native starch	FCC[a]	Food ingredient	None	Starch – native/natural
Physically modified	FCC	Food ingredient	None	Starch – physically modified
Enzymatically modified	JECFA[b]	Food ingredient	None	Starch – enzyme modified
Dextrinized	JECFA	Food ingredient	None	Dextrin
Acid treated	JECFA	Food ingredient	None	Acid-treated modified starch
Alkali treated	JECFA	Food ingredient	None	Alkali-treated modified starch
Bleached	JECFA	Food ingredient	None	Bleached modified starch
Oxidized starch	Directive 95/2/EC[c]	Food additive	E1404	Oxidized starch
Monostarch phosphate	Directive 95/2/EC	Food additive	E1410	Monostarch phosphate
Distarch phosphate	Directive 95/2/EC	Food additive	E1412	Distarch phosphate
Phosphated distarch phosphate	Directive 95/2/EC	Food additive	E1413	Phosphated distarch phosphate
Acetylated distarch phosphate	Directive 95/2/EC	Food additive	E1414	Acetylated distarch phosphate
Starch acetate	Directive 95/2/EC	Food additive	E1420	Acetylated starch
Acetylated distarch adipate	Directive 95/2/EC	Food additive	E1422	Acetylated distarch adipate
Hydroxypropyl starch	Directive 95/2/EC	Food additive	E1440	Hydroxypropyl starch
Hydroxypropyl distarch phosphate	Directive 95/2/EC	Food additive	E1442	Hydroxypropyl distarch phosphate
Starch sodium octenyl succinate	Directive 95/2/EC	Food additive	E1450	Starch sodium octenyl succinate
Starch Aluminum octenyl succinate[d]	Directive 95/2/EC	Food additive[d]	E1452	Starch aluminum octenyl succinate

[a] FCC: *Food Chemicals Codex*, 5th ed. (Institute of Medicine, 2003).
[b] JECFA: *Compendium of food additive specifications*, addendum 9 (Joint FAO/WHO Expert Committee on Food Additives, 2001).
[c] Directive 95/2/ec: *Directive No. 95/2/ec of 20 February 1995 on food additives other than colours and sweeteners*, amended by directive 2003/114/ec & 2006/52/ec (European Parliament and Council, 2006).
[d] Restricted use.

or for binding water up front. They can also be used in injected meats for particle suspension in brine.

Pregelatinized starches are unique in that they swell upon contact with any fluid; therefore, they do not need to be heat/shear activated like cook-up starches. There are two main ways of manufacturing pregelatinized starches:

Table 2.3 Chemical modifications and their functionality in meat

Modification type	Modification objective	Functionality in processed meat
Stabilization	Improve solubility	Decrease gelatinization temperature Freeze–thaw stability Shelf life
Cross-linking (inhibition)	Modify cooking characteristics	Process tolerance (high temperature, low pH)
Acid thinning	Reduction of hot viscosity	Good gel strength (firm texture)
Conversion	Create unique rheology	Low viscosity Mouthcoating (fat mimetic)
Dextrinization	Create lower molecular weight starch (dextrins)	Low viscosity Solubility Film formation (fat mimetic)
OSA (octenylsuccinates)	Impart lipophilic character	Emulsion stability Decrease fat loss

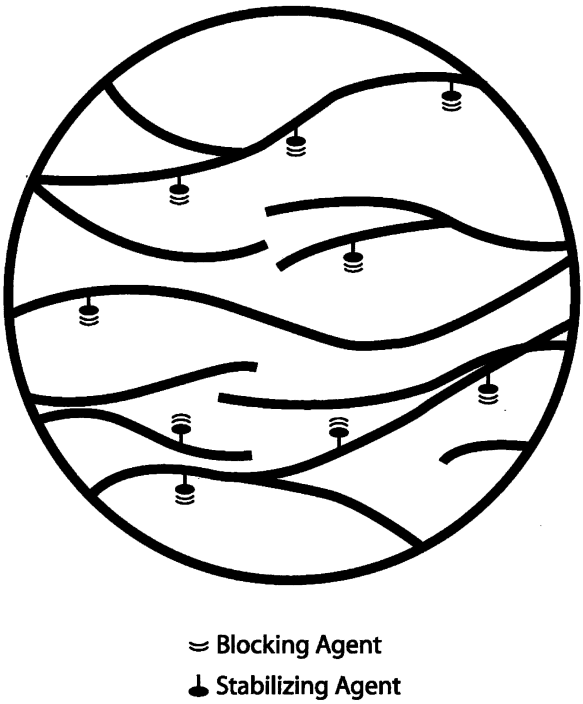

Fig. 2.2 Starch substitution or stabilization (From Mitolo, 2006. Reproduced with permission).

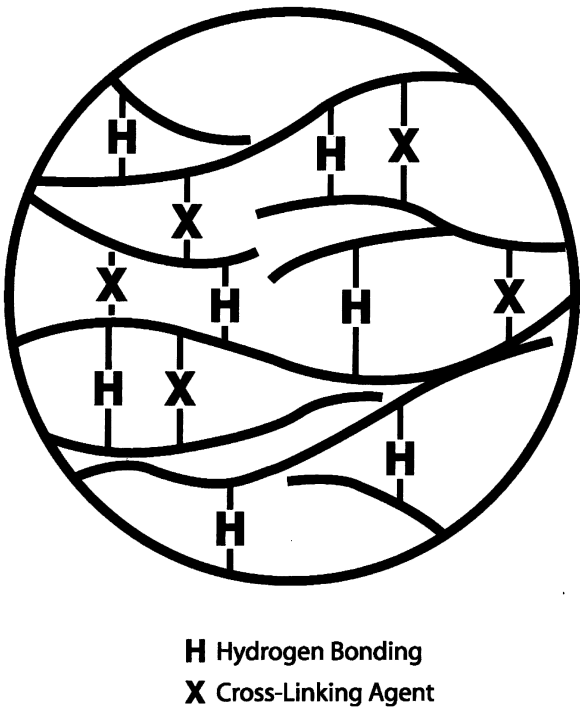

Fig. 2.3 Starch cross-linking (From Mitolo, 2006. Reproduced with permission).

a. *Drum Drying Process.* The cook-up starch slurry is "cooked" and dried on a hot drum to form starch flakes that are ground to the desirable particle size. This is the oldest pregelatinization technology and provides the most economical pregelatinized starches. These are the ones most commonly used in meats.

b. *Spray Cooking Process*. The cook-up starch slurry is "cooked" and dried through a nozzle in a hot air tower. The fine, dry starch powder collected at the bottom of the tower can be agglomerated to make the starch particle size larger and easier to disperse. This is a more recent technology that provides a smoother texture than drum-dried starches.

2. *Dextrinization.* This is a dry powder process where acid hydrolysis is performed in the presence of dry heat and agitation. The heat treatment allows for repolymerization of the glucose polymers, offering a large variety of properties. The resulting (pyro)dextrins are generally used because of their film-forming, thin-viscosity, and fat-mimetic properties. They are used in meat for fat replacement and mouthfeel enhancement, but also in meat coatings (glazes or batters and breadings) to provide a strong film (Chap. 12). If the starch is hydrolyzed even further (where an enzymatic process is typically used), maltodextrins are produced. Those also have fat-mimetic properties in meat.

3. Thermal Inhibition. This is a patented technology (NOVATION®, National Starch Food Innovation, Bridgewater, NJ, USA) that puts the starch through a physical, rather than chemical, treatment that strengthens the starch granules to provide the efficiency of chemical cross-linking without the use of chemicals. In the United States, the "native functional starches" created through this process are widely used in "natural" and organic (when made with organic NOVATION®) meat products. Product labels declare these starches simply as "corn starch," "tapioca starch," organic corn starch," or "organic tapioca starch." Thermally inhibited starches (as native starches) qualify under the "National List of Allowed and Prohibited Substances" (Code of Federal Regulations, 2007a), so their use can fall under the organic 95-5 rule. Currently in Europe, native functional corn starch is on the National list and can therefore be used in the 5% rule for an organic meat product because there is no organic corn starch available (Council of the European Communities, 1991). All other starch bases (e.g., tapioca, potato) must be organic for use in organic meats. Those starches can go through the heat, shear, and acid conditions that a native starch cannot. This process can be combined with a pregelatinization process to offer pregelatinized native functional starches, which also find use in "natural meats." Figure 2.4 presents most of the described modifications.

Regulation of Starch Use in Meat

Europe

The addition of starch in the EU is regulated for many specific meat products. Regulations can differ from country to country, so one must always check the local law for the country where the product is to be sold. If starch is permitted, either it can be a modified or a native starch and used at specific levels. Additionally, certain product standards do not permit the use of starch, unless the product is given a different name (e.g., "kind of…"). Regional terms, indications of origin, and traditional specialties are protected.

Table 2.2 shows the *E* numbers of modified starches used for label declarations.

United States

In the United States there are limitations on starch usage for some specific meat products. For instance, modified food starch is permitted to prevent purging in cured pork products, at levels not to exceed 2% of product formulation in "Ham Water Added" and "Ham with Natural Juices," and 3.5% in "Ham and Water Product- X percent of Weight is Added Ingredients" (CFR, 2007b). The percent of

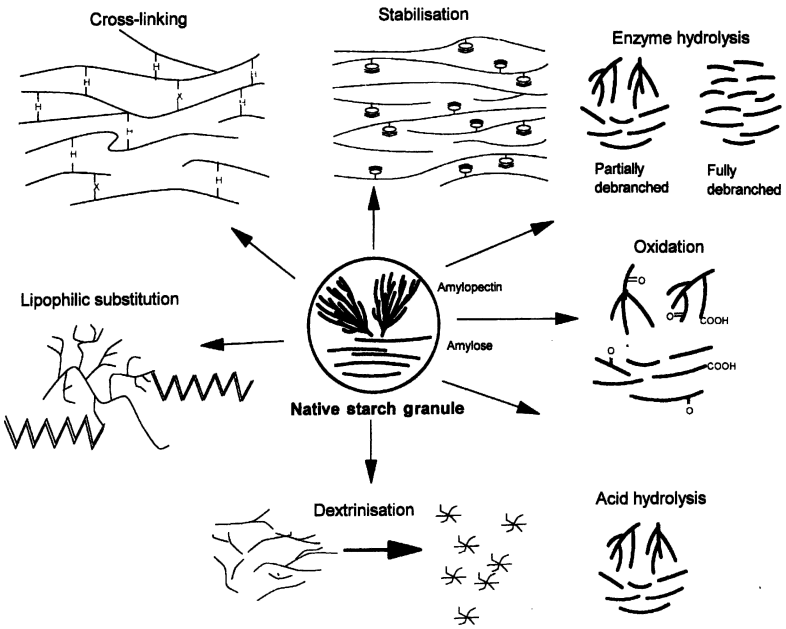

Fig. 2.4 Chemical and biochemical modifications of starch (From Taggart, 2004. Reproduced with permission from Woodhead Publishing Ltd., Cambridge, UK).

modified food starch that can be used will vary with the label declaration and the possible combination with other water-binding ingredients, such as soy protein concentrate. This will also vary with the source of meat (poultry, pork, or beef) used. The use of starch in standardized sausage products, such as frankfurters, bologna, etc., is only permitted in "modified, substitute sausage products" (e.g., reduced-fat frankfurters) to "replace fat, improve texture and prevent syneresis" (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 1995; Wade, 1995).

In the United States, some products have strict standards of identity that do not allow the use of starch, such as "beef hamburger." If the product is called a "beef patty," then starch addition is allowed (USDA-FSIS, 2005).

In terms of labeling, the term "starch" may be used to designate corn and wheat starch but not tapioca starch, which must be declared as "tapioca starch" or "food starch." The term "vegetable starch" refers to starches from any vegetable source (USDA-FSIS, 2005).

Other Countries

In other countries, regulations should be assessed carefully to make sure the finished product adheres to them. For instance, in Mexico regulations allow high

levels of starch in meat products, due mostly to economic factors. The use of high levels of water and starch (often in combination with other binders) is commonplace in many other countries, and enables processed meat products to be more affordable for the general population.

Starch Performance

Choosing the Correct Starch

In order to extract maximum performance out of a starch, it is important to choose the correct one for the application. Before choosing a starch source and modification, the formulator has to think of the key attributes of the final product to be created (Table 2.4), many of which are related to water management within the meat matrix. Water management can be expressed by yields and cooking losses, by purge and drip loss over storage, and, finally, by finished product texture, flavor, and color, all of which are influenced, to varying degrees, by the amount of water in the formula and the way in which it is bound.

Starch Performance in Meat

Once incorporated in the meat by either injection, tumbling, or simple direct addition (in a bowl chopper or blender), cook-up starches become distributed in the meat matrix. The starches are incorporated between the meat fibrils when it is injected and/or tumbled or between meat particulates in the case of ground/emulsified meat. Halden, Mathiesen, and Proctor (1992) patented the classical industrial method of "pickling" or "marinating" meat with starch at 0.5–5% of the weight of the meat, with the succession of injection and (vacuum) tumbling.

Li and Yeh (2002) looked at the possible interaction of starch with meat protein in pork ham. Using differential scanning calorimetry (DSC), they produced thermograms that did not show any chemical interactions between starch and meat protein during the heating process. This work seems to show that starch is behaving in an isolated manner, with each starch granule swelling independently when the gelatinization temperature is reached in its vicinity. A critical point is indeed reached when the granule swells with temperature and time and continues to swell to the extent that it can burst and lose its integrity. The starch granule is acting like a balloon, but instead of air it is water that contributes to its expansion. A classic diagram of a starch cooking curve is represented in Fig. 2.5, where the starch reaches its pasting/gelatinization temperature, swells, and goes through the cooking phases, categorized as undercooked, slightly undercooked, good cook (when it reaches its peak viscosity and 80% of the

Table 2.4 Key quality and performance attributes to consider when designing a meat product

Final texture (firmness, succulence, dryness/moistness, chewiness, etc.)
Eating experience (mouthfeel, bite)
Desired functionality (water binding, emulsification, texturization, fat mimicry, adhesion, etc.)
Target composition (water level, fat level)
Expected yields
Purge control
Shelf-life conditions and time (refrigeration, canning, etc.)
Process
Label declaration requirements (e.g., natural or organic)
Market positioning
Cost

starch granules are fully swollen), slightly overcooked, and overcooked (when there is a major loss of viscosity and most of the granules become fragmented and lose their water-holding capability). Figure 2.6 also shows the difference between unmodified and modified waxy maize starches at different levels of cross-linking (from slightly cross-linked to highly cross-linked). As expected, the unmodified starch loses its viscosity upon cooking, although it reaches its peak viscosity faster. While cross-linked starches continue to swell, they do not reach full swelling capacity as quickly as unmodified. The cross-linking strengthens the granule, making swelling more difficult but offering functionality in the case of long heat exposure or repeated heating of the meat.

Starch in a Manufacturing Scenario

Cook-Up Starches

Cook-up starches are used in many meat products. They should bind the water when the meat is being heat-treated (as the meat proteins release water when denaturing), as well as additional free water coming from the brine. The amount of bound water is strongly linked to the starch base used. But the starch base also has a huge impact on the related texture.

In general, water binding in a meat product is well described by the process of gelatinization. No interaction between the starch and meat protein takes place. Therefore, the most important criterion in choosing a starch for meat products is its gelatinization temperature. This gelatinization temperature of a starch has to correspond with the temperatures achieved during thermal processing of the meat product.

Gelatinization temperatures are greatly influenced by the binding forces within the granule, which vary with species (National Starch and Chemical Company, 1996) and are presented in Table 2.1.

High Amylose Corn Starch. High amylose corn has much greater bonding force than other maize varieties, due to the high degree of linearity within the granule.

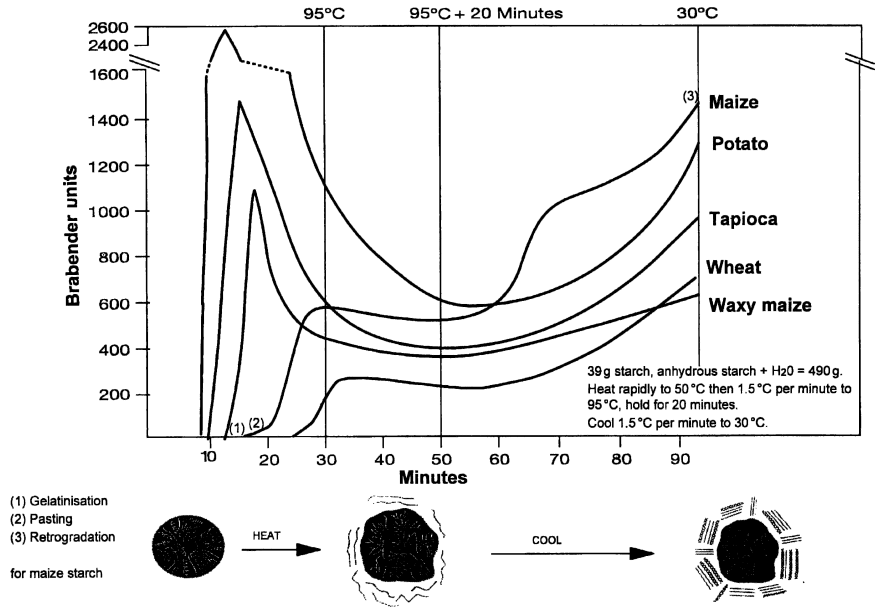

Fig. 2.5 Changes in native starch during processing (From Taggart, 2004. Reproduced with permission from woodhead Publishing Ltd., Cambridge, UK).

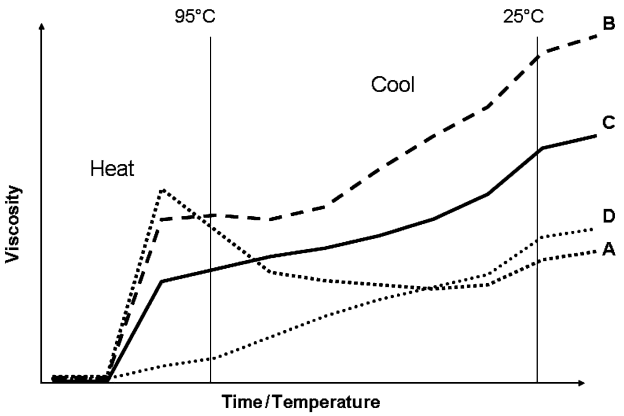

Fig. 2.6 Viscosity curve of modified waxy maize starch at different level of cross-linking (A: native, B: lightly, C: moderately, D: highly) (starch at 6% concentration in water).

Therefore, it takes much longer for the starch to gel and to be functionalized (National Starch and Chemical Company, 1996). This makes the starch unsuitable for usage in meat products with traditional expectations of yield, purge control, etc. Indeed, normal gelatinization temperatures of high amylose corn starches in Table 2.1 are not reached during a normal meat cooking process.

Potato Starch. Potato starches have orthophosphate ester within the potato granule, which tends to weaken bonding and thus lower the energy required for gelatinization (National Starch and Chemical Company, 1996). In addition, potato starches have relatively large granules, low lipid and protein content, a higher water-binding capacity than other starch types, and yield high paste viscosities. The presence of the esterified phosphate groups stabilizes dispersed phases with high efficiency. Besides water binding, it is sensory characteristics such as texture, appearance, and flavor that have made potato starch successful in meat applications. One major advantage of potato starch is that it will start to gelatinize at the same time as the meat proteins are losing the most water. It is also fully gelatinized between 72°C and 76°C, the same temperature range to which most meat products are cooked. Potato starches are very sensitive in processed meats with a higher salt content.

With the swelling of amylose-containing granules, the amylose molecules are solubilized and leach out into solution. These molecules will then re-associate into aggregates and precipitate at low concentrations or set to a gel at higher starch concentrations. This is referred to as "set back" or retrogradation but can also lead at the same time to a firmer or meatier texture within the final product. This firmness can contribute to better slicing performance.

Waxy Maize Starch. Waxy maize starch has no linear amylose molecules, so its paste will remain flowing. It will not gel or weep and will also give a much softer texture when used in a meat product. Since amylose starch chains tend to retrograde or give up water after packaging, as described above, high-amylopectin starches, like waxy maize, are best suited to achieve long-term water holding capacity.

Tapioca Starch. Having a small amount of amylose, tapioca starch gives a soft gel when pasted. It is used mainly in extremely bland meat systems and also in instances in which a "clean" label declaration is desired. Tapioca starch is generally more expensive than other starches.

To summarize, the amylose content related to the starch variety has an influence on the gelatinization temperature and on the final texture. This must be considered when choosing a starch for use in meat products. Corn starch normally needs to be heated in water to 95°C. Potato starch will absorb more water and the gelatinization temperature is lower, causing the solution to thicken quickly on heating. As shown in Table 2.1, tapioca starch gelatinizes at a temperature between that of waxy maize and corn starches, and has a slightly lower viscosity than waxy maize. The gelatinization temperature and the maximum functionality must be achieved during the heat treatment of the final product; otherwise, the starch will not be fully functionalized and its full water-binding capacity and stability will not be reached.

In a study that compared potato starch or potato flour with wheat flour in frankfurters, sensory panels rated the potato starch or flour as providing more tenderness and juiciness (Bushway et al., 1982). Other trials have confirmed the improved stability and textural firmness of comminuted meat products with added wheat flour and modified corn starch (Comer, Chew, Lovelock, & Allan-Wojtas, 1986).

Cold Swelling Starches

Cold swelling starches can be used in meat products for several reasons. They work in minced meat products as water binders and texturizers to improve the processability of the uncooked meat. They also help to form the minced meat, keep the water bound in the meat batter, and depending on the kind of starch used, will keep the water bound during processing.

Cold swelling starches can be used as well as suspension aids for insoluble ingredients like cook-up starches. By increasing the viscosity of a brine solution, they can help to avoid sedimentation of these ingredients.

As with cook-up starches, the botanical source of the starch determines the amount of bound water and the final texture. Again, potato starches are widely used due to their high water-binding capacity, where waxy maize starches lead to an improved shelf-life and freeze/thaw stability.

Starches in Brine Systems

In the manufacturing environment, starches are added to dry ingredients such as salts, spices, etc., and can be dispersed in cold water with minimal agitation. If a cold swelling starch is used in the formulation, agitation should be adjusted, so the starch hydrates without lumping.

Cook-Up Starches

Cook-up starches are used in injected and/or tumbled whole muscle or reconstituted meat products. They are used to bind the additional water added in the formulation and the inherent water existing in the meat. This occurs during the heating process and avoids high cooking losses. Savage and Chenneour (1970) patented a process where the meat is injected with raw starch granules and the result is a reduction of cooking losses compared to the no starch control. After cooking the starch granules keep the moisture in the meat and thus maintain the succulence of the product. As cook-up starches are insoluble, they will sediment, so the viscosity of the brine has to be increased to suspend the raw starch, or the brine needs to be kept agitated. The agitation can be produced by recycling of the brine. The normal dosage for tumbled

meat products is between 1% and 3% of cook-up starch in the final product. If the product is injected, better distribution of the starch in the meat is typically achieved, so the normal dosage can be reduced to 0.3–1.0% of the final product. At too high a dosage, starch pockets or so called "tiger stripes" may be observed. This is due to poor starch distribution and results in the starch slurry being visible in the finished product. Occasionally when meat is injected at high pressures, the meat fibers become displaced and brine pockets are not properly distributed. In light of this, it is obvious that more starch does not necessarily mean better yield; there is a threshold where performance will not be improved and can even be worsened by the addition of too much starch. This could be due to steric hindrances with the meat fibers.

High dosages can also lead to an earthy (potato) or cereal (waxy maize) taste in meat products. It was noticed, for instance, that European populations are more accustomed to the flavor of potato starch, whereas North American consumers are more used to corn starch flavor.

The starch itself, whatever the base, is under normal conditions fully injectable, as long as no sedimentation is created in the process.

Cold-Water Swelling Starches

Since insoluble ingredients like cook-up starches tend to sediment without agitation, viscosity-increasing ingredients like xanthan gum or cold-water swelling starches are used. By increasing the viscosity of the brine system, a network is built up that prevents the cook-up starch from settling. The amount of cold-water swelling starch in the brine should be approximately the same as that of cook-up starch. Very often, native starches are used as cold swelling starches (due to their higher swelling volume) but, of course, process stable ones like modified or native functional starches will also work. One way to experiment with the effectiveness of the suspension agent is to make brines with different levels of pregelatinized starch or gums and observe over time the settling in a graduated cylinder.

One additional effect of using cold-water swelling starches is that the drip loss between the injection and thermal process is reduced. This approach can be useful when making, for instance, injected whole birds that are left to rest overnight before roasting. Cold swelling starches help the brine remain inside the bird (by virtue of increased viscosity) much better than cook-up starches used by themselves.

Starches in Emulsified Meats

Cook-Up Starches

In emulsified meat products, such as sausages of any kind, cook-up starches are especially useful when lower quality meats or high water levels are used. The starch is added at the beginning of the initial chopping process with the other dry ingredients and binds the free available water during the cooking process of the sausage.

For initial water binding, native, modified, or functional native starches can be used. A disadvantage of native starches in this application is that they allow high drip losses (package purge) during product storage life. In addition, process stability is a problem for double pasteurized or canned products.

A typical starch dosage level would be 2–3% of the final product, but higher levels are not uncommon, especially when native starches are used.

Dexter, Sofos, and Schmidt (1993) reported a reduction in cooking and purge loss due to the addition of potato starch during the chopping process of turkey bologna, although they did not observe an increase in product hardness. Carballo, Fernández, Barreto, Solas, and Colmenero (1996) reported that a more compact and stronger heat-induced protein network resulted when starches were added to meat emulsions.

Cold-Water Swelling Starches

In emulsified meat products containing high water contents, cold-water swelling starches are used to bind the water in the bowl chopper, save the meat proteins from "drowning," and make the meat batter more processable for the sausage filler. During cooking, they ensure the water remains bound in the meat batter, resulting in higher yields and less shrinkage. The dosage of cold-water swelling starches in emulsified meats can be in the range of 1–3% of the meat batter, depending on the formulations' water content and on the water-binding ability and quality of the meat utilized.

If the meat batter is produced and stored overnight before filling, the starch prevents water separation and helps stabilize the batter.

Starches in Minced/Ground Meat Products

Cook-Up Starches

In minced meat products like meatballs or kebabs, the starch is added at the beginning of the mixing process to reach a good distribution within the meat mass. During frying of the meat product, the starch will bind the free accessible water released from the meat proteins. In this way it avoids high shrinkage of the meat product and maintains the desired moisture. A typical dosage in minced products is on the order of 2–3% of the final product.

Cold-Water Swelling Starches

In minced meat products cold-water swelling starches are used to bind the free available water, especially when high levels are used. They also help to improve processability, increase water binding during and after frying, and reduce shrinkage. The usage level depends on the amount of water added and is generally 1–3% of the meat mass.

Other Properties of Starch in Meat

Emulsification

In specific products like pâté, where the fat content is high and the fat has a tendency to render out and separate upon cooking, the use of an emulsifying starch is a good option. The emulsifying starch will favor fat stabilization in the meat emulsion. Emulsifying starches are generally not the best water binders, as their primary function is to emulsify. However, they can be combined with viscosifying starches in the same formulation, so as to optimize water binding and control all fluid (fat- and water-based) losses.

In some areas, pre-emulsions are prepared, which contain 1 part emulsifier, 5–6 parts fat and 5–6 parts water. These pre-emulsions are used to pre-emulsify fat and enable the use of lower quality fats, up to 20%. They are often stored in coolers for hours or days before use, thereby permitting increased production efficiencies. Protein emulsifiers, very often sodium caseinate or soy protein isolate, can be successfully replaced with an OSA starch or an OSA starch-containing starch composition. But as there is no cold-water-binding, these starch-containing pre-emulsions will be less viscous than those containing protein. However, when using these thinner pre-emulsions, the final result in terms of the quality of the sausage will be comparable.

Fat Mimicry

Fat has a basic effect on various physicochemical (heat transfer, etc.) and sensory (flavor, mouthfeel, juiciness, texture, bite, etc.,) characteristics and it cannot be modified and/or reduced with improper use of fat replacers, less fat, or another type of fat. The food industry has responded to consumer demand by offering an ever-increasing variety of low-fat meat choices. Many good-tasting, low-fat meats are available today, thanks to the growing use of one or more low-calorie fat replacers (Tokusoglu & Ünal, 2003).

In order to meet the demand for low- or lower-fat meat products, two options are possible: increasing moisture or mimicking fat. Very often fat replacers in the meat industry primarily bind more water, since water replaces fat. By using starches, gums, and proteins, a texture comparable to that of regular fat can be achieved. Combinations of these ingredients can be used to achieve the desired product texture. Starches for this purpose can be used here in a wide range of products, from whole-muscle meats to sausages and bologna. By using functional native starches, more water can be added and retained during the process. This will provide the desired succulence, which disappears when too much lean meat is used. The other possibility is to add starches as fat replacers. One possibility is to add rice starches, as they offer some “fatty behavior,” such as a soft gel structure and a creamy mouthfeel (due to their smooth texture). Rice starch has unique rheological characteristics due to its small particle size, the smallest of all commonly used starches (2–8 μm).

Its rheological properties are similar to those of fat, which may explain the fat-like texture it provides. Another possibility is to add specialty modified starches, dextrin, or specialty maltodextrin, all of which provide fat-like texture and mouthfeel. Even visible fat-like particles can be produced for the production of some products like bologna types (Salvage, 2006). Converted starches (those that have been prebroken down during starch manufacture) are also a solution for fat replacement. In a U.S. patent, Baumanis, Norton, Brown, and Underdown (1995) describe a comminuted or ground meat product where a gel made of starch and another "gelling network" hydrocolloid is incorporated. This gel is used like fat bits and will retain moisture.

In low-fat frankfurters, Yang, Trout, and Shay (1995) were able to improve texture with modified potato starch. Modified potato starch also was used by Claus and Hunt (1991) for texture improvement in low-fat bologna. Kao and Lin (2006) incorporated gels made of mixed Konjac and potato starch into reduced-fat frankfurters and reported that they had similar juiciness and firmness as the high-fat product.

Hoffman and Mellet (2003) studied the quality characteristics of low-fat ostrich meat patties formulated with either pork lard or modified corn starch, soy isolate, and water. They found that a trained taste panel could not distinguish ($P > 0.05$) between ostrich patties containing either 10% pork lard or 10% of a modified starch/soy protein isolate (fat replacer) mixture. The fat replacer consisted of 70% water, 20% modified corn starch (COLFLO® 67 [National Starch Food Innovation, Bridgewater, NJ], a moderately cross-linked and moderately stabilized waxy corn starch), and 10% soy protein isolate (Profam 974, Archer Daniels Midland Company, Decatur, IL). However, the panel could distinguish ($P < 0.05$) between the types of ostrich muscle/meat cuts used, with a significant number preferring ostrich patties made from meat containing a higher collagen content (around 3% vs. <1%). The total fat content of patties containing pork fat was more than 6% higher than that of those containing the fat replacer. The authors concluded that fat replacers can be used successfully for the production of low-fat ostrich patties without any negative quality attributes being perceived.

A combination of cassava (tapioca) starch and oatmeal used as fat replacers in low-fat lamb burgers did not show any differences in overall acceptability when tasted next to a 9.5%-fat lamb burger (Seabra, Zapata, Nogueira, Dantas, & Almeida, 2002). On the other hand, in some comminuted scalded sausage formulations, fat replacement with a modified potato starch did not successfully compensate for the textural side effects of fat removal. Protein and fat content in that experiment were the major determinants of texture and water binding (Pietrasik, 1999).

These studies are very typical of fat-replacement approaches. Starch by itself, or in combination with other ingredients, can substitute the mouthfeel effect of fat and can bring back richness.

Phosphate Replacement

Functional native starches (e.g., N-HANCE® 59 potato starch, National Starch Food Innovation, Bridgewater, NJ) are able to replace the water-binding capacity and tex-

ture provided by phosphates, depending on the recipe and application. In extended poultry products like injected and/or tumbled chicken breasts, improvements in terms of cooking losses and texture can be achieved by replacing phosphate with functional native potato starch (Table 2.5). When replacing phosphate in minced meat products, comparable reductions in cooking loss can be achieved, but the texture will be slightly different, having been described as "less sticky." This can lead to benefits such as higher manufacturing throughput of formed meatballs or beef burgers. In general, starch is used at higher levels than phosphate, as there is very often a minimum usage level required for starches to reach good functionality. Tables 2.6 and 2.7 list sample recipes for phosphate-free sausage and pork meatballs.

Table 2.5 Comparison of brine systems with and without phosphate addition

Ingredients	With phosphate	Without phosphate
Water	99.69	98.99
Xanthan gum	0.01	0.01
Polyphosphate	0.30	–
Native functional potato starch[a]	–	1.00
Total	100.00	100.00

[a] N-HANCE® 59 (National Starch Food Innovation, Bridgewater, NJ).

Table 2.6 Example of a phosphate-free Vienna sausage formulation using native potato starch

Ingredients	%
Beef 8% fat	35.00
Pork 20% fat	20.00
Pork 50% fat	20.55
Ice/water	20.00
Seasoning	0.60
Curing salt (0.4–0.5% sodium nitrite)	1.80
Ascorbic acid	0.05
Native functional potato starch[a]	2.00
Total	100.00

[a] N-HANCE® 59 (National Starch Food Innovation, Bridgewater, NJ).

Table 2.7 Example of pork meatballs without phosphate addition

Ingredients	%
Minced pork	62.00
Water	24.80
Potato flakes	6.00
Vacuum salt	1.20
Native functional potato starch[a]	2.00
Onion, fresh	4.00
Total	100.00

[a] N-HANCE® 59 (National Starch Food Innovation, Bridgewater, NJ).

The trend for "clean label" foods is currently increasing in North America, and phosphate removal may help in this regard. Starch can compensate for yields and texture, but will not contribute the salty note of phosphates. This has to be addressed when developing phosphate-free formulations.

Comparison Among Starches

Modified Food Starches

There is a great variety of modified food starches available on the market. Generally, starch companies have a preselected range that they know performs well in meat applications. Those starches have selected gelatinization temperature ranges and bases so textural solutions can also be provided.

Low gelatinization temperatures are helpful in many cases. A stabilized starch for use in meat is more likely to have a gelatinization temperature close to the temperature at which the meat proteins denature and release water, so that that the starch can be used to swell and hold moisture.

Joly (2002) evaluated the effect of various modified starches on the cooking yield, purge, and firmness of industrial chicken rolls made of chicken breast injected at a 45% extension level followed by tumbling. Results are shown in Table 2.8. The finished product formula, representative of what would be made in the industry, was as follows:

She found that the best performing starch in terms of cooking yields was a waxy, highly stabilized starch (FIRMTEX®, National Starch Food Innovation, Bridgewater, NJ), which increased the cooking yield 9% compared to the control (containing no starch). The other close performer to the stabilized waxy was a stabilized and cross-linked tapioca starch (NATIONAL FRIGEX HV®, National Starch Food Innovation, Bridgewater NJ). Both modified starches have gelatinization temperatures close to the denaturation temperature of the meat proteins (approx. 60°C). Freezer purge was generally higher than refrigerated purge as ice crystal formation alters the physical integrity of the muscle and favors the loss of fluid. The sample containing the modified waxy corn starch (FIRMTEX®) presented the lowest purge after 21 and 42 days. Firmness was measured both analytically and by sensory testing. Texture measurement on the sample containing native functional potato starch (NOVATION® 1900) gave the highest peak of compression before breakage, higher than the control. The other starches yielded texture measurements similar to the control.

Ingredients	*%*
Boneless skinless breast with rib meat	68.96
Water	25.88
Sodium chloride	1.65
Corn syrup solids	1.50
Sodium phosphate[a]	0.50
Modified food starch	1.50

[a] Tri- and di-phosphate blend.

Table 2.8 Effects of various modified starches on the cooking yields and firmness of 45% extended chicken rolls

Modified starch	Average cooking yield (%)	Standard deviation (%)	Firmness (compression peak, g)
Control (no starch)	88.4	1.0	1,595
Stabilized waxy corn (FIRMTEX®)	97.5	0.7	1,530
Stabilized cross-linked tapioca (NATIONAL FRIGEX HV®)	97.0	0.7	1,536
Stabilized, lightly cross-linked dent corn (NATIONAL 740)	96.3	1.0	1,519
Native Functional Potato (NOVATION® 1900)	95.8	0.6	2,039
Native Functional Waxy Rice (NOVATION® 8300)	96.3	0.7	1,608

Modified starches are from National Starch Food Innovation, Bridgewater, NJ.

Native vs. Native Functional

By changing from a native starch to a native functional starch, existing formulations can be improved by a stronger water-binding capacity and an increase in stability. In fried or boiled sausages, shrink loss in vacuum packaging is reduced. The starch also prevents changes in texture. Meat products made with native functional starches have a shorter texture, which gives the product a better "bite." Used in pâtés, the starches increase spreadability. They can also replace skimmed milk powder in sausages and improve overall product quality in minced meat products (National Starch brings innovation to potato starches, 2000).

In highly extended (110%) formed cooked ham, 10% native potato starch has been effectively replaced by 3% native functional starch (N-HANCE® 59, National Starch Food Innovation, Bridgewater, NJ) (Table 2.9). The difference in cost between these starches would probably make a 1:1 replacement of native potato starch uneconomical. However, the greater functionality of the functional native starch permits the use of much less, while resulting in a very comparable product.

Table 2.9 Replacement of native starch with a native functional starch in a 110% extended formed ham

Ingredient	Reference	Comparison
Pork shoulder, 8% fat	47.62	47.62
Polyphosphate	0.50	0.50
Water	38.88	45.68
Curing salt (0.4–0.5% sodium nitrite)	1.80	1.80
Dextrose	1.00	1.00
Semirefined carrageenan	0.20	0.40
Native potato starch	10.00	–
Functional native starch[a]	–	3.00
Total	100.00	100.00

[a] N-HANCE® 59 (National Starch Food Innovation, Bridgewater, NJ).

To match the control, addition of an inexpensive filler is recommended to account for the decreased starch level and the increase in water content.

Combination of Starches with Other Functional Ingredients

Carrageenan

There has been some discussion as to whether there is synergy between starch and carrageenan. They are often used together in formulation, especially when the extension level is high (>40%), to optimize water binding. In any case, the industry has found it beneficial to use a combination of both, in terms of texture and water binding.

Prabhu and Sebranek (1987) did not find any synergy between kappa carrageenan and starch when used together in ham in terms of moisture retention. Microscopic examination revealed that both ingredients were randomly distributed in the meat matrix, also indicating a very low likelihood of synergy (Chap. 3).

In practicality, simple formulation and experimentation on a pilot scale can provide good direction and can help in the selection of the most functional combination. For example, tests were conducted on chicken roll at 40% and 60% extension, where the formulation and protocol were as follows:

> *Manufacturing Protocol.* Grind 10% of total chicken breast to 7 mm (1/4"). Inject whole chicken breast with brine solution. Macerate chicken breast. Tumble injected chicken breast, remaining brine and ground fill for 2 h at 8 RPM. Vacuum stuff, heat-seal, and cook. Ramp steam cook to internal temperature of 74°C (165°F). Chill product at −10°C (14°F) to an internal temperature of −2°C (28°F). Determine cook yield.

The yields and purge were dramatically improved when using a combination of starch and carrageenan, compared to starch or carrageenan alone (Tables 2.10 and 2.11), especially at 60% extension. This was not necessarily exclusive of synergy but proved the benefits of leveraging the capabilities of both ingredients, especially at high extension levels.

Ingredients	%
Chicken breast meat (10% ground to 7 mm)	[a]
Sodium chloride	1.65
Dextrose	1.50
Sodium tri-polyphosphate	0.50
Carrageenan (CE 680, Quest International)	0.50
Starch	[b]

[a]75.22% (40% extension) or 50.15% (60% extension).
[b]Modified potato starch or native functional potato starch (NOVATION® 1900, National Starch Food Innovation, Bridgewater, NJ) at 0.75%, 1.00%, or 1.50%.

Table 2.10 Cooking losses in a 40% extended chicken roll containing starch and starch/carrageenan combinations

Ingredient (%)			Cooking loss (%)	
Modified starch[a]	Functional potato[b]	Carrageenan[c]	Mean loss	Standard deviation
–	–	–	9.23	0.53
–	0.75	–	7.77	0.52
–	1.00	–	7.74	0
–	1.50	–	6.08	0.51
0.75	–	–	6.95	0
1.00	–	–	6.43	0.43
1.50	–	–	5.07	0.3
–	–	0.50	6.30	0.23
0.75	–	0.50	3.80	0.14
1.00	–	0.50	3.50	0.95
1.50	–	0.50	3.60	1.42
–	1.00	0.50	4.50	0.4

[a] FIRMTEX (National Starch Food Innovation, Bridgewater, NJ).
[b] NOVATION 1900 (National Starch Food Innovation, Bridgewater, NJ).
[c] CE 680 (Quest International, Hoffman Estates, IL).

Table 2.11 Cooking losses in a 60%-extended chicken roll containing starch, starch/carrageenan combinations, and soy protein isolate/carrageenan combination

Ingredient (%)				Cooking loss (%)	
Modified starch[a]	Functional potato[b]	Carrageenan[c]	Soy protein isolate[d]	Mean loss	Standard deviation
–	–	–		12.10	0.29
1.00	–	0.50		3.80	0.56
–	1.00	0.50		3.75	0.19
–	–	0.50		4.80	0.49
1.00	–	–		8.05	0.73
–	–	0.50	1.00	5.30	1.00

[a] FIRMTEX (National Starch Food Innovation, Bridgewater, NJ.
[b] NOVATION 1900 (national starch food innovation, Bridgewater, NJ).
[c] CE 680 (Quest International, Hoffman Estates, IL).
[d] PROFAM 929 (Archer Daniels Midland Company, Decatur, IL).

Milk Proteins

According to Wade (2005) whey proteins provide a meat-like texture, while starches also supply water-binding ability and increased yield. Functional whey protein concentrates do not need heat to build up viscosity. They bind water up front upon hydration and impart a very clean flavor that is complementary to meat. The level of addition is dependent upon specific application and standards of identity. In practice, not very many meat products can be found in Europe that contain whey proteins. This is due to price and availability. The whey protein products used for meat are very often lower in protein (less than 35%) and contain a high amount of mineral salts.

Trials have shown that in deep-fried meatballs (82.7% mixed comminuted meat, 12.9% water, 1.6% salt) the addition of different water binders leads to different results (Joly, n.d.). Frying losses could be reduced by 20% when adding skimmed milk powder, 18% with whey protein concentrate 35 (WPC 35), and 14% with native functional potato starch (NOVATION® 1900, National Starch Food Innovation, Bridgewater, NJ).

A starch–protein complex having improved gel strength and emulsion stability has been disclosed by Hermansson (1979). The starch–protein complex is formed by heating starch with an aqueous dispersion of casein or caseinate at a temperature above the gelation temperature of the starch, so the starch is swollen. The casein/caseinate protein forms a complex with gelatinized starch granules. The degree of gel strength of the complex is greater than that of the casein itself, and the emulsion stability of the protein is improved. One could easily apply this understanding to a meat system to improve fat stability and firmness of the finished product. Also, choosing a starch in this system that has a tendency to retrograde, like an amylose containing source, could increase the product's firmness.

Sodium caseinate is known to improve the quality of sausages and other emulsified meat products (Chap. 6). It binds fat and water, thereby increasing yield and reducing shrinkage, while contributing high quality protein. Although highly effective, sodium caseinate is a milk-based binder, and its pricing structure is therefore unreliable and fluctuating. In 1992, specialty starches from modified waxy maize were developed (New starches replace casein in meat and dairy products, 1992) to fulfill the resultant demand for an affordable casein substitute. The starches perform well in emulsion, injection, and tumbled cooked meats in terms of binding and holding juices. Trials show that modified waxy maize starch can result in a very succulent cooked product which avoids the free moisture development often visible in vacuum-packed meats. Greater succulence can be achieved in roast beef by tumbling the raw meats in a slurry containing modified waxy maize starch before cooking. Poultry rolls with good yields and firm texture can also be achieved by tumbling or injection with a salt brine containing modified waxy maize starch. Nowadays, the tendency is more to incorporate clean label starches with further improved functionalities.

In emulsified sausages, where sodium caseinate is most often found, it can be shown that by adding specialty starches like an OSA starch composition (N-HANCE® 6, National Starch Food Innovation, Bridgewater, NJ) comparable yields and texture can be achieved. An example recipe is shown in Table 2.12.

Soy Proteins

Rogov and Dianova (1978) compared the moisture-retaining capacity of soy protein and a filler material formed of cold-blended soy protein and starch. The filler material was found to have a higher moisture-retaining capacity, and, therefore, was determined to be the preferred material for forming meat emulsions.

Table 2.12 Recipes used to compare processing losses and texture in emulsified Vienna sausages

Ingredients	Reference	Test recipes		
Beef trimmings, 8% fat	20.0	20.0	20.0	20.0
Pork cuttings, 11% fat	25.0	25.0	25.0	25.0
Pork fat, 90% fat	20.0	20.0	20.0	20.0
Curing salt (0.4–0.5% sodium nitrite)	1.8	1.8	1.8	1.8
Dextrose	2.0	2.0	2.0	2.0
Spices	0.4	0.4	0.4	0.4
Onion	1.0	1.0	1.0	1.0
Ascorbic acid	0.1	0.1	0.1	0.1
Native potato starch	–	4.0	4.0	4.0
Soy protein isolate	–	2.0	–	–
Sodium caseinate	–	–	2.0	–
OSA starch composition[a]	–	–	–	2.0
Water	29.7	23.7	23.7	23.7
Total	100.0	100.0	100.0	100.0

[a]N-HANCE® 6 (National Starch Food Innovation, Bridgewater, NJ).

Good results can be achieved when comparing soy protein isolates with products like OSA starch composition (see Table 2.12) in emulsified products like bologna-type sausages. The OSA starch composition (N-HANCE® 6, National Starch Food Innovation, Bridgewater, NJ) emulsifies and binds water at the same time. As mentioned above, emulsifying starches alone offer lower water-binding capacity and are therefore often added in addition to water binders. No differences were observed in terms of texture, processing losses, and shelf-life stability (Anderstein, 2003).

When comparing starches with soy protein isolate in cooked ham systems, studies have shown that at low water addition levels soy protein isolate results in better yield and texture. But it was seen as well (Brammerloh, unpublished data) that, at high extension levels, the possibility of meat-protein to non-meat-protein interactions is lower and therefore starch, and especially starch/carrageenan combinations, can achieve comparable yield and texture.

Egg Proteins

Carballo et al. (1996) looked at the effects of fat, starch, and egg white on the microstructure and texture of bologna sausages. A relationship was found between increased levels of starch and fat and an increase in the number of pores in the sausage, which produced a more “aerated” product. In addition, the use of egg white and starch increased the chewiness and firmness (hardness) score in low-fat bologna. Colmenero, Barreto, Fernández, and Carballo (1996) found that high-fat (20%) sausages exhibited better water-binding properties than low-fat (7.2%) sausages after freezing. In general, the addition of starch in those products increased freeze–thaw stability and penetration force, decreasing elasticity. Egg white did not change the water-binding ability of bologna, but affected texture.

Egg white has perfect gelling characteristics when it comes to setting a firm texture. When selected properly, starch performs in a similar way. Modified amylose-containing starch (which retrogrades and sets back) and egg white could be combined. The modification that could be chosen should favor gel formation, such as a thin boiling starch where the amylose is free to retrograde (see Table 2.3).

Troubleshooting in the Plant

PSE Meat

Pale, soft, exudative (PSE) pork meat is a major problem that the industry faces in terms of quality and yields compared to normal meat. If PSE meat is extended, the additional water cannot be held like it would in normal meat when it is cooked, thus reducing quality and shelf life. Starch has been explored as a remedy to reduce loss and re-establish texture in PSE meat. Zhang and Barbut (2005) looked at the effect of native and modified starches on PSE meat compared to normal meat. They noticed that the starch could compensate for some of the loss of functionality of the meat protein, with higher cooking yield and better firmness.

Starch Pockets/Tiger Stripes

Starch pockets, or "tiger stripes," can occur when too much starch is used in injected meat products and the starch is not evenly distributed within the meat structure. This can be seen especially when injecting meat with the skin intact. The starch lies in one special area and swells upon cooking. The cooked starch slurry becomes visible and may look unappetizing. If this happens, two solutions are possible. One can either change the process by adding a tumbling step after injecting, or reduce the starch dosage. Where a starch dosage of above 1% is usually recommended, in these products good results can very often be reached with levels of 0.3–0.5%. Sometimes, decreasing speed and pumping pressure can help reduce the defect, as evidenced by tests on an 80% extended ham (0.3% polyphosphate, 2% nitrite salt, 2% and 4% starch) (Joly, n.d.).

Soft Texture

Too soft a texture can occur when replacing higher amounts of low-functionality ingredients like native starches with lower dosages of more functional ingredients, like N-HANCE 59. This softness can occur because of differences in dry matter content. It is recommended that dry matter be adjusted to a higher level, even if it is with inexpen-

sive non-water-binding fillers. In an 80%-extended formed ham (2% salt, 0.5% semirefined carrageenan, 0.5% polyphosphate) the difference in dry ingredients when reducing starch from 7% to 2% was made up with 5% maltodextrin.

Another reason for a soft texture can be an overly high dosage of the starch. Contrary to liquid applications like soups or sauces, trials have shown that the texture of meat products at either too-low or too-high starch dosages tends to be soft.

Sedimentation of Cook-Up Starch

Since cook-up starches are not soluble, they have a tendency to sediment. When a starch-containing brine is made, it must be kept constantly agitated. When using the brine for injecting, the entire processing line should be checked to see if there is a tank or pretank, or any other piece of equipment where settling could occur. That equipment should be monitored and constant agitation is recommended. Another way to avoid sedimentation is through the use of cold-water swelling starches or gums like xanthan. By increasing the viscosity of the brine, these ingredients can prevent, or at least slow down, the sedimentation of the starch. A typical ratio of cold swelling and cook-up starch is 1:1.

When used at the right levels, the addition of these starches to increase viscosity creates no problems with injector needles. In addition, they offer the advantage of decreasing drip before cooking.

Starch Distribution and Cook Status

When the proper distribution of starch in the meat is in doubt, a simple iodine staining technique can be used. By spraying a slice of turkey ham, for example, with a dilute solution of potassium iodide (KI) and iodine, one can detect where the starch is localized. Amylose-containing starch will stain blue and amylopectin-containing starch (>99% amylopectin) will stain pink. The density of the staining can be indicative of the relative amount of starch present in each area of the meat. This can help determine if the starch is as well distributed as desired.

This staining technique can be also used by taking a piece of meat, grinding it, and mixing it with water and the KI/I solution. A drop of the liquid phase of the mixture on a slide can be observed under a microscope, and the degree of starch cook (undercook, good cook or overcook) can be determined by the size of the starch granule, the integrity of the granule (a fragmented granule is generally overcooked), and the intensity of the iodine staining (the lighter the more cooked). This allows for the optimization of starch selection for better water-binding performance, generally by obtaining a good cook.

Conclusion

As discussed in this chapter, starch is widely and successfully used in meats, either by itself or in combination with other nonmeat ingredients, to achieve desired yields and specific product attributes. It is also commonly used to replace fat and expensive, as well as allergenic, protein.

In terms of adding value to meat products, starch is often utilized "around" the meat as a major ingredient for coating and enrobing, as in traditional fried tempura or glazes (Chap. 12). These applications call for specialty starches, ranging from dextrins (film-forming and crisping agent) to high-amylose starches (film formers and crunching agents), which change the organoleptic attributes and eating quality of the meat, thus offering a vast range of opportunities into age-specific, convenience or ethnic markets.

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Chapter 3
Nonstarch Hydrocolloids

James W. Lamkey

Introduction

Meat formulas differ over time and across regions, the most predominant reason being economic conditions. Processors strive to provide products that are affordable by the majority of the population, replacing meat proteins with less expensive nonmeat ingredients. Raw material availability can also be a reason for varying formulations. Animal fat, for example, is less available in some countries, requiring formulators to make adjustments for the lack of fat. Fat replacement in other countries is based on consumer trends for taste and texture of traditional products, but with a lower fat content. Food safety concerns have also caused processors to reformulate products to withstand treatments that promote a safer food product. Postpackaging pasteurization can cause a high degree of moisture loss, requiring the addition of an ingredient that aids in the retention of moisture. Organic acids are being used as an added hurdle for food safety. When adding the sodium salts of these acids, sodium levels approach the upper limit of nutritional labels. Substitution with potassium salts to adjust for this increase in sodium may have effects on other ingredients in the formula, requiring an additional substitution to recover the taste and texture desired. As the industry grows and markets reach beyond domestic borders, formulations will need to change to meet the requirements of their targeted regions.

Ingredients added to meat can enhance or supplement meat protein functionality. Proteins are the primary component in meat that aid in the retention of moisture. However, in the area of processed meat, proteins may be required to retain more moisture than they are intrinsically capable of retaining. Optimizing the proper use of basic ingredients, such as salt and phosphate (Chap. 1), as well as processing conditions, enhances the proteins' ability to retain moisture. In many cases, however, this is not enough to meet the desires of the processor and, ultimately, the consumer. Therefore, ingredients that aid in the retention of excess moisture are needed, with the selection based on the cost of the ingredients as much as on their overall contribution to the product.

When selecting ingredients for a particular function, consideration must be given to how these ingredients perform in the presence of other components, as competition for moisture can occur. One ingredient having a higher affinity for water may not allow for the proper hydration of other components and a noticeable loss in yield will

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ensue. When given the opportunity, some ingredients will take on more water than they can comfortably manage, releasing it during heat processing or storage. Optimal use of ingredients will give the best overall functionality at the best cost.

A greater understanding of ingredients can be obtained if the hydrodynamic properties are known (Lee, 2002). Starches (Chap. 2) and some gums are highly active when added to meat products, as evidenced by changes observed during thermal processing. Soy protein isolate (Chap. 5), being moderately active, aggregates during the initial stages of processing and is set during the thermal process. This matrix can then assist in the retention of moisture and contribute to texture. Oils add to the overall palatability of food products, but do not change structure during heating and are, therefore, considered inactive.

Although meat proteins are very functional, there are times when meat products can benefit from the addition of other ingredients. These additional ingredients do not enhance protein functionality but work with the proteins to help retain moisture and modify texture. This chapter will focus on the most common hydrocolloids used in the meat industry and how they function.

Carrageenan

Origin and Manufacture

Carrageenan is a high molecular weight linear hydrophilic polysaccharide comprising repeating galactose units and 3,6-anhydrogalactose (3,6 AG) units, both sulfated and nonsulfated, joined by alternating α-(1,3) and β-(1,4) glycosidic linkages.

The main carrageenan types, kappa, iota, and lambda, can be prepared in pure form by selective extraction techniques from specific seaweeds such as *Euchema* species, *Chondrus crispus*, and *Gigartina* species (Fig. 3.1). Mu and nu carrageenans are the precursor structures for carrageenan without a 3,6-anhydro ring. These precursors can be converted by enzyme action or chemical treatment into the 3,6-anhydro forms of kappa- and iota-type carrageenans (Fig. 3.2).

Very strong and brittle gels characterize kappa carrageenans. Iota, on the other hand, is less strong and more elastic. Lambda carrageenans do not gel and are used primarily for suspension of particulates in sauces and dressings. Kappa and iota are the carrageenans showing the most benefit in meat applications, performing as water managers and texture modifiers. Lambda may show benefit for retention of moisture, but is detrimental to texture.

Commercial carrageenan used in the food industry comes in many forms. Extracted or refined carrageenan has been used extensively in all aspects of food manufacture. After the seaweeds are identified and chosen to make a particular extract, they are washed to remove sand and stones before applying appropriate amounts of various alkalis to swell the seaweed and extract the carrageenan. After extraction and structural modification which completes or advances the naturally occurring precursor transformation, the dilute carrageenan extracts are filtered and

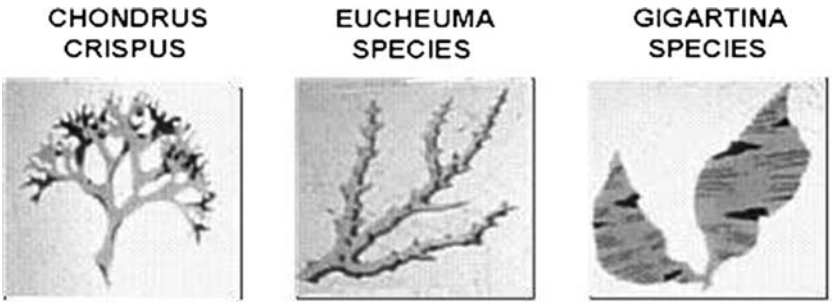

Fig. 3.1 Typical seaweeds used for the extraction of carrageenan (Courtesy of FMC Corporation, Philadelphia, PA)

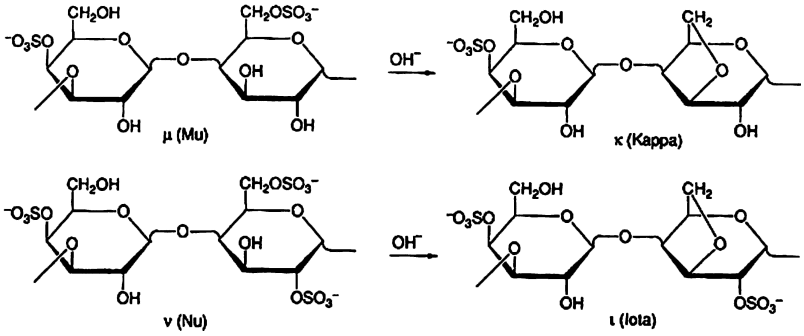

Fig. 3.2 Structures of mu and nu with the corresponding carrageenan after the formation of 3,6-AG unit (courtesy of FMC Corporation, Philadelphia, PA, USA).

clarified by high-speed centrifugation and concentrated by a variety of methods. The concentrated solutions are then precipitated with isopropyl alcohol to give a fibrous mass that is squeezed to remove the alcoholic liquor, dried, and finally ground to the appropriate particle size.

An alternative carrageenan recovery process is specific for kappa-type carrageenan and utilizes potassium chloride (KCl) to promote the gelling of kappa carrageenan. As the kappa carrageenan solution is extruded into a concentrated solution of KCl, a fibrous gel mass is formed. The precipitated gel mass exhibits syneresis (exudation of free water as molecular structures aggregate and tighten) and is further dewatered under pressure to make "gel press" carrageenan. The precipitated carrageenan may be frozen and thawed to assist this dewatering step, as this action increases the tightening of molecular structure and the resulting syneresis. The pressed fibers are then dried and ground to the appropriate particle size (Fig. 3.3)

In addition to extract carrageenan, processed *Euchema* seaweed (PES) is also available to food processors. Processed *Euchema* seaweed (PES) is the regulatory name for this grade of carrageenan, but it is known under a variety of synonyms:

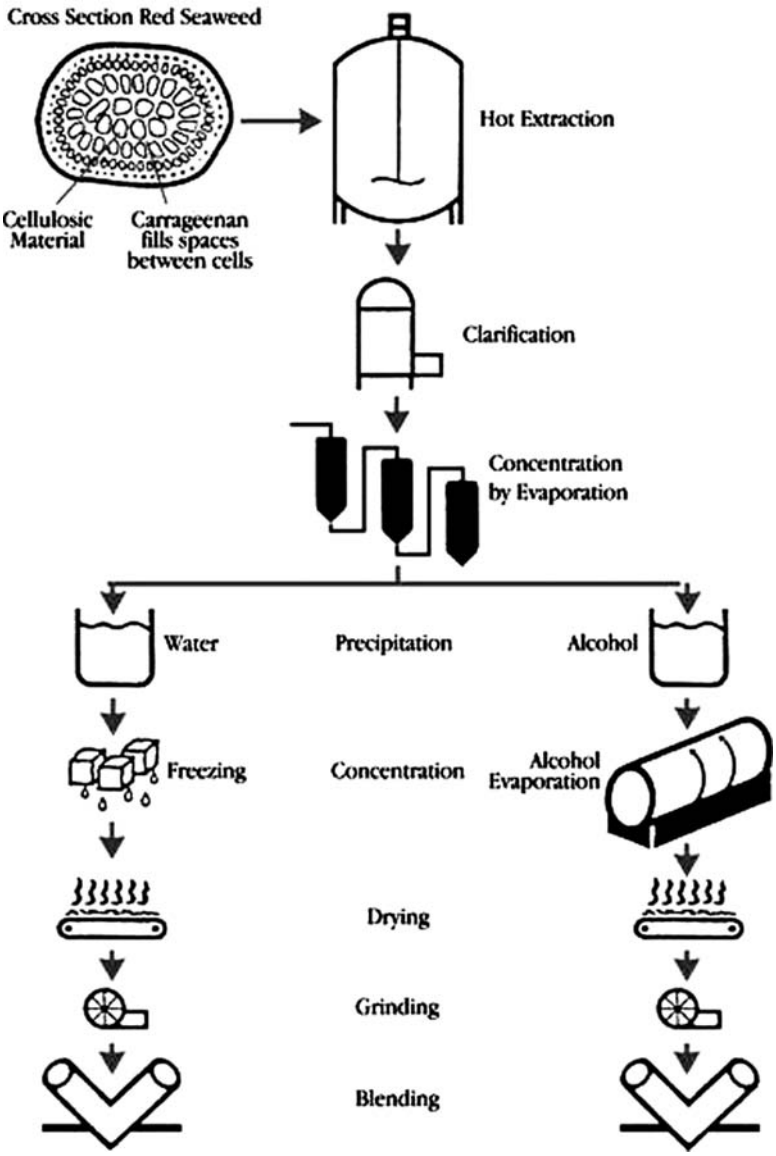

Fig. 3.3 Manufacturing steps for the extraction of carrageenan from seaweed (courtesy of FMC Corporation, Philadelphia, PA, USA).

Philippines natural grade (PNG), semirefined carrageenan (SRC), and alternatively refined carrageenan (ARC). Another synonym historically used for this type of carrageenan, alkali-modified flour (AMF), is now reserved for a grade of carrageenan used in retorted pet foods due to its dark color, strong sea odor, and high microbial levels.

PES differs from traditional extract carrageenan in that it contains 8–15% acid-insoluble matter (AIM), compared to 2% maximum for an extract. The AIM mainly comprises the structural network of plant cellulosic and proteinaceous materials, which maintain their integrity during the PES process. Unless processed correctly, applications using PES must have enough inherent energy in the process to break down the AIM structure and release the carrageenan. This means that dispersion, hydration, and solubility profiles are different for extract and PES. In meat applications, swelling of carrageenan is the primary characteristic for functionality. In many applications, PES has proven to be as functional as extract. For applications requiring full clarity, however, extract carrageenans need to be used.

Functional Characteristics and Properties

The primary carrageenan characteristics of which a meat processor should be aware are type, composition, and concentration. The composition of carrageenan used in meat processing can vary widely. Of the components frequently found in a carrageenan blend, the most common are KCl, sodium chloride, sugars, and various other hydrocolloids that include locust bean gum (LBG), guar gum, and xanthan gum. Other hydrocolloids are added to promote unique characteristics based on known synergies with carrageenan. When included in meat products, however, these synergies may not be realized. The high level of ions and the insulating effect of the proteins subdue most interactions that may be possible. Xanthan is often added to a carrageenan blend to promote suspension of carrageenan and other macromolecules in a brine for extended periods. Guar is often added to carrageenan blends for the purpose of increasing viscosity of meat batters. LBG has shown benefits for aiding in the retention of moisture in injected products. However, if the product is tumbled after injection or heat-processed directly after injection, the benefits of LBG are not as visible. In all cases, kappa carrageenan alone will normally give the highest cook yield and firmest texture.

The type of carrageenan selected is dependent on the desired finished product characteristics. Kappa carrageenan is the "workhorse" of the meat industry. They improve cook yield, reduce purge (syneresis), and in some cases provide a firmer texture. Iota carrageenans will increase cook yield, reduce purge, and provide a more elastic texture. Under processing conditions where the product is cooked in an impermeable container, kappa carrageenans will generally have a higher cook yield and firmer texture when compared to iota or lambda carrageenans. However, under conditions where the product is open to the cooking environment, the differences in cook yield between types of carrageenan are lower. In fact, Xiong, Noel, and Moody (1999) found lambda carrageenan to have the highest yield when compared to iota and kappa in beef sausages. Mills (1995), Prabhu and Sebranek (1997), and Bater, Descamps, and Maurer (1992b) found that differences between kappa carrageenan and starch were smaller in smoked hams than in hams cooked in impermeable casings. Usually, cook yield and textures will depend on the level of moisture added to the meat product.

KCl is the most common ingredient in carrageenan because potassium is required for the gelling of kappa carrageenans. As indicated earlier, KCl is used for the precipitation of carrageenan in gel press manufacturing processes and becomes a component of the carrageenan particle. In some cases, however, carrageenan will be blended with additional KCl for improving water gel firmness. Cations enhance gelation of carrageenans through the stabilization of the helical conformation of carrageenans by shielding the charge of the sulfate groups, followed by intermolecular (ion–dipole) coordination binding with the polysaccharide into aggregates (Braudo, 1992; Morris & Chilvers, 1983). Kappa carrageenan will generally show an increase in gel strength with up to 20% KCl added, but that can vary between carrageenans. This improvement in gel strength, however, does not transfer to the meat product. In fact, the addition of KCl may interfere with the function of carrageenan, as reported by Trius, Sebranek, Rust, and Carr (1994a). The presence of potassium causes the temperature at which carrageenan solubilizes to increase, which in turn restricts the swelling of carrageenan and reduces its ability to manage water. Unpublished data have revealed that as little as 0.16% KCl will induce a measurable reduction in cook yield in products cooked in an impermeable casing (Table 3.1). When 1% KCl is added to the formulation, cook yields can decrease by as much as 11%. This effect of potassium will have a dramatic impact on formula changes to lower sodium in products. Kappa carrageenans are affected more than other types of carrageenan, with extracts being more sensitive to potassium than semirefined carrageenans. The potassium has a direct effect on the carrageenan and there are no known processing changes that would overcome this.

Discussions about carrageenan always include gelling characteristics, allowing for the ability to differentiate between carrageenan types. For the most part, gelling characteristics are very important, especially from a quality assurance standpoint. However, comparison between carrageenans of the same type should stop short of discussing gel strength as an indicator of functionality in meat applications. Kappa carrageenans will give firmer texture and better sliceability when compared to iota carrageenans. Within carrageenan type, however, there is very little evidence to suggest gel strength has a significant influence on the overall texture of meat. Within a meat system, evidence suggests that carrageenan does not go completely

Table 3.1 Addition of KCl to turkey breast and effect on cooking loss

Treatment	Added KCl (% of F.P.)[a]	Cookout (%)	Comments
1	0.00	0.2	Excellent protein adhesion to cooking film
2	0.16	0.7	Acceptable. Minimal protein adhesion
3	0.32	2.3	Unacceptable. Gelled cookout on meat surface
4	0.64	8.5	Unacceptable. Excessive soft gel cooked out
5	1.28	11.6	Watery cookout. Carrageenan did not swell
6	2.55	10.6	Same as treatment #5

From Lamkey (2006).
[a]F.P.: Finished product

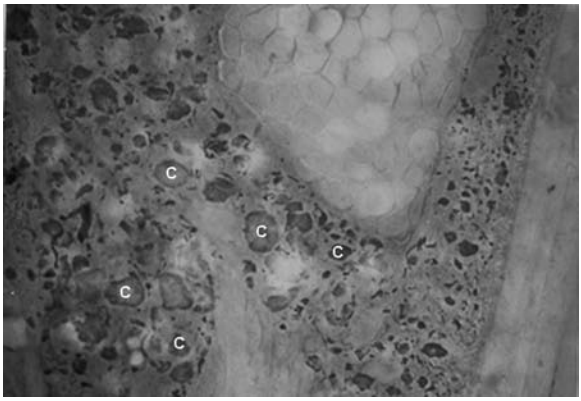

Fig. 3.4 Microscopic view of carrageenan within a muscle food system – turkey breast muscle with brine added equivalent to 50% of green weight (40× magnification). C: swollen carrageenan particles (from Lamkey, 2006).

into solution, a condition necessary for the development of a gel structure (Fig. 3.4). In a study designed to investigate the gelling mechanism of a simulated protein solution similar to the aqueous environment of turkey breasts, Bater, Descamps, and Maurer (1992a) observed that the temperatures at which kappa carrageenan began to swell increased with increasing salt level. The authors found that at 0% salt, kappa carrageenan began swelling at 28°C, while at 4.4% salt swelling did not begin until 67°C. Proteins began gelling at 52°C. Bater et al. (1992a) concluded that an increase in salt concentration could cause an increase in swelling temperature and a decrease in kappa carrageenan swelling in meat products. This suggests that in the presence of salt, carrageenan will not swell until after the proteins have begun gelling, isolating the carrageenan particles within the protein gel matrix. Prabhu and Sebranek (1997) observed that carrageenan particles were dispersed throughout a ham product without evidence of gel formation. The authors hypothesized that protein gels before carrageenan become fully soluble and therefore the carrageenan gets trapped within the protein gel structure. These same researchers also observed that starch and carrageenan were found in localized regions separated from each other. From these data it is highly unlikely that the synergism known to exist in water gels is manifested within the meat product, due to the isolating effect of the protein gel (Chap. 2). Pérez-Mateos, Hurtado, Montero, and Fernández-Martín (2001) looked at the interactions of kappa carrageenan with various other hydrocolloids in fish myosystem gels. The value of using fish myosystem is that endpoint temperature is usually higher than the solubility temperature of most hydrocolloids. No synergistic effects were found as evidenced by measured functional properties, except for a slight synergistic effect between kappa carrageenan and LBG, a galactomannan. The use of guar gum, another galactomannan, yielded lower hardness and cohesiveness scores. These differences are likely due to differences in galactose:mannose ratio, which is much higher for guar gum. Lee and Kim (1985)

postulated that the lack of synergies is due to a strong competition for moisture. Bernal, Smajda, Smith, and Stanly (1987) concluded that although carrageenan may help to improve moisture retention in meat emulsions, it does so by holding water in the interstitial spaces of the gel network rather than by true interactions with the proteins in the formation of the network. Based on the evidence that carrageenan does not form a continuous gel network within the meat product, it supports the contention that variances in gel strength with carrageenan types are not a direct indicator of functionality in meat applications.

Carrageenan improves water retention, consistency, sliceability, and texture of poultry products with high levels of added brine (Bater et al., 1992b; Trudso, 1985). In regions of the world where 8–12% starch is added to produce economical products, carrageenan is added at quantities of 0.5–0.8% to improve syneresis control and sliceability. Globally, a primary use for carrageenan is cured hams. Mills (1995) showed that 1.5% kappa carrageenan in a 38% added ingredient (AI) cured pork ham exhibited the highest cook yield for cook-in-bag products. The ingredient that most closely approximated carrageenan in this respect was 2% sodium caseinate, which exhibited a cook yield 8.2% below that of carrageenan (96.4% for carrageenan vs. 88.2% for sodium caseinate). The USDA does allow up to 1.5% carrageenan in cured pork products, but it should be noted that this level of use is often too high for the level of added ingredients used in this study. Adding too much carrageenan can result in detrimental effects, such as a gel-like texture or, in the case of injected products, visible gel lines within the muscle.

Usage level of carrageenan will vary depending on the quality of the carrageenan, the amount of carrageenan in the blend and the extension level of the meat product. For all carrageenans, the characteristic measured will increase for each unit increase in carrageenan up to an optimum use level. Beyond that optimum level, adding more carrageenan will not give better results (Fig. 3.5). The optimum

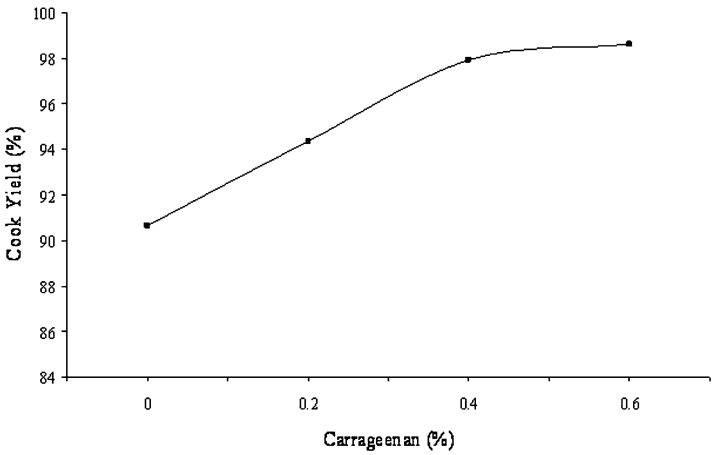

Fig. 3.5 Carrageenan concentration and effect on cook yield in turkey breast in impervious casings with 50% injected brine (from Lamkey, 2006).

review paper that suggests a relationship between breast cancer and consumption of carrageenan, the European Commission's Scientific Committee on Food (SCF) issued an opinion in which it concluded that "there is no evidence of any adverse effects in humans from exposure to food grade carrageenan, or that exposure to degraded carrageenan from the use of food-grade carrageenan is occurring" (Scientific Committee on Food [SCF], 2003). The opinion goes on to state that "[t]he Committee does however consider that, if feasible, a molecular weight limit of not >5% below 50 kDa should be introduced into the specification, in order to ensure that the presence of any degraded carrageenan is kept to a minimum" (SCF, 2003).

Presently, there is no validated analytical method available to accurately measure the low molecular weight tail (LMT) of carrageenan, thereby rendering the European Commission's molecular weight specification very difficult to enforce in a consistent manner (Marinalg International, 2007). As a general matter, updates on the work done thus far to establish a validated analytical method for the quantifying the LMT can be found in the web site of the World Association of Seaweed Processors, *Marinalg International* (http://www.marinalg.org/).

Alginate

Increasing the value of under-utilized muscles of food animals has been the target of many published reports involving alginate. Many of these muscles, usually originating from the hind leg or shoulder area, are under utilized due to lower palatability. Connective tissue removal, tenderization, and comminution improve the raw material. Restructuring allows for the recombination of the meat into a shape that is easily recognized and handled. Early reports on restructuring meat products promote the use of salt and/or phosphate to aid in the extraction of proteins which hold the meat particles together once the product is heat processed. However, in the raw state, a product made in this way must be frozen in order to retain its integrity. Marketing an unfrozen or uncooked restructured product processed using only salt and phosphate is virtually impossible. Storage stability and visual appearance of these products are also a concern, since products containing salt have a tendency to oxidize at a faster rate.

To overcome the need to freeze or cook restructured products, a binder was sought that becomes active without heat and holds the pieces of meat together in both the raw and cooked state. Sodium alginate, a common ingredient in the food industry, is a hydrocolloid that gels in the presence of multivalent ions and forms a heat-stable gel. Utilizing this process has the added advantage of not requiring the inclusion of salt or phosphate for protein extraction and, because of the heat-stable gel formed, the integrity of the structured product withstands normal cooking temperatures for meat products (Clarke, Sofos, & Schmidt, 1988; Means, Clarke, Sofos, & Schmidt, 1987; Means & Schmidt, 1986).

addition of carrageenan, with higher moisture reducing the firmness of the product. At moisture:protein (M:P) ratios below 5.5, the addition of carrageenan will actually reduce firmness due to an increase in moisture retention. At M:P ratios above 5.5, firmness is slightly increased with the addition of carrageenan.

Specific Applications

Turkey Breast

In the United States, turkey breast is the most common application for carrageenan. Products made with turkey breast can be "whole muscle" or ground and formed. The most common mistake made by turkey processors is using too much carrageenan for the application. This not only increases costs but can also result in a quality defect referred to as "stretch marks" or "tiger striping." This is a condition where the gel is visible between the muscle fibers.

Ham

On a global basis, carrageenan is most often used in ham applications, although poultry applications are on the rise. In the United States, binders are approved for use in any cured pork product that is labeled "Natural Juices," "Water Added," and "X% Added Ingredients" (Code of Federal Regulations [CFR], 2007a).

It is common among cured pork processors, as it is with turkey processors, to use too much carrageenan. This gives the final product a glassy appearance and promotes the appearance of "stretch marks" or "tiger stripes." This is more of a problem with ham processors, as more fibrous muscle tends to increase the visibility of carrageenan between the fibers.

Roast Beef

Using carrageenan in beef, and particularly roast beef, is a relatively new application that is less frequently seen in the US market due to regulatory requirements. If used as an ingredient in roast beef, or any product with a standard of identity, a declaration on the name panel is required.

Aside from regulatory issues, using carrageenan in roast beef applications has proven useful. Unpublished data (Lamkey, 2006) suggest that a slight increase in cook yield can be obtained with the addition of carrageenan to semimembranosus muscle. In firmer muscles, such as the biceps femoris, however, cook yields will not be as high. Carrageenan can increase the retention of moisture during extended heating times or when the product is held at high temperatures for long periods of time, such as in foodservice applications.

Seafood

A common use for carrageenan is in imitation seafood products, such as crab leg and shrimp analogs. These products use surimi as the raw material. Surimi is the name given to purified protein obtained from fish, most commonly pollack. Carrageenan is added to surimi applications to improve firmness, elasticity and reduce costs. Kappa carrageenans are preferred when firm textures are desired. Iota carrageenans result in more elastic textures.

In a study by Llanto, Bullens, and Modliszewski (1989), penetration and compression forces were highest with iota carrageenan, followed, in order, by lambda and kappa. Syneresis after three freeze/thaw cycles was lowest with iota carrageenan. Iota carrageenan appears to be a viable carrageenan for surimi and surimi-based analogs when elasticity and freeze/thaw stability are top priorities.

Regulatory Status and Toxicological Safety

In the United States, no regulatory distinction is made between carrageenan and PES. Both are described as "carrageenan" and have Generally Recognized As Safe (GRAS) status in accordance with Food and Drug Administration regulations (CFR, 2006). The USDA restricts the use of carrageenan to 1.5% in the finished product (CFR, 2007b), but in the United States usage levels are generally less than 1.0%. In the European Union, carrageenan (E407) and PES (E407a) are listed as "generally permitted food additives" in Annex 1 of the European Parliament and Council Directive 95/2/EC (European Parliament and Council, 2006) for use "quantum satis," i.e., the level required to achieve a given technological benefit.

Carrageenan for food is defined as having a molecular weight (MW) greater than 100 kilodaltons (kDa), this being successfully monitored for decades by a viscosity specification of not less than 5 cP for a 1.5% solution at 75°C. Poligeenan (MW 20 kDa), a chemically degraded derivative of carrageenan, is prepared by deliberate acid hydrolysis, is used only clinically, for example, in barium sulfate radiological contrast solutions and stomach ulcer therapy, and is completely nonfunctional in food applications (W. R. Blakemore, personal communication, 2007).

Poligeenan at high dosage levels has shown some specific adverse colonic effects in specific animals, in particular guinea pigs, and when administered in drinking water rather than in food. Carrageenan above 100 kDa (i.e., viscosity > 5 cP) has shown no adverse toxicological effects under these same conditions. Unfortunately, many researchers over the years have missed the clear distinction between "carrageenan" and "poligeenan," and have incorrectly associated carrageenan with the adverse toxicological effects of poligeenan, resulting in unfounded positions and false criticisms of carrageenan's safety record in food use (W. R. Blakemore, personal communication, 2007). After reviewing a recent

review paper that suggests a relationship between breast cancer and consumption of carrageenan, the European Commission's Scientific Committee on Food (SCF) issued an opinion in which it concluded that "there is no evidence of any adverse effects in humans from exposure to food grade carrageenan, or that exposure to degraded carrageenan from the use of food-grade carrageenan is occurring" (Scientific Committee on Food [SCF], 2003). The opinion goes on to state that "[t]he Committee does however consider that, if feasible, a molecular weight limit of not >5% below 50 kDa should be introduced into the specification, in order to ensure that the presence of any degraded carrageenan is kept to a minimum" (SCF, 2003).

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Alginate

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Description and Origin

Alginic acid is a copolymer of two uronic acids, mannuronic and guluronic, is extracted from brown seaweed, and has the unique property of forming a gel in the presence of a divalent cation (Fig. 3.6). When producing alginates, the uronic acids are converted into their salt forms, mannuronate (M) and guluronate (G) through a neutralization step. In the natural state, M and G units can be linked together in one of three blocks: MM, GG, or MG (GM). The proportion, distribution, and length of these blocks determine the chemical and physical properties of the molecules. The reactivity with calcium and the consequent gelling capacity is a direct function of the average length of the G blocks. Alginates containing high levels of GG fractions possess the ability to form strong gels. These fractions are more abundant in the stems of the seaweed, while the leaves contain alginic acids with higher proportions of M fractions.

Functional Characteristics and Properties

Alginates are soluble in cold water and therefore do not require heating to form gels. These gels develop upon exposure to multivalent cations. Because of its commonality and safety, calcium is usually the cation of choice.

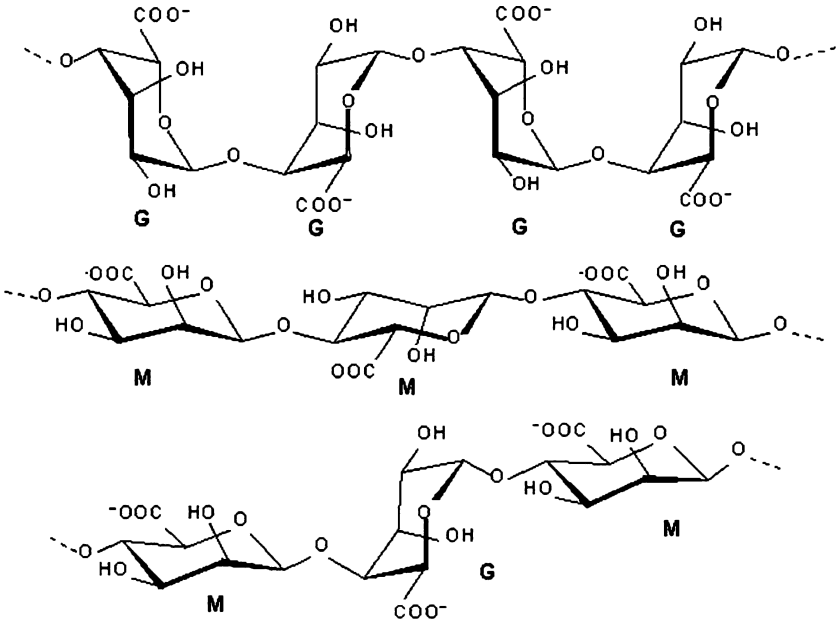

Fig. 3.6 Basic structures of G-block and M-block components of alginic acid (courtesy of FMC Corporation, Philadelphia, PA, USA).

The solubility of the calcium source is one of the criteria used to control the rate of gel formation. A calcium salt solution will cause alginate in solution to gel on contact. Continuous exposure of the alginate to the calcium solution will increase the firmness of the gel, as more calcium diffuses into the gel and binds to the G blocks within the alginate structure. This process, used for encapsulating liquids, can be used for forming a gel layer, or skin, on the surface of meat products. The addition of alginate to a meat blend followed by bathing or showering in a calcium chloride solution results in the formation of skin on the surface. Extended exposure time to the calcium chloride solution will increase the thickness of the skin and, if long enough, will eventually create a gelled structure throughout the product due to calcium diffusivity. Time of complete gelling will be dependent on the thickness of the structure. This process of forming an alginate gel is referred to as external gelling.

For internal gelling, sodium alginate and the calcium source are added directly to the meat mixture and allowed to form a gel over time. The success of this process is based on ensuring the complete hydration of alginate and the proper timing of calcium release, which can be controlled by the solubility of the calcium source or by the addition of a sequestrant. A gel formed too quickly has the potential to break apart during the mixing or stuffing process. Because calcium alginate gels do not re-heal, if the gel is broken during the structuring process, it could appear that it did not form in the first place. Calcium chloride would not be the calcium source of choice for this type of application. More commonly, a less soluble calcium source is used.

The process for making structured meat using alginate gels was patented by researchers at Colorado State University (Schmidt & Means, 1986). The patent describes a method to form structured meat products using sodium alginate, calcium carbonate, and glucono-delta-lactone (GdL). Sodium alginate, once added to the meat block, is hydrated by the moisture in the meat. Calcium carbonate has low solubility and gives the processor time to stuff the meat mixture into casings. GdL decreases pH over time, which causes the solubility of calcium carbonate to increase, thus releasing calcium and resulting in the formation of an alginate gel able to entrap the meat particles. Once the gel has set, the product is sliced into steaks or chops. Since the calcium alginate gel is heat stable, the product will not fall apart during cooking.

To assure optimum gelling properties, it is essential that the alginate be fully hydrated and evenly distributed in the mixture. Adding alginate directly to the meat source allows the alginate to hydrate by drawing moisture from the meat. To speed up gel formation, sodium alginate should be hydrated with water prior to addition to the meat mixture.

The action of a sequestrant is to chelate calcium, with the objective being to slow down the gelation mechanism of sodium alginate. There is evidence that slowing gelation can also increase gel strength, most likely due to a more ordered formation of the gel structure. Conditions that retard intermolecular interactions will result in a more homogeneous and regular network and consequently a stronger gel (Bernal et al., 1987). The sequestrant most often used is phosphate. The effectiveness of the

sequestrant is influenced by solubility. For example, at equal phosphate levels, sodium tripolyphosphate is more effective than tetrasodium pyrophosphate at retarding gel formation.

The texture of structured meat is a primary focus among meat processors. Structured meat should be superior to ground meat, without having a gel-like texture. The mechanical properties of structured meat are influenced by size and shape of the raw material (Berry & Civille, 1986) as well as sodium alginate/calcium ratio (Trout, Chen, & Dale, 1990). Devatkal and Mendiratta (2001) found the mechanical properties of structured pork rolls were superior for salt-phosphate rolls in the cooked state but were better for alginate/lactate gels in the raw state.

Raharjo et al. (1995) found bind force to be higher for calcium alginate-structured meat made with sliced meat and added water than for a no additive control. As Schaake, Means, Moody, Boyle, and Aaron (1993) found, calcium alginate steaks had significantly lower bind force compared to salt and phosphate restructured steaks. Interestingly, however, sensory panels rated calcium alginate steaks equivalent to those made with salt and phosphate, the only exception being sliced meat with added water (Raharjo et al., 1995) being lower in sensory ratings.

Tensile strength of structured products is a function of both alginate and calcium. In a study by Trout (1989), cooked tensile strength of restructured pork chops was measured on factorial arrangements of calcium and alginate concentrations. Sodium alginate alone decreased tensile strength, as it interferes with protein interactions. A slight increase in tensile strength was observed when 0.13% calcium was added to 0.7% alginate. There was no increase in tensile strength with further increases in alginate. However, an increase in calcium at both levels of alginate tested (0.7% and 1.4%) resulted in an increase in tensile strength.

Means and Schmidt (1986) indicated the ideal alginate:calcium ratio was 2.5 g alginate:0.18 g calcium ion. Ensor, Ernst, Sofos, and Schmidt (1986) made acceptable structured turkey meat using 0.4–1.0% sodium alginate, 0.075–0.1875% calcium carbonate, and 0.6% lactate. Later, Ensor, Sofos, and Schmidt (1990) found 0.4% sodium alginate, 0.075% calcium carbonate, and 0.6% lactate were optimal for use in structured meat products. Basic differences in optimal use level will result with different raw materials. As the divalent ion concentration increases, there is an increase in the strength of the alginate gels, resulting from multiple molecular interactions. Along with the increase in gel strength, however, increases in calcium level can also result in an increase in syneresis or purge, as stronger gels will tend to push more water out of the structure. Decreasing the amount of calcium added to the formula will lower the gel strength and reduce the associated syneresis.

The gelling mechanism for alginate is well known. However, the extent to which the gelling mechanism is influenced by the presence of muscle proteins has not been elucidated. A few studies have been conducted to determine if proteins are part of the gelling mechanism in meat applications. Imeson, Ledward, and Mitchell (1977) observed considerable changes in UV–visible absorption of myoglobin and serum albumin caused by alginate and certain other polysaccharides. These absorption spectra indicated some type of interaction between alginates and proteins,

which the authors attributed to electrostatic forces. Bernal et al. (1987) later confirmed the involvement of electrostatic forces with crude myofibrillar protein. However, Xiong and Blanchard (1993) showed no change in absorption spectra when scanning various combinations of salt-soluble proteins (SSP) and alginate but did find a reduction in the gelling suspension. Offering an explanation for this unpredicted result, the authors surmised that there may be minimal hydrocolloid–protein interactions in the initial phase of SSP gelation (structural unfolding) but, as the gelling point is approached, alginate interferes with the gel network formation. It could also be that alginate has no influence on hydrophobic groups within the protein molecule but has some influence on electrostatic bonding. This influence of alginate could also be observed in the final gelled product as lower gel firmness. Schaake et al. (1993) reported that dried egg albumin did not enhance the performance of the alginate binding system in structured beef steaks, while Ensor, Sofos, and Schmidt (1991) suggested a change in the physical state of proteins in a structured meat system with the addition of algin/calcium as evidenced by differential scanning calorimetry (DSC). Mei, Chan, and Lin (2002) indicated that alginate appeared to protect the myosin heavy chain through heat denaturation, suggesting that the alginate surrounds and protects the protein, and may, consequently, interfere with the protein gelling mechanism. This was also seen in surimi, where alginate provided protection against frozen storage. The interaction between protein and alginate has yet to be completely defined. However, in most cases, higher levels of protein will interfere with the alginate gelling mechanism.

Meat products using alginate gel technology should be treated like ground meat from a food safety standpoint (Ortega-Valenzuela, Phebus, & Thippareddi, 2001) and, therefore, should be cooked to internal temperatures that ensure product safety. This could limit their acceptance as replacements for intact muscle. Making products using batch-type systems is another limitation for the acceptance of alginate gel technology. There are no known methods to process structured meat products on a continuous basis. Wotherspoon (1988) was granted a patent teaching the continuous production of reconstituted pet food chunks from comminuted materials. Modifications of this procedure may be used to make raw materials for further processed meat products.

Besides increasing the value of under-utilized muscles, meat processors can take advantage of alginate characteristics in other ways. Mechanically separated muscle has limited use due to its contribution to a reduction in firmness of the final product. Using alginates to structure these products improves the texture of the final product, with the added advantage of improving cook yield (Lamkey, 2006). Sodium alginate can be used to structure mixtures of protein and water, where the protein source can range from 25% to 70% of the formulation. The boundaries are limited only by the texture desired. Reducing the amount of protein creates a firm, gel-like texture, while increasing the protein results in softer but more meat-like texture.

Lin and Keeton (1998) suggested a combination of sodium alginate and carrageenan can replace fat in precooked beef patties with similar textural properties. In this case, alginate is used in its nongelled form. Means and Schmidt (1986), however, found off-flavors associated with higher alginate levels and attributed it to

nongelled alginate. It is, therefore, recommended that sensory attributes on nongelled alginate products be evaluated.

Alginate, like most carbohydrates, can also form films used to protect the surface of fresh meat products. A patent by Earle and McKee (1976) describes the use of alginate in combination with starch to form a coating for the protection of fresh beef carcasses. Williams, Oblinger, and West (1978) concluded that a calcium alginate coating significantly decreased shrink in coated vs. uncoated steaks and also helped maintain the oxymyoglobin color for a longer period of time. Warmed-over flavor was eliminated in precooked, alginate-coated patties as judged by sensory scores and TBA values (Wanstedt, Siedeman, Donnelly, & Quenzer, 1981). Patties having a calcium alginate coating were juicier and more desirable in texture and overall palatability than raw or precooked patties without calcium alginate. A reduction in oxidation as well as an improvement in moisture retention were shown to be advantages of having alginate in a presoak for rabbit muscle that was irradiated with 5 KGy.

Regulatory Status

Based on current USDA regulations, a mixture of sodium alginate (not to exceed 1% of product formulation), calcium carbonate (not to exceed 0.2%), and calcium lactate/lactic acid (or GdL) (not to exceed 0.3%) is permitted for use in restructured meat food products to bind meat pieces. The entire mixture is not to exceed 1.5% of product at formulation and it must be added dry (CFR, 2007b). For ground and formed raw or cooked poultry pieces, sodium alginate cannot exceed 0.8%, calcium carbonate cannot exceed 0.15%, and calcium lactate/lactic acid cannot exceed 0.6%. The entire mixture cannot exceed 1.55% of product formulation and it also must be added dry (CFR, 2007b).

The USDA also allows for the application of an alginate film on freshly dressed meat carcasses to reduce cooler shrinkage and help protect surface (CFR, 2007b). A mixture containing water, sodium alginate, calcium chloride, carboxymethylcellulose, and corn syrup solids may not exceed 1.5% of hot carcass weight when applied and chilled weight cannot exceed hot carcass weight (no added water).

In the European Union, sodium alginate is listed in the group of approved emulsifiers, thickeners, stabilizers, and gelling agents for use "quantum satis," and has been assigned the reference number E 401 (European Parliament and Council, 2006).

Konjac

Konjac is the generic name for the flour formed from grinding the root of the *Amorphophallus konjac* (elephant yam) plant (FMC Biopolymer, 1994). It is a food ingredient containing a high-molecular weight polysaccharide classified as a glucomannan. The molecular structure is comprised of mannose and glucose chains in a

molar ratio of 1.6 to 1, respectively, with -(1,4) glucosidic linkages. The glucomannan molecule, the functional component of konjac flour, has short side branches and acetyl groups randomly present at the C-6 position of a sugar unit (Tye, 1990).

This natural ingredient has been used in Asia for centuries in traditional foods such as noodles and other food products requiring stability in boiling water. Its rate of hydration is controlled by the particle size distribution whereas the degree of gelling is controlled by the presence of the acetyl groups. Deacetylation of the molecule using a weak base allows the formation of thermostable gels that can withstand retort temperatures.

Konjac is cold soluble in water and will not form a gel even when heated. Introducing a mild alkali to the konjac solution cleaves the acetyl side groups, which then allows for aggregation of the chains and the formation of a strong, elastic, heat-stable gel. To form a heat-stable gel with konjac requires a minimum concentration of 1.5–2% konjac flour and a minimum system pH of 9.0. Although heat stability of this caliber is sought for many meat applications, adjustment of pH to this level within a meat system will destroy meat protein functionality. Alternatively, creating the gel outside the meat system and then adding it back does not promote heat stability to the same level as a continuous gel. However, there are other applications where konjac can contribute useful functional properties.

When attempting to process low-fat meat products, replacing fat with water retains the moistness of the product but does not replace the lubricity or mouth coating that fat supplies. When mixed with water, konjac creates a mucilaginous sensation and is then able to work as a fat mimetic. It is for this mucilaginous effect that konjac is added, at levels not exceeding 0.25% of the formula weight. There are very few studies where konjac has been used in a meat product by itself. In low-fat bologna model systems, a 1% konjac/modified corn starch blend at moisture:protein (M:P) ratios of 5.5 and 6.0 was similar in texture to a 30% fat control. At the 0.5% konjac blend level, only the samples with added carrageenan at the M:P ratio of 6.0 had textures similar to the control (Chin, Keeton, Longnecker, & Lamkey, 1998). In general, konjac by itself will tend to decrease the firmness of meat products at high concentrations. However, the addition of other substances, such as starch or carrageenan, can counteract the effects of konjac. Yang, Keeton, Beilken, and Trout (2001) found that firmness was highly significant and directly proportional to the acceptance of low-fat meat products.

As indicated earlier, taking advantage of konjac gels will require forming the gel separately from the meat mixture. Once the gel is formed, it can be chopped, ground, or cut into the sizes needed for the desired effect. Although this gel is heat-stable at retort temperatures, it will not extend that property throughout the meat product. However, using it as a replacement for fat can help maintain the appearance of products where visible fat is traditional, such as salami, mortadella, pepperoni, etc. Gels made in this way will not bind to the protein matrix and processors may find it advantageous to add a protein source, such as soy protein isolate, to the gel blend to improve adhesion.

Regulatory Status

In the United States, the Food and Drug Administration (FDA) considers konjac flour a GRAS ingredient. It is permitted in meat and poultry products in which starchy vegetables flours are permitted, not to exceed 3.5% of the product formula individually or collectively with other binders. The European Commission lists konjac with the group of approved emulsifiers, thickeners, stabilizers, and gelling agents and has been assigned the reference number E 425 (European Parliament and Council, 2006).

Xanthan Gum

Xanthan gum is produced by a biotechnological process involving fermentation of glucose or sucrose by the *Xanthomonas campestris* bacterium. Xanthan is a long chain polysaccharide composed of the sugars glucose, mannose, and glucuronic acid (Fig. 3.7). The backbone is similar to cellulose, with added side chains of trisaccharides. It has been used in the meat industry as a viscosifier for marinades, its unique property being its ability to effect a large increase in the viscosity of a liquid at very small concentrations, on the order of 1%. It is often used for suspending particulates in brines as well as food products such as dressings and sauces. Hsia, Smith, and Steffe (1992) found xanthan gum assisted in batter pick-up, adhesion, and improvement in overall yield (Chap. 12).

Xiong and Blanchard (1993) investigated xanthan for texture modification in an SSP model system. The authors found that xanthan interfered with protein binding, more from a physical entanglement than a chemical reaction. The overall result was a reduction in gel strength of the protein gel. This was also found to be true in frankfurter batters (Foegeding & Ramsey, 1987; Whiting, 1984;). As with many cold-soluble hydrocolloids, the addition of xanthan can also reduce cook yield. However, evaluation of cook yield will be different depending on the processing

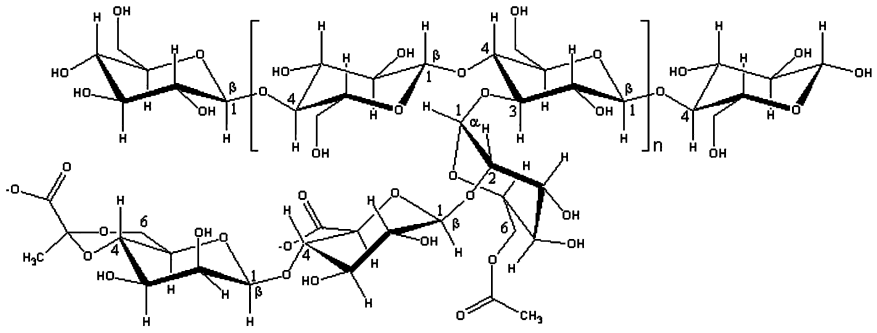

Fig. 3.7 Basic structure of xanthan (from Chaplin, 2007c. Reprinted with permission).

method and product. Cook losses between xanthan gum and both kappa and iota carrageenan were quite similar and favorable for frankfurter batters cooked in polycarbonate tubes (Foegeding & Ramsey, 1987). Fox, Ackerman, and Jenkins (1983) also found xanthan very effective in retaining water during various cooking demonstrations. However, incremental increases in levels of xanthan in turkey breast products cooked in impermeable casings were found to decrease cook yield. As indicated by Foegeding and Ramsey (1987) evaluating one of the properties without the other can prove detrimental to the processor.

Regulatory Status

Xanthan has been shown to be a safe food additive and is self-affirmed as GRAS under US regulations. Its use as a food ingredient is without limitation, providing that current good manufacturing practices (GMP) are used. Xanthan has the European Commission reference number E 415 as an approved additive. The bacterium used to produce it is not considered a genetically modified organism (GMO), so, therefore, it can be used in GMO-free products.

Locust Bean Gum

LBG is a galactomannan vegetable gum extracted from the seeds of the carob tree. Also called carob gum or carubin, it is commonly used as a thickener and gelling agent in food products. LBG is less soluble and has lower viscosity than guar gum, as it has fewer galactose branch points. It needs heating to dissolve, but is soluble in hot water. LBG differs from guar gum in that it does form weak thermo-irreversible gels by association of the galactose-deficient regions and, therefore, has poorer freeze–thaw behavior. Being nonionic, LBG is not affected by ionic strength or pH, but will degrade at pH extremes at high temperatures.

LBG is often used in canned meat formulations because of its heat stability. The temperature at which it goes into solution is about 80°C, with maximum viscosity reached at 95°C. Because it does swell in cold water, it has been found to increase brine retention in injected products prior to cooking. In most meat applications, LBG is often used in conjunction with carrageenan, as it can improve brine retention while carrageenan improves cook yield. It is true that many carrageenan blends do contain different levels of LBG, usually up to 10%. Reportedly, the advantage of having both hydrocolloids in the blend is to take advantage of this known synergy. However, as indicated earlier, synergies between hydrocolloids do not manifest themselves to their full potential due to the isolation effect of the meat proteins. The hydrocolloids are carried by the water and the water is entrapped in the protein matrix during processing and heating. This entrapment, along with higher solubility temperatures, decreases the interactions that would otherwise take place.

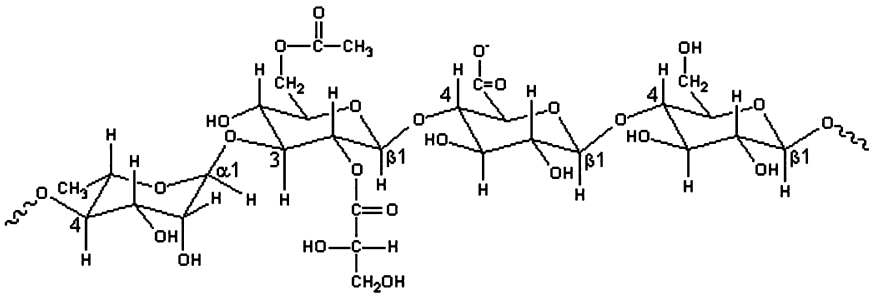

Fig. 3.8 Basic structure of gellan (from Chaplin, 2007b. Reprinted with permission).

Regulatory Status

In the United States, LBG has GRAS status and its use is not limited when following GMP. The USDA permits its use in cured pork products in combination with carrageenan and xanthan gum, not to exceed 0.5% of product formulation (CFR, 2007b). In the European Union it has been assigned reference number E 410 under the approved additives list (European Parliament and Council, 2006).

Gellan Gum

Gellan gum is a high-molecular weight extracellular heteropolysaccharide produced by fermentation of a carbohydrate with a pure culture of the bacterium *Sphingomonas elodea*. Gellan is a high molecular weight linear tetrasaccharide of one rhamnose, one glucuronic acid, and two glucose units (Fig. 3.8). Once the fermentation is complete, gellan gum is isolated and dried. The unique characteristics of gellan gum are its ability to gel in the presence of ions and the diverse properties of the gels produced by its interaction with different ions.

Shand, Sofos, and Schmidt (1993) showed that gellan gum helped increase the cook yield of alginate-structured meat, but had no effect on a salt/phosphate system. In addition, as the amount of water increased, the firmness decreased, which the author attributed to a decrease in ionic strength. Tang, Tung, and Yanyin (1995) found that gellan gels were strongest in the presence of calcium, which could create some competition in alginate-structured steaks.

Regulatory Status

Gellan gum has been shown to be completely safe for use in food. In the European Union it is a generally permitted additive (E 418). It has GRAS status in the United States, and is not limited when used following GMP.

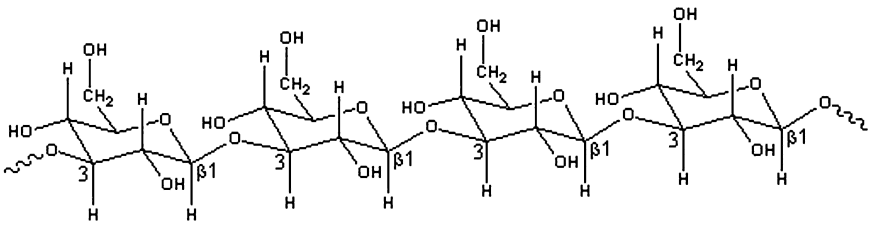

Fig. 3.9 Basic structure of curdlan (from Chaplin, 2007a. Reprinted with permission).

Curdlan

Curdlan is a linear homopolymer of D-glucose with β-(1, 3) glucosidic linkages (Fig. 3.9), and is produced commercially from the fermentation of glucose by the bacterium *Alcaligenes faecalis* var. *myxogenes* (Harada, Masada, Fugimori, & Maeda, 1966). Curdlan is capable of forming gels with characteristics determined by the temperature to which it is heated. When heated to 60°C, curdlan forms a heat-reversible gel. When heated to 80°C, curdlan forms a heat-stable gel. At 60°C, crosslinking within the gel network is structured with hydrogen bonding, whereas hydrophobic bonding is responsible for crosslinking when heated to 80°C (Konno et al., 1994). Although reportedly being used in Japan as an ingredient in various types of processed foods, including processed meats (Harada, Terasaki, & Harada, 1993; Miwa, Nakao, & Nara, 1994; Nakao et al., 1991), it has not found wide use in other areas of the world.

Funami, Yotsuzuka, Yada, and Nakao (1998b) found that nonfat pork sausages containing curdlan had textural properties similar to those of a control 20% fat control. These properties were even closer when the sausages were reheated. Funami et al. (1998b) also observed that the temperatures normally used in meat processing (≤75°C) allowed for the formation of heat-stable curdlan gels. As with most gelling hydrocolloids, more crosslinking results in stronger gels, resulting in increased syneresis. This is also true with curdlan. The addition of starch to curdlan will help reduce syneresis (Nakao et al., 1991).

Prehydrating curdlan is more effective than adding it to the meat in dry form. Funami, Yada, and Nakao (1998a) reported that the addition to meat of 1% prehydrated curdlan was more effective than the addition of 2% dry curdlan. The authors suggest a more complete gel is formed when the curdlan is prehydrated and this allows for the gel to surround the meat. This is also true of other hydrocolloids as well.

Regulatory Status

In the United States, curdlan is approved by the FDA in foods as a formulation aid, processing aid, stabilizer, thickener, or texturizer. It is not currently approved for

use in standardized meat and poultry products but can be used in nonstandardized products. At the time of this writing, curdlan has not been recognized as an approved food additive by the European Commission.

Conclusion

Hydrocolloids are important in today's meat processing industry. Economic conditions, variety, convenience, regional differences, and health concerns all play a role in the formulation of processed meat products. Hydrocolloids can assist meat proteins in the retention of moisture, and can make products more economical and/or more palatable, while fulfilling consumer needs and preferences.

An understanding of both the capabilities and the limitations of hydrocolloids will give processors a higher probability of success. Combinations of two or more hydrocolloids can give desirable results in processed meat, even though known synergies may not be realized.

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Chapter 4
Fiber

Jon M. Bodner and Jürgen Sieg

Introduction

A United States Department of Agriculture (USDA) survey shows that the average American consumes only 15.4 g of dietary fiber per day (United States Department of Agriculture, Agricultural Research Service [USDA-ARS], 1997). Using a 2,500 cal per day diet as a reference, this is only 52% of the United States Food & Drug Administration's (FDA) Daily Reference Value for dietary fiber of 11.5 g of fiber per 1000 cal (Food and Drug Administration [FDA], 1999). Although a thorough discussion of the eating habits that led to this fiber "deficiency" is beyond the scope of this chapter, Cordain et al. (2005) identified seven nutritional characteristics, including a reduction in fiber consumption, that have changed over the course of time, in the human diet. These changes have occurred over time as society has shifted from a primarily hunter/gatherer base thru an agricultural period and into an industrialized era. The types of food we eat and the methods used to prepare and process these foods changed dramatically during this time. The increased use of refined sugars and starches along with finely milled flours has resulted in a decrease in fiber consumption compared to the diet of early man.

Throughout the years, fiber containing ingredients such as whole grains, flours, and bread crumbs have been used in a wide variety of food products, including processed meats. But it was not until the 1980s that consumer awareness of dietary fiber came to the forefront. In 1987, in response to increasing scientific data showing a link between diet and disease, the FDA initiated a change in its long standing policy barring health claims on food labels (Farley, 1993). This change ultimately culminated in the passage of the Nutrition Labeling and Education Act of 1990, which reaffirmed and defined the FDA's authority to regulate the health claims made on food labels.

This new marketing ability, however, did not completely transfer to meat products regulated by the USDA. The USDA has a long standing policy prohibiting nutrient fortification of meats (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 2005b). This policy has at its origin the FDA position that fortification of fresh produce, meat, and fish is not appropriate. Unlike meat producers in other areas of the world, American meat companies have not

R. Tarté (ed.), *Ingredients in Meat Products: Properties, Functionality and Applications*
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been able to incorporate fiber into their products for the purpose of nutrient fortification. They have, however, discovered that the functional properties of certain dietary fibers, such as increased water and fat absorption, can be used to improve the economic and quality profiles of meat products irrespective of fiber fortification.

Types of Dietary Fiber

Finding agreement between various scientific groups and regulatory agencies on a definition for dietary fiber has proven difficult. In a report submitted to the Board of Directors of the American Association of Cereal Chemists (AACC) (AACC International, 2001), the AACC Dietary Fiber Definition Committee defined dietary fiber as follows:

> Dietary fiber is the edible parts of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation. (p. 1)

As noted in the report, the difficulty in defining dietary fiber is finding a balance between the physiological effect of fiber and the analytical methods used to detect and quantify it in foods. Since the USDA prohibits fiber enrichment of meats, the details of the fiber definition are of limited importance to US meat producers. It is, however, possible to fortify meat products with fiber in other areas of the world. The more important consideration for the inclusion of fibers in meat products is an understanding of the functional attributes of the various available fiber sources. In order to better grasp the functional properties of dietary fiber it is helpful to categorize the fibers into groups. Historically, dietary fibers have been classified based on their relative solubility in water. Fibers that are composed primarily of cellulose, hemicellulose, and lignin, such as oat fiber and wheat bran, are primarily insoluble. Fibers that include substantial portions of gums, polyfructoses, pectins, and mucilages, such as psyllium, fruits, and oat bran contain significant fractions of soluble fiber. Within the categories of insoluble and soluble fiber it is also helpful to further classify the fiber ingredients as native or refined. This is a more subjective classification and refers to the level of processing or extraction the fibers undergo relative to their starting substrate.

Some common insoluble, native fiber sources used in food products include wheat bran and corn bran. These bran ingredients are produced through the dry milling of cereal grains. Although these fibers find wide use in items such as breakfast cereal and bakery products for fiber enrichment, their use as a functional ingredient in meat is limited. As shown in Table 4.1, the water and fat absorption of these ingredients is significantly lower compared to the more refined fibers. In addition to the reduced functional attributes, the native, insoluble fibers typically contribute flavor and color components of the raw substrate which may not be acceptable in

Table 4.1 Properties of various fibers

Type	Dietary fiber, %			Water Absorption, %[a]	Oil Absorption, %[a]
	Total	Insoluble	Soluble		
Cellulose (300 μm)	95	95	<1	740	470
Cellulose (20 μm)	95	95	<1	350	210
Oat fiber (minimal extraction)	85	81	<5	350	240
Oat fiber (fully extracted)	93	90	<3	800	580
Wheat fiber	93	91	<3	830	600
Soy fiber(from hulls)	90	89	<1	300	200
Soy fiber (cotyledon)	70	62	8	1,000	280
Pea fiber (cotyledon)	70	65	5	1,100	300
Carrot fiber	85	65	20	1,500	300
Citrus fiber	88	68	20	2,000	290
Potato fiber	69	56	6	1,500	250
Sugar beet fiber	68	48	20	500	230

[a] Modified centrifuge method.

certain meat products. They can also impart a more gritty texture, owing primarily to the larger particle sizes available in commercial trade.

Powdered Cellulose

One of the first commercially available, insoluble, refined fibers was powdered cellulose. Cellulose, a glucose polymer, is one of the most abundant organic compounds on earth. It is the major structural component of green plants. To manufacture food grade powdered cellulose, organic plant material is cooked in a caustic solution, usually with sulfur compounds, at high temperatures and pressure. This hot caustic solution dissolves the lignin structure and other extractives which are then removed in subsequent filtering and washing steps. The resulting fibrous pulp is bleached to remove color, dried, and ground. Powdered cellulose can be produced from a number of raw material sources. As a result, the Food Chemicals Codex definition for powdered cellulose (Institute of Medicine, 2003) is not specific to a particular substrate. Any plant material which has been processed adequately to meet the purity and quality standards of the Codex can be labeled as powdered cellulose. Due to availability of supply and cost considerations, most powdered cellulose is sourced from either wood-, cotton-, or bamboo-based plant material. Commercially, powdered cellulose is available in a number of variations, the primary differences being fiber length. The absorption capability of the cellulose fiber is largely based on capillary action. The ability to absorb more or less water through the capillary action is at least partially dependent upon fiber length. Longer fibers tend to absorb more water than shorter fibers. In commercial trade water absorption is typically measured using a centrifuge-type method similar to that used to measure protein absorption. In the test, the fibers are over hydrated with

water, centrifuged, and decanted. The mass of water held by the fiber after centrifugation divided by the mass of the starting fiber expressed as a percent of the starting fiber gives the absorption. While this method is useful for comparing the relative absorptions of various insoluble fibers, it is less useful for comparing fibers with high levels of soluble fiber or gel-forming properties. For oil absorption, the same methodology is used, only substituting vegetable oil in place of water. Powdered cellulose is available in fiber lengths ranging from less than 20 μm to over 500 μm. While the longer fibers can impart increased water absorbing capability to meat systems, they can also result in detrimental changes in texture, depending upon the usage level. In practice, the length and water absorption of the fiber must be balanced against the textural changes the fiber causes. Table 4.1 gives an example of the impact of fiber length on absorption. Powdered cellulose is bright white in color and very bland in taste. In addition to its use as an ingredient, cellulose has also been widely used for many years in the production of casings for sausage products. Powdered cellulose is listed in Food Safety and Inspection Service (FSIS) Directive 7120.1 (USDA-FSIS, 2007) as an approved ingredient for use in comminuted poultry, at a level not to exceed 3.5% in various nonstandardized products. Cellulose can also be further processed and modified to produce cellulose ethers such as carboxymethyl cellulose or methyl cellulose.

Oat Fiber

When considering oat fiber, it is important to differentiate between the dry-milled grain products such as oat bran or oat flour and the refined insoluble oat fiber extracted from oat hulls. The first commercial varieties of refined, insoluble oat fibers appeared on the market in the 1980s1987. In two US patents, Gould (1987) and Gould and Dexter (1987) describe an alkaline peroxide treatment of agricultural residues, including oat hulls, which yielded a higher absorbing insoluble fiber. Just a few years later, Ramaswamy (1991) patented the application of a process similar to soda pulping used in paper production to oat hulls, which resulted in a very high absorbing oat fiber product. Oat hulls had long been a low value by-product of the oat milling industry. Although oat hulls naturally have a high total dietary fiber content (75–80%), they also contain silica. This silica results in an abrasive mouthfeel and texture, which limits the use of oat hulls in food or meat products. In the above patents, the inventors both applied process conditions that partially (Gould) or fully (Ramaswamy) removed the lignin from the oat hulls. As the lignin is removed, the silica is also washed from the oat hulls, resulting in a fiber with a much softer texture. The extraction of the lignin also allows the individual cellulosic fiber strands to separate. This increases the surface area and allows the fibers to swell and absorb larger amounts of water. In the case of the Ramaswamy process, the high temperature and pressure removes most of the lignin and silica from the oat hulls. The rapid decompression process from the high pressure cooking also adds a mechanical shear action which further defibrillates the fiber strands, leading to absorption enhancement.

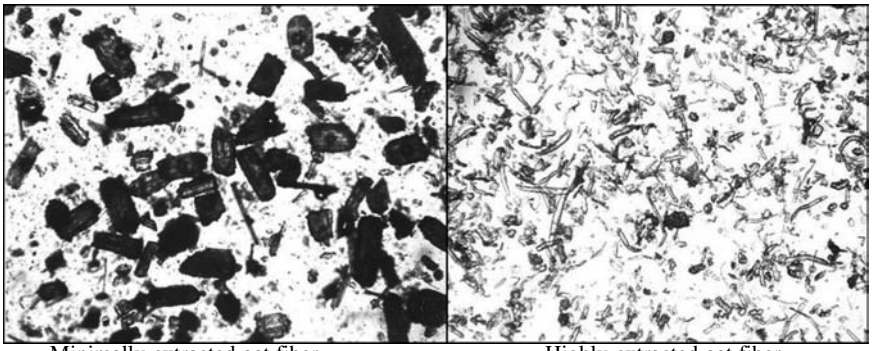

Fig. 4.1 Comparison of oat fiber structure at 100× magnification (courtesy of J. Rettenmaier & Söhne GmbH + Co KG, Rosenberg, Germany. Reprinted with permission)

Oat fiber absorption levels can range from 250% to over 800%, depending upon the level of extraction applied in the manufacturing process. Once the oat fiber is fully extracted, the absorption can be further manipulated through milling, as with powdered cellulose. Figure 4.1 gives an example of the structure differences between a minimally extracted and highly extracted oat fiber. Oat fiber is available in colors from light tan to white. The more extracted versions have very little taste. Oat fiber is listed as an ingredient in the FSIS *Food Standards and Labeling Policy Book* (USDA-FSIS, 2005a). In keeping with the USDA policy forbidding nutrient fortification of meat products, the handbook states that oat fiber should be labeled as "Isolated Oat Product" on meat products.

Wheat Fiber

The processes described for the production of powdered cellulose can also be applied to other agricultural materials. In Europe, an insoluble wheat fiber made from wheat straw has been widely used in meat products. This material is produced using a process identical to that used for powdered cellulose. The resultant wheat fiber has very similar characteristics to a fully extracted oat fiber. In fact, in most applications, wheat fiber and oat fiber can be used interchangeably with little formula alteration. Wheat fiber is bright white in color with very little taste. At present, wheat fiber is not listed as an approved ingredient for use in meats by the USDA. It is, however, allowed for use in certain meat products outside the USA.

Soy and Pea Fiber

Soybeans and peas are both legumes and have very similar properties relative to the fibers produced from them. In both cases there are two types of fiber available,

either from the outer hulls or from the cotyledon portion. In the case of the hull-based fibers, these can range from simply a dry-milled material to a fully extracted material. A two-step process to produce a fiber from legume hulls has been described in a patent by Vail (1991). The extracted fibers of the pea and soy hulls are shorter and more cube-like rather than the long thread-like structures seen with oat and wheat fiber. As a result, the absorption characteristics of these materials tend to be lower. The cotyledon-based fibers are typically produced as a by-product of the protein extraction process. The cotyledon-based fibers generally have a higher level of soluble fibers, which can boost the water absorption capability, but they tend not to have the oil/fat-binding capability of the higher insoluble fraction varieties. The extracted versions of the legume hulls are white to off-white in color and carry very little flavor. The cotyledon and minimally extracted hull versions are tan/yellow to white in color and have a definite taste. In some cases, the taste profile may be significant enough to limit application of the material.

Carrot Fiber

Carrot fiber is a relatively new fiber to find application in meats. A recent US patent describes a process for producing a carrot fiber from the cuttings and peelings of carrots (Roney & Lang, 2003). This process uses benzoyl peroxide as bleaching agent to reduce color and flavor. The resulting fiber from this process is off-white in color and most of the typical carrot flavor has been removed. The high-water-absorption capability (1500%) of this fiber makes it useful for many meat applications, but like many mixtures of soluble and insoluble fibers, the oil absorption is relatively modest at 300%. Carrot fiber is listed in the FSIS *Food Standards and Labeling Policy Book* (USDA-FSIS, 2005a) and is approved for use in meat products in the USA. The specified labeling for carrot fiber in meat products would be as “isolated carrot product”.

Citrus and Fruit Fibers

There are a number of variations of citrus and fruit fibers available in the market. The source of these materials is usually the by-products of juice and pectin manufacturing. Fibers sourced from the juicing process, like apple pomace, tend to contribute significant color and flavor properties that can limit their application. Fibers derived from pectin manufacturing are normally higher in fiber content and more consistent in their nutritional profile. As seen in Table 4.1 the absorption of a citrus fiber is actually extremely high, likely based on the high soluble fiber content. An important consideration when formulating with citrus fibers is the taste profile. Some of these fibers have a very low pH which can cause an acidic/bitter

taste when applied in meat products. Citrus fiber is not specifically listed in the FSIS *Food Standards and Labeling Policy Book* but would be covered under the vegetable extract guidelines.

Potato Fiber

Potato fiber is manufactured from the cuttings and peeling by-products of the potato processing industry. The cuttings and peelings are washed in a water solution, which may or may not include other extraction chemicals, to remove residual sugars and other solubles. The resulting fiber is a mixture of fiber and starch. It is interesting to note that potato fiber contains a portion (12%) of resistant starch. Resistant starch is the starch fraction that is resistant to digestion in the small intestine, but which is available for bacterial fermentation in the large intestine. In potatoes, the resistant starch is largely due to the high amylose starch content. The tightly bound nature of the amylose starch granule that provides the resistance to digestion also results in a low water absorption capability. In the case of the potato fiber, the low absorption nature of the resistant starch fraction is offset by the nonresistant starch content (16%). This results in a fiber with good water absorption capability (1500%), but relatively low oil binding capability (250%). The residual starch content should also be taken into consideration when formulating products as well. While in the initial cooking phase the gelatinization of the starch granules will increase viscosity and absorption, these granules can retrograde upon cooling and storage, leading to syneresis. Potato fiber is tan to off-white in color and has some residual potato flavor. The water-only-extracted potato fiber would meet the guidelines described in the FSIS *Food Standards and Labeling Policy Book* for "vegetable extract" and be labeled as "potato extract".

Sugar Beet Fiber

Sugar beet fiber is derived from the fibrous pulp remaining after the extraction of sucrose from sugar beets. During the sugar refining process, the beets are thinly sliced and washed to solubilize and remove the sugars. In the most common process, the leftover pulp is washed, dried, and milled to form sugar beet fiber. In other processes, more complicated washing steps, including the use of further extraction and bleaching chemicals, can be used. Sugar beet fiber has a high level of soluble fibers. The water absorption and oil absorption of sugar beet fiber is low compared to other fiber sources, limiting its use in meat product. Sugar beet fiber also has a flavor, best described as "earthy," which can also limit its application in food products. The color of sugar beet fiber ranges from tan/gray to off-white. Like potato fiber, sugar beet fiber would be labeled as "sugar beet extract" in meat products.

Soluble Fibers: Inulin and Hydrolyzed Oat Flour

The functionality and application of soluble fibers in meats encompasses a wide array of ingredients. Modified cellulose ethers like methylcellulose and carboxymethyl cellulose, along with hydrocolloid ingredients like xanthan and acacia, can also be considered soluble fibers under some definitions. Since a thorough review of hydrocolloids is beyond the scope of this chapter (see Chap. 3 for an in-depth discussion), the present discussion will be limited to those soluble fibers that are also typically used as fiber sources. Inulin is a soluble fiber extracted by a washing process from chicory roots. It contains both oligo and polysaccharides. The polymer is composed of fructose connected by β -(2,1) links and usually terminates with a glucose molecule. The degree of polymerization (chain length) ranges from 2 to about 60 (Orafti Active Food Ingredients, 2006b). The use of inulin in meat products has been especially focused on fat replacement. Inulin has the ability to form a stable gel network which can be used to mimic some textural properties of fat when applied to low-fat meat products. By substituting the inulin gel for fat processed meat applications, it is possible to achieve acceptable textural and sensory properties in low-fat products (Orafti Active Food Ingredients, 2006a). Inulin is a fine off-white to white powder with little flavor or odor and is listed in the FSIS *Food Standards and Labeling Policy Book* (USDA-FSIS, 2005a) as approved for use in meat products.

Another soluble fiber that has been used in meat products is hydrolyzed oat flour. In two US patents, Inglett (1991, 1992) describes a method for producing a hydrolyzed cereal flour with increased content of soluble fiber in the form of β -glucan. This product, developed by the USDA Research Labs in Peoria, IL, was licensed under the trade names "Oatrim" and "TrimChoice." β -Glucan is another glucose polymer (like cellulose) but soluble in nature, forming thick gels. β -Glucan is widely associated with its cholesterol-lowering benefits. The FDA currently permits a "reduced risk of coronary heart disease" health claim for food products which contain soluble fiber from oats at a level of at least 0.75 g per serving. In addition to its use as a soluble fiber supplement, hydrolyzed cereal flours are also useful in meat applications for water absorption and their impact on texture. In particular, the gel-forming capability of the hydrolyzed cereal flours can be used to mimic the textural properties of fat in meat products. A 1994 US patent (Jenkins & Wild, 1994) describes a food composition comprising hydrolyzed cereal flour, a hydrocolloid, and a comminuted meat product. The patent further points out that the high-water-binding and gel-forming characteristics of hydrolyzed cereal flour, when used alone, contribute to a weak or mushy texture. In fact this effect is seen when applying many of the very high absorbing fibers previously discussed. Hydrolyzed oat flour is listed in the FSIS *Food Standards and Labeling Policy Book* (USDA-FSIS, 2005a) and must be labeled as "hydrolyzed oat flour."

Colloidal Fibers

Like the soluble fiber β -glucan, mentioned above, colloidal fibers also form gels. In this case, however, the gel is not due to soluble fiber, but to the formation of a colloidal dispersion of very small insoluble fibers. When insoluble fibers are wet-milled to an extremely small fiber diameter, they can form a stable thixotropic gel. As early as 1961, Battista, Hill, and Smith (1961), in a US patent, describe a method for producing an insoluble gel from microcrystalline cellulose. In that case, hydrolyzed cellulose was subjected to shearing in a blender, forming a stable gel. It is also possible to produce this fibrous gel via microbial fermentation. The technology for this type of material, called Cellulon, was developed by the Weyerhauser Company using *Acetobacter xylinum* (Deis, 1997). The small microreticulated fibers form an extremely stable gel which exhibit reversible shear thinning. The wet milling technology used to produce insoluble fiber gels can be applied to a number of substrates, including oat fiber, wheat fiber, cellulose, and corn bran. From a logistics point of view it is impractical to ship and store these very high water content gels. As a result, the gels are normally dried for commercial sale. In these cases it is necessary to include a dispersing agent (e.g., hydrocolloid, protein, polysaccharide, etc.) to coat the fibers and prevent re-agglomeration upon drying. Although high shear is still necessary to re-activate the colloidal gel, the coating reduces the needed activation force. Activation in a high-shear mixer or bowl chopper is usually adequate to re-form the gel. These colloidal gels exhibit many of the same fat replacement properties seen with the soluble fibers, including smooth texture and water-holding capability. Approval for use in meats of the colloidal gel products would be dependent upon, and the same as, the fiber substrate from which they were derived.

Application of Fibers in Meat Products

As previously noted, the existing group of commercially available dietary fiber concentrates display an incredible variation in functionality. In nature they play a role as structural components and aid in the binding or transport of water. These characteristics, along with fat binding and functionality as a fat mimetic, offer interesting concepts for the food industry. Ongoing economic pressure forces meat processors to look for reliable solutions to produce competitive, high-quality, products. This also opens the door for an economic-driven use for most of the fiber concentrates.

During the last 20 years, articles have been published covering low-fat meat products using different fiber types to increase water dosages. In these investigations, the technological and functional diversity of the fiber types has been only partially highlighted. Comparing three existing studies (Aleson-Carbonell, Fernández-López, Pérez-Alvarez, & Kuri, 2005; Chang & Carpenter, 1997;

Steenblock, Sebranek, Olson, & Love, 2001), totally different systems have been utilized for the commonly described "oat fiber." With dissimilar fiber extraction levels, variations in water and/or fat binding impacted the results. While two of these research groups applied a traditionally produced bran, Aleson-Carbonell et al. (2005) utilized oat bran containing higher β -glucan content to push the health aspects of the soluble fiber fraction. From a nutritional point of view it was a noble intent, but the low fiber content in oat bran makes it very difficult to reach adequate nutritive fiber levels without serious detrimental effects on the product's organoleptic properties. Another crucial attribute is the fiber particle size. Chang and Carpenter (1997) found that graininess was more detectable and juiciness was reduced with increasing bran dosage. An option to handle this organoleptic challenge was to increase water dosage. However, too large of an increase could exceed some regulatory limits. Much like the in the USA, in Germany, for example, when legal limits are exceeded, the additional water has to be labeled in the ingredient list. As discussed earlier, low/no extraction fibers such as oat bran usually have limited water-holding capacity. Increasing the water dosage to compensate for the missing fat will be evident as purge in the package. Furthermore, the gritty mouthfeel is a real problem. Large particle sizes with rough edges do not fit into low-fat systems, where the reduced fat content amplifies sandiness. Comparable results were obtained by Claus and Hunt (1991). In low-fat, high added-water bologna trials, they observed that 40 mesh (425 μm) sugar beet pulp fiber attained an unpleasant profile in graininess, whereas, by comparison, a highly extracted oat fiber at 3.5% dosage scored very close to the control product. Their final recommendation was a defined particle size reduction

In recent decades fiber manufacturers have offered new tailor-made products for processed meat applications, and have promoted additional health benefits. When defining grain fibers the extraction level is vital. Studies by Steenblock et al. (2001) and Claus and Hunt (1991) cast a first light on this topic. Steenblock et al. (2001) chose two varieties of oat fibers reflecting different processing levels. More recent research has proven that highly extracted, long-chain cellulosic fibers offer increased functionality for processed meat.

The broad range of nutritionally important fibers offers additional health concepts to the meat industry. The diet of the Western world suffers from a distinct lack of dietary fibers, and processed meats could be a vehicle for increased consumption. Public awareness of the benefits of fiber is continuously growing and many marketing concepts in other food applications are using this strategy to boost consumer acceptance. US and Canadian meat regulations inhibit this concept, while health-oriented meat products are being actively marketed in Europe and South America. Over the last two years several German meat products marketed as "enriched with fibers" and "rich in fibers" have been launched. For success of these products, taste and organoleptic properties are crucial. Customers are not willing to change their traditional quality expectations and a comparable price level has to be achieved. Marketing concepts based on digestive health and protection against colon cancer are examples of the way fibers can be applied to take advantage of their prebiotic effect (i.e., improvement of the microbial flora in the large intestine).

Besides the nutritive aspects of fat reduction or fiber enrichment, the second model is driven by economics. High water binding and significant water retention can help decrease cooking losses or purge in vacuum packages. Further benefits can be obtained from the structural characteristics of the fiber. Improved meat product quality reflects an optimized bite, texture, springiness, hardness, or snap. An understanding of the overall technological model of fiber incorporation can be constructive in order to maintain the standard properties of the product, even with a reduction in the quality of the starting meat block. The fiber portfolio offers concentrates with overlapping functionalities, diverse production processes, numerous raw material sources, and unique chemical compositions.

Fiber Application Goals

With product options continuing to grow, it is vital to understand the functionality of the various fiber sources. There is no standard recommendation. The conceptual possibilities cover a wide range of nutritional, technological, economical, and marketing aspects. Table 4.2 summarizes the most important physical and chemical aspects of fibers used in meat products.

One single fiber is not able to provide all possible features, but new progressive strategies combine the unique characteristics of different fibers. In a recent review article on the use of fat replacers in meat products, Tokusoglu and Ünal (2003) stated that "to obtain any reformulated processed meat with desirable characteristics, several of the available technological aspects have to be combined." In the last five years numerous German processed meat products have been launched containing two different fiber sources. These fat-reduced products offer combinations of soluble and insoluble fibers and offer a combined fiber content of 3–6%.

The German Federal Research Centre for Nutrition and Food (BfEL), located in Kulmbach, developed comprehensive analytical concepts to evaluate the total and singular fiber content. Münch (2006) analyzed inulin with an enzymatic method and insoluble fiber with a gravimetric procedure. In the detection of the total and single fiber content, inulin interfered with the overall results. Reality shows there is still a need to improve the analytical methodology used to quantify the different sources of fibers in all kinds of foods. Analytical results are also influenced by the different ratios of protein, fat, and carbohydrates, as pointed out by Hadorn, Piccinali, and Suter (2007).

Regional Meat Quality

With the growing use of mechanically deboned meat (MDM) or mechanically separated chicken meat (MSCM), products reveal different textural and structural

Table 4.2 Overview of fiber grades commonly used in processed meats

	Grain (oat & wheat)	Potato	Pea	Citrus	Inulin	Soy	Sugar beet	Carrot
Price	$$	$$	$$	$$$$	$$$	$$	$$	$$
WBC[a]	1: 11	1:15	1:3 – 1:12	1:20	1:4	1:3	1:4	1:18
Fat binding	1:8		1:3	Low	–	Low	Low	1:4
Fiber length	>500 μm			<250 μm	DP 2–60	>80 μm		15–160 μm
Particle size		> 4 mm	70–300 μm				32 μm–2 mm	
Flowability	+/–	++	++	++	++	++	++	++
Taste/Flavor	Neutral	Sweet/potato flavor	Neutral to mild pea flavor	Neutral	Slightly sweet	Neutral	Off-taste	Sweet
Composition	Cellulose, hemicellulose, lignin	Cellulose, hemicellulose, pectin, resistant starch, protein	Cellulose, hemicellulose, resistant starch, starch, protein	Cellulose, hemicellulose, pectin	Oligo- and polysaccharides	Cellulose, hemicellulose, lignin	Hemicellulose, pectin, cellulose, lignin, protein, sugar	Cellulose, pectin, protein
Insoluble fiber	90	60	80	70		90	50	78
Soluble fiber	5	14	2	20	97	2	24	14
pH value	6.0–8.0	5.0–7.0	4.0–7.0	3.5–4.5	5.0–7.0	6.5–7.5		4.5–5.5
Extraction	Very high	Mid	Low to mid	Very high	High	Low	Low	Mid
Color	White to Off-white	Brownish	Green gray to bright	White	White	White	Brownish	Off-white

[a] WBC: water-binding capacity.

properties. In markets like the USA, MSCM is often used in low-fat comminuted meat products (i.e., bologna and frankfurters). MDM can also be found nowadays in dry sausages. When using MDM, manufacturers have to address its negative effects on final cooked product texture (soft or "mushy" texture), fluid loss during storage (purge), and reduced palatability (marginal taste). As a result of the mechanical deboning and storage process, the primary MDM meat protein, myosin, loses some of its ability to bind water and has reduced interaction with the myosin from the other lean meat trimmings used in the product, resulting in inferior texture. The incorporation of some fiber sources offers an alternative to recover product texture, resulting in an improved bite or snap, and fit into the economical concept. The best performing fibers for structural enhancement are primarily cellulosic, such as potato, wheat, oat, or citrus. In contrast, inulin, with its short-chained molecule dimension, is not able to improve the texture. A number of insoluble fibers are limited by particle size and structure. Higher dosages could result in a grainy or rough mouthfeel, while the highly refined cellulosic structure from citrus fiber contributes a hard texture without sandiness.

Allergenicity and GMO

In a world of growing numbers of food-related diseases consumers are becoming increasingly concerned with the status of ingredients in processed food. The new European legal standards for labeling intend to inform consumers about potential allergens. To avoid a negative image and to reach the widest possible range of potential consumers, manufacturers are attempting to substitute relevant ingredients. The needed recipe modification can be a technological and economical challenge. The properties of these allergenic ingredients cover a wide range of functionality, including water binding, fat holding, or textural improvement. Extracted fiber concentrates can be used in the replacement of some allergen-containing ingredients. Based on the raw material selection and the severity of the extraction process, many refined fiber concentrates have allergen-free status. Even with wheat fiber, where gluten is a concern, ELISA (enzyme-linked immunosorbent assay) analysis has shown this material to be gluten-free. A Scandinavian processed meat producer labeled "wheat fiber (gluten free)" in the ingredient list of a ground meat product.

In recent years the media has documented increased anxiety and concern regarding the topic of gene modification. Especially, in Europe, and increasingly in North America, food producers and consumers are trying to avoid genetically modified ingredients. Even when soy fiber producers can prove their GMO-free status by using certified GMO-free raw material, markets like central Europe are resistant to accept soy-related products. A different situation is seen in regions like Eastern Europe and South America. The long-term use of soy isolates, concentrates, and texturized vegetable proteins has resulted in an advantage point for this type of fiber.

Nutritive Fiber Usage

Fiber Enrichment

The market for fiber-enriched food is fairly large. Although the bakery and cereal industries have been the traditional platforms in this area, the development of extracted and refined fiber concentrates has opened the door for new food applications. Based on tradition and geographic preferences, research scientists and producers have focused on distinctive product types. The European market has developed products with fibers from wheat, potato, inulin, pea, and other sources, while the US market has shown a preference for oat sources and, in a few products, isolated carrot.

When discussing fiber applications in meat worldwide, it will become obvious that the regulatory and labeling requirements are not consistent. In the European market the high-fiber dosages required in the Codex Alimentarius for a fiber claim are not easy to achieve. Furthermore, the properties of different fibers have to be reconciled with consumer expectations. High-fiber inclusions are easier to handle in coarser, structured ground meat products than in finer emulsions. Often the structure and dimensions of the particle limit the possible dosage and the range of processed meat products to which it can be added. Combined fiber concentrates – soluble and insoluble – are very effective for achieving Codex requirements. A different model could involve the use of a rounder structure, like in sugar cane fiber or soluble fibers, offering a smearing effect. While insoluble fibers struggle with organoleptic limitations, many soluble fibers display restrictive digestive tolerance. With low water binding, a bland taste profile, and small particle size, inulin would be an ideal solution with minimal technological difficulties. A limiting factor is its potential digestive side-effects. At high concentrations there would be, in addition, the possibility to claim both prebiotic benefits and improved calcium uptake, but many consumers would complain about flatulence and abdominal pain. Some studies have already involved this combination strategy. In dry sausage studies, Mueller (2006) used both inulin and wheat fiber to limit the negative properties of each. He was able to add 3%, and even 6% (on a dried finished product basis), and meet the WHO recommended combination of soluble and insoluble fibers. In Hadorn et al. (2007) and Münch (2006) a comparable strategy can be found. Comparable processed meat products can be found in the German and Swiss markets. In the US market, fiber enrichment of meats is not possible due to USDA regulations. In Canada only a few refined fibers, specifically those approved under the novel fiber regulations, are allowed to be used for fiber and calorie claims (Canadian Food Inspection Agency, 2007).

Fat Reduction

Fat reduction in processed meats is another area where the functional properties of fibers can make important nutritional contributions. Commercial low-fat products can be found in the USA, Germany, Switzerland, and Scandinavia. Because fat is

not just a simple caloric filler hidden in a protein matrix, a fat replacement strategy for processed meats must address the diverse influence of fat on structure, texture, and mouthfeel. Since the addition of a single fiber cannot solve the problem, combinations of fibers and other ingredients with unique and complementary properties may be used in order to take advantage of synergistic effects, in terms of water binding, creaminess, and structure.

Fibers from different sources exhibit varying degrees of water binding and holding. Based on fiber content and structure, citrus and wheat fiber display the highest water absorption and inulin the lowest. Potato and pea fiber show high values, due to their significant starch and protein content. A disadvantage of this digestible carbohydrate content is recrystallization and, consequently, water leakage in vacuum-packaged products. In addition to water binding and juiciness, other essential quality factors are graininess, springiness, hardness, and cohesiveness. Chang and Carpenter (1997) showed that insoluble fibers, like bran, and added water have complementary effects on springiness, hardness, and cohesiveness of frankfurters. However, large fiber particle sizes and increased dose levels led to increased graininess. Since the early 1990s, several new fiber ingredients with better organoleptic properties than bran have been launched. Nevertheless, sandiness is still an issue for several cellulosic fibers. Dose levels up to 1.5% are generally possible without creating sandiness. Customized milling and advanced lignin extraction can also improve the organoleptic profile of cellulosic fibers. In the case of citrus fiber, the cellulose and pectin combination, combined with a high-extraction production process, creates a nice round mouthfeel, but high price and low pH values limit its usage level. The combination of cellulosic fibers with a low dose of psyllium is a good option. Psyllium's extremely high-water-binding and gel properties could aid in overcoming the sandiness of cellulosic fibers. Higher psyllium dosages are a challenge, since further water binding continues to occur during storage. This makes it difficult to control the texture and structure of the processed meat product throughout its storage life. A second disadvantage of psyllium is that it melts during reheating and releases a brownish, slimy purge.

In the European processed meat industry inulin has captured much attention. Based on the market growth of prebiotic dairy products, customers have become aware of inulin's nutritional benefits. Long-chained inulin in water under high-shear forces can create a particle gel (Jánváry, 2006) that contributes a smooth mouthfeel that resembles the characteristics of fat. With inulin, two usage scenarios are possible: Preactivation in water or addition at the beginning of the bowl chopper process. When working with crystalline inulin, 24 h are required for complete gelling. In a comparison of fat replacers with a 24 h swelling activation modus, maltodextrin and inulin were used in 10%-fat frankfurters, with both resulting in a white exudate in vacuum-packaged product (Orafti Active Food Ingredients, 2006a). The water-holding capacity of inulin and maltodextrin was not strong enough to immobilize the water for the duration of shelf life. Based on its molecular weight and particle size, inulin responds to the osmotic pressure and migrates from the meat batter into the purge. To avoid this scenario, lower doses of inulin in combination with other high-water-holding fibers can be applied. Commercially,

products in the German market have been launched with combinations of inulin and wheat or citrus fiber. A second reason to use lower levels of inulin has to do with digestive tolerance problems (consumption of inulin at levels higher than 4 g per serving can lead to the formation of unpleasant amounts of gas). Hadorn et al. (2007) tested a combination of inulin and wheat fiber in boiled sausages to control both problems. An even higher dose of wheat fiber in this study could be possible since, at a 1% level, no roughness in mouthfeel was noted. The functional benefit of an insoluble fiber would improve the sausage quality. In this test, sliceability was improved by the addition of the long, insoluble wheat fiber.

Combinations of fibers with hydrocolloids also offer a potential solution for low-fat meats (Tokusoglu & Ünal, 2003). A commercial product line introduced in Europe takes this approach. It utilizes a patented (Christensen & Mogensen, 1995) combination of ingredients (modified or native starches plus dietary fibers) and a defined preparation process. An example of this product line, shown in Fig. 4.2, uses a combination of fiber, starch, and carrageenan.

Fig. 4.2 The *Den Grønne Slagter* product line contains 3% fat and relies on a combination of modified starch, potato fiber, and carrageenan. Ingredient line for product Jægerpølse (shown in figure): Lean pork meat (52%), water, *modified gluten-free starch*, salt, *potato fiber*, acid regulating component (sodium lactate), stabilizer (*carrageenan*), spring onion, antioxidant (sodium ascorbate), garlic extract, dextrose, spice extracts, glucose syrup, preservatives (sodium nitrite) (courtesy of Tulip Food Company, Randers, Denmark. Reproduced with permission).

Nutritionally Enhanced Processed Meat Products

Nutritionally functional food concepts have been considered for some time. Consumer demand for these products has been increasing, spurring new and more creative developments. Based on their technological functionality and nutritional benefits, fibers can be used, either alone or in combination with other ingredients, for fiber enrichment, as sources of prebiotic fiber, to improve calcium uptake, to reduce fat, saturated fat, and/or cholesterol, to reduce salt or phosphate, and to enable the use of plant oils rich in omega three fatty acids. Unfortunately, current US regulations limit health claims in processed meats, thus limiting the potential application of fibers primarily to fat and cost reduction. The European market, however, has already seen the launch of several of these more advanced concepts, with fat reduction, as well as fiber enrichment and other benefits.

These functional food concepts include a wide range of ideas, where the nutritive as well as the technological properties of fibers are very useful. Two of these strategies highlight the idea of replacing or reducing added salt or phosphates. The current impetus to reduce sodium levels across processed foods poses a particular challenge for the meat industry, given the key functional role of salt in meat systems, as it relates to protein solubilization and water binding (Chap. 1). Because of the peculiar challenge this poses, many different approaches to reduce sodium in processed meats have been attempted. The use of high-water-binding fiber concentrates constitutes one more tool to address this important issue. A comparable strategy can be discussed in processed meats for phosphate reduction. Due to concerns that the modern human diet contains excessive amounts of phosphorus, attempts have been made to replace phosphates in meat products. Unlike phosphates, fibers are not able to work at the molecular level on the actin–myosin complex, but their high ability to bind water and improve texture can help achieve partial phosphate replacement in different types of processed meats.

In addition to aiding in fat replacement and calorie reduction, some soluble fibers have the ability to bind bile acids in the small intestine, thus blocking their recycling through the ileal mucosa and forcing their excretion in the stool. The body is, consequently, forced to consume more cholesterol to regenerate the lost bile acids. As in the previously mentioned German product, animal fat may be partially or totally replaced by more polyunsaturated vegetable oil. In these instances, insoluble fibers may help stabilize the product batter and avoid fatting out. To achieve the best fat binding, these fibers could be premixed with the oil. The recipe below demonstrates a German “healthy” cooked poultry sausage concept developed by the company J. Rettenmaier und Söhne. It illustrates the combined use of soluble and insoluble fibers, poultry white meat, carbonate as a cutter process aid, and vegetable fat.

The second German recipe, a 65% fat-reduced Wiener sausage with a 65% fat reduction, contains 10% fat and enough fiber to permit the use of the claim “source of dietary fiber,” based on the requirements of the Codex Alimentarius (Codex Alimentarius Commission, 2007). For fat replacement, two different concepts are possible. The first option involves the use of a combination of different fibers to

Wellness concept poultry boiled sausage (Lyoner type)

Ingredients	%
Poultry meat	48.30
Rape seed oil	22.75
Water/ice	28.95
Wheat fiber	1.8[a]
Inulin	1.5[a]

[a] Calculated on meat block basis.

avoid the labeling of *E* numbers, while the second option takes advantage of the functionality of cellulose gum and colloidal microcrystalline cellulose. With either application, high-shear force equipment is essential to open the co-processed particle structure and achieve the needed dispersion. Preactivation of the fiber alone with shear could be done, but it is also possible to achieve the same result by adding the fiber blend at the beginning of the chopper step. The mechanical shear in the bowl chopper is normally adequate to fully activate the gel from the inulin or colloidal microcrystalline cellulose. The activated fat replacer exhibits thixotropic behavior, and resembles the mouthfeel of fat and provides juiciness.

Wiener sausage – 65% fat-reduced (10% fat) and dietary fiber-enriched (3%)

Ingredients	%
Beef trimming 80/20	15
Pork trimmings 80/20	44
Pork fat	9
Pork back fat	3
Water	29
Oat fiber	1.2[a]
Fat replacer	1.4[a]
Inulin	0.4[a]

[a] Calculated on meat block basis.

Process Implementation of Fibers

Most fiber concentrates have a strong tendency to bind and hold water, which allows them to influence, or even modify, production parameters. Regardless of the manufacturing equipment used, the following must be considered and controlled: fiber hydration time, fiber dispersion in the meat batter based on mixing or comminution time, handling or timing during production, protein solubilization, batter extensibility, batter viscosity, temperature control, emulsion stability, cooking and smoking yields, and peelability. In the case of high-water-binding and holding fibers, an adequate fiber:water ratio is crucial to sufficiently hydrate the batter components; otherwise the meat matrix could be too dry and its process temperature could

rise faster than expected. In low-water environments, the stickiness of soluble fibers, like pectin, could make it difficult to disperse them since they show a strong tendency to lump. Some fibers or fiber systems require a pretreatment, usually with shear, to achieve their full technological potential.

Wherever fibers are added in the process, it is critical that they be well dispersed in order to gain maximum technological benefit. Fibers can be added either in combination with other ingredients or functional seasoning compounds, or in a single step at the beginning or end of comminution or mixing. The process timeline may need to be adjusted based on physical energy input from the blender, cutter, or the brine agitation. Another consideration is whether the process is continuous or in batches.

In most production scenarios the fibers are combined with the seasoning blend or the other dry ingredients. When combined with the other dry ingredients, the fibers will compete for water in the system. It is important that all formulation constituents be adequately hydrated. Due to differences in water affinity and fiber structure which can affect the timing of water uptake, diverse technologies or adaptations have to be considered. Pure cellulosic fibers absorb water very fast without the need for special processing. Mixed fiber/starch systems, like potato or pea fiber, require heating to gelatinize the starch fractions. Long and thin fiber versions, like wheat and oat fiber, have to be thoroughly dispersed during the mixing and/or comminution steps in order to set up a network-like system. In this system, fibers are able to create a drainage system, holding and dispersing the water in the meat system. Inulin can be handled as a dry powder with a 24 h set-up time or as a preactivated paste. At higher concentrations (20–25% solution) the long inulin molecules create a particle gel that mimics fat. This solution incubated overnight in a cooling chamber will result in a heat- and shear-resistant paste. Psyllium husk fiber has extraordinary water-holding ability but needs hours to reach full hydration. During this time, the dry powder is transformed into a system that exhibits plastic rheological behavior. The dry powder has a tendency to stick, so care must be taken during mixing. Good preblending with other dry ingredients is necessary in order to ensure that the material is sufficiently dispersed in the meat batter. Under high-shear forces, the water binding of highly extracted citrus fiber increases and the fiber thickens into a creamy paste.

When using blender or mixer systems, fibers should be added early in the process to allow maximum hydration. During the initial grinding step, a reduction in the size of the meat pieces and a first release of soluble protein take place. After transferring the ground meat into the mixer, protein solubilization increases by the mechanical forces of the rotating shaft. In order for the fibers to form a well-dispersed fiber network, they should be added early in the process, before batter viscosity increases dramatically.

At higher fiber dosages, sufficient water for hydration has to be taken into consideration. An improper fiber:water ratio can have a negative influence on batter viscosity, as insufficient water could result in a very viscous, dry, and dense batter. With more friction in between batter and mixing tools, a faster temperature rise is to be expected. An additional consequence of this scenario could also be an insufficient dispersion of fibers. The worst case would be a sandy mouthfeel based on

fiber agglomerates and an uneven water distribution in the meat batter. To avoid this, the addition of sufficient extra water or water/ice is recommended, as is a combination of different fiber types. The prehydration of the fibers would be an extra step in production which is not necessary in most cases.

In comparison to blenders, the high-shear forces from a bowl cutter enable different production schemes, including fiber addition at a later stage of comminution. This flexibility makes it possible to achieve different technological aspects and enables the use of colloidal fiber products as fat replacers. However, during comminution and mixing, the friction of the knives causes a gradual increase in batter temperature, which is controlled by using combinations of water and ice or nitrogen. If temperature control is inadequate, viscosity-increasing ingredients like starch or fibers should be added in the last third of the process. High-water-absorbing concentrates, like citrus fiber, must be handled with care. If added too early in the process, the fiber will bind all the available water and disturb the emulsification process.

When using a bowl chopper, the high-absorption fibers can modify the appearance of the batter obtained during and at the end of the process. During or after chopping, the surface of products which contain phosphate as a cutting processing aid are glossy and the emulsion displays a long structure with good extensibility. When a high-absorption fiber is applied, the free water on the surface is reduced and the meat batter that results is not as glossy and has a shorter texture.

Premixes and Compound Mixes

Fiber concentrates vary greatly in terms of particle size, bulk density, flowability, ability to lump, and dusting. Since many meat processors often work with premixes or automatic dosage systems, these characteristics need to be considered in order to avoid stratification and separation during handling and storage.

Table 4.1 gives a good overview of the particle size and shapes of the existing fibers. The sizes range from a very fine powder (e.g., inulin) to particles of up to 2 mm in diameter (e.g., potato fiber). Depending on their extraction level, cellulosic fibers from wheat and oat have a length up to 500 µm. All powders show good flowability and are easy to mix. Fine powders or fibers have a great tendency to generate dusting. Many fiber manufacturers have produced low dust versions of their fibers by the addition of very small amounts of food grade oils or lecithin. These oils bind the very fine particles and prevent dust during handling. These oils are applied at very low levels and have no impact on fiber functionality. A second option would be granulation through compaction. For most applications, however, compaction granulation is not recommended, as the agglomerated particles formed by this process can greatly increase the hydration time of the fibers. Any remaining agglomerated particles that are not fully hydrated could also lead to textural problems.

Specific Product Applications

Cooked Sausages

There is a large variety of processed meat products worldwide. Despite the use of common labeling names (e.g., frankfurter), products with vastly different formulations and concepts have been launched based on historical and economical distinctions. Below, three different recipes, from Germany, Mexico, and Russia, are given, all of which represent one product commonly described as *Wiener*.

In higher meat quality markets like Germany, the conceptual fiber strategy highlights fiber enrichment, fat reduction and, of course, cost control. High quality meat cuts in the formulation guarantee a stable emulsion and, consequently, a finished product with strong bite, expected snap and superior mouthfeel. With reduced meat content, lower meat quality, and higher fat percentage, the technological benefits of fiber increase significantly. Fibers are added to these products in doses ranging from 0.4% to 2.5%.

German Wiener formulation

Ingredients	%
Beef trimmings 80/20	22
Pork trimmings 80/20	20
Pork cheeks	15
Fat	15
Water/ice	28
Wheat fiber	1.5[a]

[a] Calculated on meat block basis.

After cooking, color evaluation sometimes reveals a slight reduction in redness. Depending on the ratio of fiber to added water, the hemoglobin content in the overall recipe may have been diluted. The use of coloring agents is not universally approved; hence a shift in meat composition can alternatively help. A higher proportion of beef can help regain some of the lost red color.

In Russia, low-cost recipes, binders, and extenders are more common than in Central Europe. This reflects the economic environment of Eastern European countries. Meat substitutes, binders, and extenders are used at higher levels to cope with the technological and cost challenges. High amounts of water and fat need to be bound in the meat batter to reduce losses during heating and storage. Highly extracted, cellulose-based fibers (e.g., wheat fiber, oat fiber) work best under these circumstances. The structural hardness and high fat-binding ability of the highly extracted wheat fiber have resulted in strong market acceptance in Eastern Europe.

Citrus fiber, with its extremely high-water-binding ability, would also be a good fiber option but, in addition to its the acid content, its paste-like structure is not able to create the desired bite or texture in these high-fat recipes. MDM use dominates the product's organoleptic profile; therefore, formulation adaptations need to address the soft and mushy texture associated with it, since most consumers prefer a denser structure and snap.

Russian "Urkainskie" sausage formulation

Ingredients	%
Beef trimmings 90/10	20
MDM	12
Pork trimmings 30/70	13
Skin emulsion	10
Pork fat	20
Water	25
Soy isolate	3[a]
Tapioca starch	3[a]
Plant fiber	1.5[a]

[a] Calculated on meat block basis.

In this product a skin emulsion is used to avoid a soft bite, since skin's high collagen content is effective for improving texture. During skin processing, high temperatures and friction result in a burnt flavor. Cellulosic fibers, which offer a neutral taste and structural support, work well with skin emulsions at acceptable addition levels, without sacrificing taste.

Fibers and Hydrocolloids in Sausage Products. Hydrocolloids like carrageenan, soy protein (texturized, concentrated, and isolated), and native or modified starches are also commonly used in sausage products. Fibers show good synergistic behavior with these ingredients. As opposed to kappa carrageenan or native starch, most fibers do not need a heating step to activate their full technological potential. This is a huge processing advantage. Fibers are easily incorporated to control meat batter viscosity before and during stuffing and also during the formation of meat patties or in the batter and breading of restructured items. Fibers bind water immediately, thus guaranteeing optimum batter consistency during transfer, handling, and stuffing procedures. In low-cost patty formulations, where softness and stickiness cause problems during molding using high-pressure forming equipment, insoluble fibers from potato, sugar beet, pea, grain, or citrus can reduce free water and create a drier, less sticky product surface. A stable fiber network, at dose levels higher than 1.5%, provides the additional benefit of maintaining product shape (Chap. 12). At high concentrations, carrageenan can lead to rubbery or gummy consistencies. Cellulosic fiber grades can economically help reduce the hydrocolloid content and create and maintain a meat-like texture, counteracting this effect. When using citrus and sugar beet fiber, the soluble fiber portion is able to stabilize the emulsion and improve bite. Comparable results can be obtained with pea cotyledon fiber or potato fiber containing protein and starch. In these, a portion of their functionality is delayed until the final heating process, but the fiber and protein components are active from the start of the mixing process.

Several starch types are commonly used in processed meat products worldwide. In the European meat industry, potato starch is common. Its high amylopectin and phosphate content give it a lower gelatinization point and a lesser tendency to retrograde. The higher amylose ratio in other starches leads to faster recrystallization, but purge in the package is often unacceptable. Fibers, with the exception of inulin, can help improve this situation. The high-water-binding and -holding capacity of fibers can be utilized to

reduce free water. Corn starch, often used in combination with MDM, creates a gel with increased firmness. A disadvantage in this case is its high gelatinization temperature and its tendency to lose water during storage. Fibers with good water-holding capacity can reduce water losses and help achieve the desired textural characteristics.

Alternative protein sources are often used to replace expensive skeletal muscle meat. In Central Europe soy protein is not accepted; however, milk, wheat, and other proteins have been used. The high-water-binding properties of fibers allow for the partial or total replacement of these additional protein sources. Milk protein is one of the best emulsifiers available to the meat industry (Chap. 6), but, when dairy prices are high, many manufacturers seek alternatives. Empirical feedback from the Eastern European markets shows that 50–100% of the milk protein could be reduced by addition of wheat fiber. In these cases, manufacturers in these markets work with systems called fat emulsions, which use protein:fat:water ratios of approximately 1:7:7. Due to their sterical structure, insoluble cellulosic wheat or oat fibers are able to bind fat and water in a three-dimensional network, which can help stabilize the emulsion and allow for reduced protein use. A unique processed oat fiber with a higher degree of extraction allows it to bind and hold more fat, due to the removal of most of the lignin during fiber production.

Potato fiber or sugar beet fiber also cause a rough sensation on the tongue and soft palate, and its particles may stick between the teeth. Furthermore, they can also provide sweet potato or dull earthy off-flavors. Using carrot fiber, Nitsch (2003) observed similar flavor issues as well as color changes. At the higher concentrations required for a fiber claim, brownish spots in the meat product could be found. Citrus fiber does not suffer the same mouthfeel limitations as potato or sugar beet fiber, but the low pH level and high price level severely limit its application in sausage.

Ground Meat Products

The use of fibers in ground meat products offers all possibilities: nutritive, economical, and technological. Due to their coarser structure as compared to emulsified sausage, these types of products typically tolerate the use of higher levels of fiber, even up to levels that meet health claims. While fiber enrichment, alone or in combination with fat reduction, is a new trend, fat-reduced products have been on the market since many years. At reduced fat levels, ground meat patties become harder and drier, leading to an unpleasant mouthfeel. Insoluble fiber's ability to bind high amounts of water and to release it under pressure could help overcome these organoleptic drawbacks. The lubricity of some soluble fibers could be another solution. When exposed to high-shear forces these fibers form a thixotropic gel that resembles fat.

Production adjustments are not necessary when using fibers in ground meat products. The fibers should be added early in the mixing process, together with sufficient water to control meat viscosity. The fast water-binding characteristics of fibers aid in high-speed production. As opposed to other binders and extenders, which need to be heated in order to absorb water, cellulosic and pectin-containing

fibers are able to control the water immediately upon mixing. This binding characteristic helps reduce stickiness of the mixture, thus enabling patties to be formed at faster rates. The use of high-water-holding-capacity fibers also helps control frying yields, which is advantageous in the production of prefried ready-to-eat products, as in the foodservice area, for example. A higher postfrying moisture content will generally result in a juicier product. In addition, water bound in the cellulosic structure is not influenced as much by freeze–thaw cycles, as evidenced by observations of reduced ice crystal growth.

Restructured and Injected Ham

In restructured hams, fibers can be added to the tumbler either mixed in with the brine or separately in a later step. Fiber dosage should be adjusted based on the size of the meat chunks and pieces that make up the product's meat block: As the amount of finely ground material is increased, so should the fiber level. Since the fiber molecules are too large to penetrate into muscle, the mechanical action of the tumbler simply scatters the fibers in and around the matrix. By becoming incorporated into the solubilized protein matrix that covers the surface of the meat chunks, the fiber can impact texture and reduce cooking losses and purge.

When injecting fibers, needle diameter as well as size and number of needle holes are critical. To prevent needle blockage and further enable injection applications, fibers with smaller particle size distribution have been developed. Insoluble fibers have a tendency to settle, while soluble ones could increase viscosity and stickiness. Simple solutions to avoid settling are agitation or increased brine viscosity. Specialized brine systems with a defined viscosity are obtained, for example, when using xanthan or cold-soluble carrageenan. After injection, insoluble fiber particles are not able to migrate deeper into the muscle or even penetrate muscle bundles. To overcome particle agglomeration, which can lead to a rough mouthfeel, fiber levels are limited to 0.5–1% of the meat block, and an injection rate of at least 60% is necessary. The good water-holding capacity will help increase the amount of brine.

Dry Sausage

With drying times up to 8 weeks and distinctive weight losses, the production of dry or raw fermented sausages requires good processing understanding. Over the past decade, dietary fiber concentrates have emerged as economical alternatives in this area. Partial meat replacement and faster drying cycles using cellulosic fibers have sparked much interest. In 1999, W. Voesgen, together with the German company J. Rettenmaier, developed the first strategy using different grades of wheat fibers, achieving a 25% reduction in drying time. Long cellulosic fibers well dispersed in the meat batter create a three-dimensional network, first inside the meat sol, and then in the solid meat matrix. The fibers build a "channel" that functions

like a drainage system able to take up water and guide it more rapidly from the area of high humidity, the sausage center, to the surface. This results in faster and more even drying. Comparable results were obtained by Roth (2002), who used wheat fiber, with additional water, to obtain higher yields without any negative influence on pH development and water activities. Harsher drying conditions led to case hardening in the control products, but not in the fiber-containing sausages. The best way to add fibers with additional water is by premixing them. The additional humidity can thus be bound by the fiber in advance and will not influence water activity. Huber, Voesgen, and Le Mintier (2003) have shown comparable results using carrot fiber (i.e., shorter ripening time, improved yield, and increased firmness).

Based on the German regulations for raw fermented sausages, Mueller (2006) highlighted the option of enriching dry sausages with 3%, or even 6%, dietary fiber. Half and half combinations of soluble inulin and insoluble wheat fiber showed good results. The fiber use was not combined with additional water. This functional food concept was expanded with a reduction in fat content and the use of rapeseed oil as a plant fat rich in omega 3 fatty acids, as well as of prebiotic lactic acid bacteria as starter cultures. pH development and water activity in these modified products were not much different than in the standard product.

Conclusion

Growing competition and price expectations have made cost control a never-ending topic in the entire food industry. This has placed enormous pressure on manufacturers to handle the price demands of grocery chains and customers. Therefore, meat quality adaptations utilizing fillers or extenders have found a place in the meat industry as processors search for ingredients with high water-binding and -holding capacities that enable them to develop products that are at the same time cost-effective and of high quality. This has brought many fibers into the spotlight. This combined strategy of cost control and product development with nutritive aspects sounds promising and presents a good alternative to unnecessary caloric intakes. The wide range of fibers available to processors offers technically viable and market relevant options to optimize processed meat products.

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Chapter 5
Plant Proteins

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Introduction

Plant proteins are used to replace the functional and nutritional properties of skeletal muscle proteins in a variety of processed meat products. Throughout the world the most common plant proteins found in meat products are those derived from soybeans or wheat. There are a variety of other plant proteins that are or could be commercially available in the future including pea, potato, corn, canola, rice and various other proteins from legumes and oilseed sources. Worldwide, soy proteins are widely used in the meat industry for their emulsification, gelation, textural/structural, water binding, and nutritional properties. Wheat proteins are also frequently used in processed meat applications; functional properties of wheat proteins include structural, emulsification and water binding. Pea proteins are popular in Europe because they are currently produced from nongenetically modified organisms (non-GMO). Potato proteins are relatively new to the food processing industry and are currently in the early commercialization process in Europe. Plant proteins are available as powdered protein ingredients as well as in the dry textured form.

The use of plant proteins in processed meat systems is regulated in most countries, but regulations differ greatly from country to country. The specific regulations for each country should be consulted prior to using plant proteins in any processed meat application. Specialty low-nitrite and -nitrate plant proteins are produced for use in uncured red meat and poultry applications. These products are manufactured under specific processing conditions to ensure that very low nitrite and nitrate levels are achieved in the plant protein in order to avoid the occurrence of cured meat color reactions in uncured meat applications such as roast beef and pork, chicken and turkey breast, beef patties, chicken patties and nuggets, pizza topping, meatballs, and meatloaf.

Functional Properties of Plant Proteins

Processed meat products are typically formulated with a combination of lean (skeletal muscle) and fat raw materials. The ratio of lean to fat raw meat materials that are used in formulating a processed meat product will determine the fat and protein

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content of the finished product. In the manufacture of processed meat products, the lean meat component is responsible for fat emulsification, product structural integrity, water binding, and finished product color. Plant proteins are most commonly used in processed meat products for their functional replacement of the lean meat component for economic reasons. Soluble plant proteins can be used to emulsify fat in finely comminuted meat products such as frankfurters and/or to bind fat in coarse ground meat products such as beef patties. Some plant proteins have gelation or structural properties and provide textural integrity as well as emulsification properties to processed meat systems. Soluble plant proteins can also be injected into whole muscle meats to provide structural integrity; increase water binding; improve product sliceability; and/or enhance the eating qualities (i.e., succulence). These would include hams, roast beef and pork, pastrami, corned beef, and bone-in, boneless and whole carcass poultry products. Plant proteins can also simply be used as water binders or extenders in processed meats. Products such as toasted soy flour, traditional soy protein concentrates (SPCs), wheat flour, or vital wheat gluten represent a group of protein ingredients that are typically used primarily for their water absorption properties. Soy protein, wheat gluten, and pea products, alone or in combination, are currently available as textured or structured pieces that can be used to replace lean meat in coarse ground products such as taco fillings, pizza toppings, meatballs, smoked and/or cooked sausages, and beef patties. These textured products are manufactured through thermoplastic extrusion of the protein under moist heat and high pressure using single-screw or twin-screw extrusion equipment. Textured proteins are typically sold in dry form and are hydrated with water prior to their use in processed meats. These textured proteins can be purchased in a variety of shapes and sizes, uncolored or colored (i.e., caramel or malt), unfortified or fortified (vitamins and mineral), and with varying protein contents (textured soy flour versus textured SPC).

Hydration of Soluble Plant Proteins

The functional properties of soluble plant proteins are maximized through proper hydration of the protein during the manufacturing process of a given food. Improper hydration of the protein can result in an overall decrease in the functional properties of the plant proteins including decreased emulsification capacity and stability, less structural and textural integrity, and insufficient water holding, resulting in decreased yields upon cooking and/or freezing or purge issues during storage. Salt can have a major effect on the functional properties of many plant proteins if the salt is present in the processed meat system prior to the hydration of the plant protein. Therefore, it is important to properly hydrate the plant protein in the absence of salt whenever possible. The solubility and resulting degree of hydration can be significantly reduced in ionic environments.

There are several methods to ensure the proper hydration of the plant proteins in the various processed meat systems. In injection applications where proteins are

used, proteins are usually hydrated in the formulation water prior to the addition of salt. This allows for the protein to hydrate in an environment where water is not competing with the sodium and chloride ions for the water bonding sites on the protein. The most common practice for preparation of injection brine solutions is to first dissolve the phosphates in the brine formulation water followed by the hydration of the plant protein. Plant proteins should be hydrated in cold water for a minimum of 10–15 min prior to the addition of the remaining ingredients. High shear is required to initially disperse the protein, but agitation should be reduced after dispersion of the protein to avoid air entrapment and foam formation.

Hydration of plant proteins in emulsified and coarse ground meat systems has traditionally been accomplished through the production of protein gels. These gels are manufactured in bowl choppers where one part protein is chopped with four to five parts water and/or ice until the protein gel develops a high sheen (indication that the protein is sufficiently hydrated). These protein gels can then be incorporated into emulsified and coarse ground meat systems; or can be used to form fat emulsions that can later be used in product manufacture. Many meat processors throughout the world prepare protein gels and fat emulsions as a method to obtain the maximum functionality from plant proteins. The development of high throughput processing equipment has resulted in the need for less labor intense processes for hydration of the protein. Today, protein hydration is commonly accomplished in large ribbon or paddle blenders through the addition of the protein directly to the coarse ground lean meat components of emulsified or coarse ground meat formulations. Addition of the protein to the lean meat results in an increased surface area for protein hydration, facilitating rapid hydration of the protein upon the addition of the hydration water (five to eight parts per one part protein). After proper hydration of the protein, salt, phosphates, and curing ingredients are added to the meat mixture. This meat mixture is continuously blended until the desired protein extraction of the lean meat component has been achieved. This is followed by addition of fat raw materials, carbohydrates (i.e., sugars and starches), and seasonings; the meat system is further blended until the added raw materials and ingredients are evenly distributed. Emulsified products are then passed through an emulsification system and coarse ground products are typically ground to a finer particle size prior to further processing.

Fat Emulsification

Fat emulsification is one of the key functional properties of plant proteins in processed meat systems. Emulsions are simply defined as dispersions of one liquid inside of another (Kinsella, Whitehead, & Bringe, 1989). In the case of processed meat products, soluble proteins are used at the water and oil interface to surround oil droplets within the meat system. These soluble proteins are considered emulsifying or stabilizing agents. The unique properties of emulsifying agents are that they have the ability or affinity to interact with both the water phase and the oil or fat phase of

the meat system (Judge, Aberle, Forrest, Hedrick, & Merkel, 1989). The emulsifying characteristic of plant proteins can vary based on protein source, solubility, and concentration, as well as the processing conditions under which the protein was manufactured. Specifics in regard to emulsification properties of plant proteins from different sources will be covered later in this chapter.

Water Binding

Water binding is another critical functional property required in plant proteins that are used in processed meat systems. Most conventional meat systems contain at least 50% moisture and up to as much as 80% moisture; good water binding is, therefore, essential in these processed meat products. Consumers typically avoid packaged meat products that contain purge (freestanding water). Processed meat products that have poor water holding capacity and/or fat binding properties have the tendency to lose liquid during the cooking and/or freezing processes, resulting in increased production costs for the manufacturer. There are various other terms that have been used to describe water holding capacity, such as water binding, hydration capacity, water absorption, water embedding, and water retention (Evergreen, 1961). It has been suggested that composition as well as the conformational structure of a protein play a major role in its water holding capacity (Hawley, Frederiksen, & Hoer, 1972). Water binding properties of proteins can also be influenced by a variety of factors within the meat system, including pH and salt concentration. Proteins bind the least amount of water at their isoelectric point, which tends to be in the acidic pH ranges that can be approached in some processed meat systems. The sodium and chloride ions of salt can interfere with water binding at various sites on the protein molecule. In the case of functional soy proteins, it is suggested, as previously described, that an attempt be made to hydrate the soy protein prior to addition of salt to the meat system to maximize functionality. The water holding capacity of the plant protein used in processed meat is critical to the overall eating quality as well as shelf life of the product.

Structural and Textural Integrity

Lean skeletal muscle is responsible for providing much of the textural and structural properties associated with processed meats. Plant proteins can be used to (1) enhance the textural properties of processed meat systems through the incorporation of additional protein into the product matrix or (2) supplement the textural properties of a processed meat product when plant proteins are used to replace lean skeletal muscle. Plant proteins are available as fine protein powders or as dried fibrous textured products which have been manufactured through the use of thermoplastic extrusion technology.

Many of the powdered protein products have thermal gelation properties similar to the thermal gelation or coagulation properties of the myofibrillar proteins found in lean skeletal muscle. Protein gelation is typically thought to be the result of the formation of partially associated polypeptides, three-dimensional matrices or networks, in which water is entrapped and the network exhibits structural rigidity (Catsimpoolas & Meyer, 1970; Kinsella, 1979). This gelation or coagulation of protein increases the rigidity of the product and is perceived as hardness or firmness in the finished meat product. The desired textural characteristics of each processed meat product can vary (1) between product categories, (2) with product quality, and (3) from region to region throughout the world. For example, the textural characteristics of a frankfurter might be considered firm and elastic, whereas a pate is soft and smooth. The textural characteristics of each processed meat product are dependent on product formulation (i.e., raw meat materials and nonmeat ingredients) as well as the processing conditions employed in the manufacture of the final product.

Each plant protein will have unique gelation properties associated with its use. Plant protein isolates will typically have higher gelling properties than plant proteins with lower protein content. In the case of soy proteins where products are compared in a model gel system, isolated soy proteins (ISPs) produced for use in emulsified meat systems form very firm elastic gels compared to functional soy protein concentrates (FSPCs) which form softer, less elastic gels. This is thought to be the result of the insoluble fiber compounds present in FSPC, which interfere with formation of the protein matrix. When these two proteins are used in an emulsified meat system at a level of 2–3% of the product formulation (10–15% of lean meat replaced; protein and water) there is typically little, if any, difference in the textural integrity of the finished meat product. However, a textural difference in finished product quality would probably be perceived between meat products produced with ISP compared to FSPC at lean meat replacement levels of greater than 20% (protein and water equate to the use of greater than 4–5% protein ingredient). There are also differences found among the plant proteins in gelation properties. ISPs have been found to have greater gelation properties compared to pea protein isolates (O'Kane, Happe, Vereuken, Gruppen, & van Boekel, 2004). The impact of differences in gelation properties can usually be compensated through the use of lower protein to water ratios in the finished meat product (e.g., use of 1 part protein to 3 parts water instead of a 1:4 ratio).

Textured or structured plant proteins can be made from many protein sources including soy flour, concentrate, or isolates; pea protein isolates; and wheat gluten. These textured proteins are produced in many sizes, shapes, colors, and flavors. Bacon colored and flavored products are among the most popular products made from texturized soy flour. Unique textured protein products can be manufactured through the use of combinations of plant proteins or other powdered ingredients such as various carbohydrate sources (e.g., starches or fibers). Textured products manufactured through the use of thermoplastic extrusion technology are distributed throughout the world in dry form. Textured protein products are typically hydrated with water prior to their incorporation into coarse ground meat systems such as beef patties, meatballs, and cooked sausages. These products can hold between two and five times their weight in water, depending

on the product choice, and once hydrated can be used on a one to one basis to replace a portion of the lean meat component in product formulations.

Product Storage and Handling

Many of the plant proteins are highly functional ingredients that require a high degree of solubility in order to function properly. These proteins possess their greatest functional properties the day they are manufactured and are typically given a shelf life of one to two years. Plant proteins are packaged in materials that provide maximum functionality over time when held under good storage conditions (below 25°C and 60% relative humidity). Storage conditions of high heat and/or humidity can result in the rapid deterioration of the functional characteristics of plant proteins, regardless of the quality of the packaging materials. This decrease in functionality has been found to be closely associated with a rapid decrease in protein solubility. As discussed previously, solubility is closely related to the emulsification, gelation, water binding, and viscosity properties of plant proteins. Processed meat manufacturers should take storage conditions and proper ingredient rotation into consideration when using functional protein ingredients in their production facilities. Product developers should make sure that they are working with fresh protein samples and ensure that these protein samples are stored in closed containers under the proper storage conditions, as noted above, or under dry, refrigerated conditions if possible, to minimize loss of functionality. These protein samples should also be replaced on a regular basis every 6–9 months.

Nutritional Properties of Plant Proteins

Plant protein ingredients are typically added to meat products for their functional benefit and as an economical replacement of lean meat raw materials. When plant proteins are used at low levels they have little impact on the overall nutritional protein quality of the finished meat product. As the concentration of plant protein increases in the product formulation there can be a significant decline in the nutritional protein quality of the finished product, depending on the plant protein used. Nutritional protein quality or nutritive value of the protein varies from protein to protein depending on the essential amino acid profile of the particular protein. For many years, Protein Efficiency Ratio (PER) was the accepted method for determining the nutritive value of proteins. This method measures the ability of a given protein to support the growth of young, rapidly growing rats. The essential amino acid requirements for these young rats were found to be quite different compared to that of humans. In 1991, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (FAO/WHO, 1991) issued a report recommending that Protein Digestibility Corrected Amino Acid

Score (PDCAAS) be used as the accepted method for determining the nutritive value of proteins for human consumption. PDCAAS can be used to evaluate food protein quality based on essential amino acid requirements for humans after adjusting for the digestibility of the protein. The PDCAAS is based on the amino acid requirements for a 2–5-year-old child and compares the essential amino acid pattern of a given food protein with the human requirements for the essential amino acids. The PDCAAS value is calculated as follows:

$$\text{PDCAAS} = \frac{\text{amino acid pattern of protein}}{\text{amino acid requirement for human}} \cdot \text{protein digestibility}$$

The PDCAAS values for some of the plant proteins discussed in this chapter are as follows: ISP, 0.92; SPC, 0.99; pea protein concentrate, 0.73; rapeseed proteins, 0.83–0.93; and wheat gluten, 0.25 (FAO/WHO, 1991).

Protein quality plays a major role in processed meat products that are produced and sold into the US School Lunch Program. The USDA Food and Nutrition Service (FNS) allows for the use of alternative protein product (APP) in meat products to be used in meal plans, but the products must meet various criteria in order to be used in the National School Lunch Program, School Breakfast Program, Summer Food Service and Child and Adult Care Food Programs (United States Department of Agriculture, Food and Nutrition Service [USDA-FNS], 2000). These criteria include that the APP must have a biological protein quality of at least 80% that of casein, as determined by performing a PDCAAS. The PDCAAS value for casein is 1.0 (FAO/WHO, 1991); thus the APP must have a PDCAAS of at least 0.80. From the PDCAAS scores noted above, some of the plant proteins do not meet the minimum nutritive protein content required for the National School Lunch Program, School Breakfast Program, Summer Food Service and Child and Adult Care Food Programs. Given their high PDCAAS, soy proteins are the most widely used proteins to meet the FNS requirements for APPs.

Protein nutritive quality could also play a role in developing countries where minimum daily requirements for protein are not being met.

Plant Proteins

Soy Proteins

Soy proteins have been used in the processed meat industry for over 40 years. They were first used as extenders to lower the cost of various processed meat systems. These protein products absorbed water but provided little other benefit to the overall quality of finished meat products. The first textured soy proteins were introduced in the early 1970s after Archer Daniels Midland Company developed and commercially produced the first textured soy flour products through thermal extrusion

(Atkinson, 1970). The meat industry embraced the use of these new textured vegetable proteins (TVP®) to lower the cost of coarse ground meat systems, such as beef patties for the School Lunch Program. The cost savings for using these new textured protein products were so attractive that meat processors extended their products to such an extent that product quality suffered. As a result of the poor quality, sales of these meat products declined, leading to a reluctance by processed meat manufacturers to use soy proteins in their products. In the USA, it took many years for processed meat companies to re-introduce soy protein into their products. Today, the quality and functional properties of soy proteins have improved significantly, and, as a result, soy proteins have become widely used in processed meat systems worldwide. In fact, the worldwide processed meat industry is the largest consumer of soy protein products for food use in the world today.

Soy proteins are produced from the defatted meal stream of commercial soybean (*Glycine max* L.) crushing operations. Today's commercial soybeans contain approximately 20% oil, 40% protein, 30% carbohydrate, with the remaining 10% being moisture and ash. Soybean processing typically involves cracking the soybean to remove the hull, followed by rolling of the cracked halves into full-fat flakes (Fig. 5.1). This rolling process disrupts the oil cells and helps facilitate solvent extraction of the oil from the full-fat flakes. After the oil has been solvent-extracted, the solvent is removed from the flakes through controlled drying conditions, creating defatted soy flakes. Based on drying conditions, defatted flakes can be produced that vary in protein solubility. The protein solubility of soy flour products is typically measured using a method known as Protein Digestibility Index (PDI), while protein solubility in SPCs and ISPs is measured by the Nitrogen Solubility Index (NSI) method (Firestone, 1993). Commercial crushing operations that are extracting oil for food use, but where the defatted soybean meal is intended for animal feed, process defatted flake that has a PDI of less than 30%. Operations that are producing soy proteins for food use typically produce defatted flakes that fall into three PDI ranges: less than 30%, 60–70%, and 80–90%. These different PDI ranges are achieved through the use of specific temperature and relative humidity (RH) conditions. Lower PDI material is produced through the use of higher temperature and lower RH, while higher PDI material is produced through the use of lower temperatures and higher RH. Each of these types of defatted flakes is produced for specific applications within the food industry. The defatted flakes with a PDI of <30% are known in the industry as toasted soy flour products and can be purchased in forms ranging from flours to grits or bits having a protein content of 50%. From a functional standpoint these proteins absorb water but have little ability to bind fat.

In addition to soy flour/grits, there are other protein ingredients that can be produced from the defatted soy flakes. SPCs (70% protein, moisture free basis) and ISP (90% protein, moisture free basis) can be produced through removal of the carbohydrate components from the defatted flake. Defatted soybean flakes contain two carbohydrate fractions: an insoluble cellulose-based fraction and a soluble fraction composed mainly of sucrose, stachyose, and raffinose. SPCs are composed of the protein and insoluble carbohydrate fractions of the soybean. SPCs are produced through the removal of the soluble sugars either through extraction via aqueous

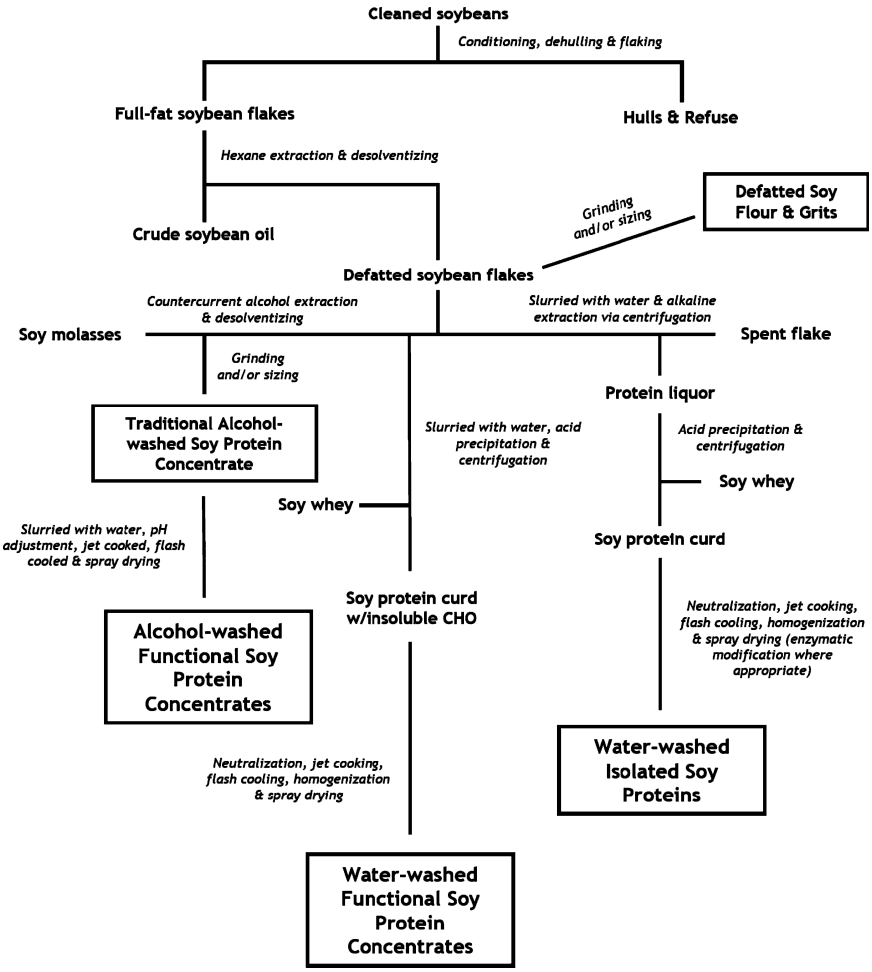

Fig. 5.1 Soy protein processing schematic

alcohol (typically ethanol) or through acid precipitation known as water washing (Fig. 5.1). For purpose of discussion here, SPC products that are produced from alcohol washing will be referred to as traditional SPC products. These traditional SPC products, as with the toasted soy flour products mentioned above, bind water but have very low fat binding properties. FSPCs can be produced through neutralization of water-washed SPC slurries or through the mixing of traditional SPC with water to form protein slurries that are then processed by jet-cooking, flash-cooling, homogenization, and spray-drying. Jet-cooking is the process where extracted protein slurries are heated almost instantaneously under pressure through injection of live steam into the product stream. These steam injection systems are commonly

referred to in the manufacturing industries as Jet Cookers. The injection of live steam into the protein slurry results in rapid temperature elevation as well as severe physical disruption of the protein matrix. Flash cooling is the process of discharging the pressurized heated slurry into a lower pressure zone, typically under vacuum. This sudden drop in pressure results in the instantaneous reduction in temperature as well as the release of volatile unwanted flavor and odor components (Hawley et al., 1972). These FSPCs typically have protein solubility between 40% and 80%, have the ability to gel when heated, emulsify fat, and bind water. Worldwide, FSPCs are used widely throughout the meat industry for their superior fat emulsification properties.

ISPs are produced through the removal of both carbohydrate fractions from the highly soluble (>80% based on PDI), defatted soybean flakes (Fig. 5.1). The insoluble carbohydrate is separated from the defatted flake through centrifugation of the soluble protein slurry. The soluble sugar fraction can be removed through water washing, aqueous alcohol washing, or membrane separation. The most common practice in the soy protein industry today for the manufacture of ISP is to first remove the insoluble fraction through centrifugation followed by final separation of the protein from the soluble carbohydrate fraction through water washing of the protein. The protein slurry is then neutralized, jet-cooked, flash-cooled, homogenized, and spray-dried in a manner similar to the FSPC process. The functional and physical characteristics of both FSPC and ISP can be modified through controlling the final pH of the protein prior to drying as well as through modification of other processing parameters. A wide range of ISP products are produced that possess specific functional properties for use in processed food systems. Highly soluble protein products with high gelling, viscosity, and emulsification properties (ISP A, Table 5.1) are manufactured for emulsified meat applications, whereas ISP products with moderate to high solubility, gelling, and viscosity properties are manufactured for use in injected meat applications (ISP B and C, Table 5.1).

Table 5.1 Functional characteristics of various soy proteins

		Solubility	pH[a]	Viscosity	Dispersibility	Gelation	Emulsification	Water binding
Isolated soy protein	A	9	9	9	3	9	7	9
	B	5	6	5	6	5	5	7
	C	8	8	4	4	5	7	7
Soy protein concentrates	D	8	9	7	4	4	9	7
	E	9	9	3	6	5	7	6
	F	7	9	5	3	2	6	4
	G	1	8	1	9	1	1	1

9 = very high; 5 = moderate; 1 = very low.
[a]pH range ~4.5 to 7.5.

Wheat Proteins

Wheat glutens were once a by-product of the wheat starch industry and initially were used as animal feed. Simplistically, wheat gluten isolation results from mixing wheat flour and water, kneading the mixture to form dough, and then gradually water washing the dough to remove the starch from the matrix. There are several variations on this process (Day, Augustin, Batey, & Wrigley, 2006). The wet gluten protein is then carefully dried to maintain functionality through ring or flash drying. Wheat gluten proteins range in molecular weight from 30,000 to more than 10 million (Weiser, 2006), indicating that they are made from a collection of different proteins. The gluten proteins have been further classified into groups based on their solubility in aqueous alcohol. Wheat proteins that are soluble in aqueous alcohol are gliadins and the insoluble fractions are the glutenins. Glutenin proteins are responsible for the strength, cohesion, and elasticity of gluten while gliadins are responsible for viscosity and extensibility. The two protein fractions in combination allow the gluten collectively to have a firm, viscoelastic texture that can stretch while maintaining strength.

Wheat gluten is primarily used in the manufacture of bakery products, where it acts to trap air and forms the light cell structure that gives bread its classic texture. Because of the strength of their disulfide bonding and entanglement, collectively the gluten proteins are very good at increasing texture and bite characteristics. While native wheat glutens themselves are not particularly soluble in water, once hydrated and/or modified they possess excellent film-forming abilities and adhesive properties (Kolster, de Graaf, & Vereijken, 1997), and once hydrolyzed they are effective at producing and stabilizing foams (Day et al., 2006).

Wheat gluten has not found the widespread use in the US meat industry as other plant proteins; however, it finds more widespread use in Oceania. This is at least partially due to nutritional inadequacies when compared to other proteins, as well as celiac concerns. While gluten is available in many functional forms, including (1) native vital wheat gluten, (2) solubilized forms, manufactured by deamidation or various degrees of enzyme hydrolysis, or (3) de-vital, these proteins do not typically have the gelling characteristics necessary for use in many processed meat systems. Devital gluten is a gluten that has been denatured to the point that it possesses little if any viscoelastic behavior (Codex, 2001; Wookey, 1979). This is generally done by heat treatment. In this way the protein portion can be used in liquid applications where the viscoelastic function is unnecessary. Gluten proteins are roughly one-third glutamic acid, which makes them useful in producing hydrolyzed proteins for flavorings. Recent research indicated that gelling properties of wheat gluten may be improved through combinations of transglutaminase and heat treatment (Wang, Zhao, Yang, Jiang, & Chun, 2007).

Although studies have been conducted using wheat gluten in mortadellas (Hand, Terrell, & Smith, 1983), meat batters (Patana-Anake & Foegeding, 1985), and restructured beef steaks (Hand, Crenwelge, & Terrell, 1981; Miller, Davis, Seideman, Wheeler, & Ramsey, 1986), among other products, most of the studies

demonstrated no compelling reason to use wheat gluten in the aforementioned products. Gluten has found more widespread use in coarse ground meat applications in the form of texturized pieces that mimic meat. Gluten is extensively used in combination with soy proteins to produce meat analogs. It is also used in meat analog applications where, because of its contribution to firming, it acts to give textured protein pieces a more meat-like bite characteristic. Similarly, powdered vital wheat gluten can also help form the chewy matrix necessary to replace meat eating characteristics. This is true when firmness of a product is needed when replacing lean meat in a product formulation.

Pea Proteins

Pea proteins are produced through the separation of the nonprotein component from field peas (*Pisum sativum* L.). Peas come from the legume family and contain between 20% and 30% protein, over 65% of which is made up of two storage proteins, legumin and vicillin (Schroeder, 1982). Pea proteins can be produced through air classification of pea flour to separate the starch fraction from the protein fraction. The pea flours are manufactured from whole or dehulled field peas through a pin milling process (Tian, Kyle, & Small, 1999). Pea protein concentrates produced through this physical process have been found to possess poorer functional properties in comparison to pea proteins produced through a wet process (Gueguen, 1991). The most commonly used wet process uses pin-milled pea flour and water to produce a slurry, which is adjusted to pH 9–10. This slurry is centrifuged to separate the carbohydrate solids from the soluble protein solution. In a process similar to soy protein, pea protein is acid-precipitated through acidification of the soluble protein solution to the protein's isoelectric point (~pH 4.3–4.5). The precipitated protein extract is then neutralized to pH 7 and spray dried to produce 90% pea protein isolates (Sumner, Nielsen, & Youngs, 1981; Swanson, 1990; Tian et al., 1999). These pea protein isolates are soluble proteins with good gelation, emulsification, and water binding properties that are needed for applications in processed meat systems.

Other Plant Proteins

There are a variety of other plant proteins that have been studied and evaluated for their functional properties and characteristics; however, only a few of these protein sources are under active development by commercial manufacturers. Plant proteins that are under development for use in food applications include canola (rapeseed), potato, and rice.

Incorporation of Plant Proteins into Processed Meat Systems

Emulsified Meat Products

Worldwide, plant proteins are most widely used in the meat industry in emulsified meat systems. Although these plant proteins help provide part of the nutrient protein requirements for some countries, the primary use of plant proteins is to provide functional benefits in a processed meat system at an economical cost. Functionally, plant proteins provide effective fat emulsification, structural and textural integrity, and water binding. Plant proteins can also reduce purge and improve product yield in these systems.

Soy proteins, including ISP and FSPCs, are widely used in emulsified meat applications. ISP products are typically used in emulsified meat systems where greater than 15% (~3% ISP) of the lean meat component is replaced with hydrated soy protein, but can be used at lower levels. In these emulsion systems, ISP helps maintain textural properties of meat products while providing fat emulsification and water holding properties. ISP used in emulsified meat systems is highly soluble and has moderate to high gelling characteristics similar to ISP A or C (Table 5.1). Most countries have regulations which either regulate minimum meat content and/or minimum protein content requirements in their processed meats. In South Africa, there are minimum protein requirements for meat products and few restrictions on the amount of plant protein used in many processed meats. Emulsified meat products in South Africa typically contain 5–10% ISP, which equates to 25–50% on a hydrated basis. In this case, soy protein is the most economical source of protein and provides textural and structural integrity to the product. FSPCs are also widely used in emulsified meat products throughout the world, primarily for their ability to emulsify fat and help maintain the textural integrity of the finished product.

FSPCs work well in emulsion systems that have a high percentage of meat in the product formulations, as is the case in the USA. In Latin America and Eastern Europe, FSPCs also work well in emulsified meat systems where other water binders, such as starch (Chap. 2) and carrageenan (Chap. 3), are used to extend the meat system. Worldwide, the use of FSPC in emulsion systems continues to grow each year and has resulted in the development of a variety of FSPC products with specific functional properties and cost structures (see D–F in Table 5.1). In Europe, pea proteins are used in a similar manner to soy proteins. The use of pea protein products has grown significantly in this market as a result of the introduction of genetically modified soy products throughout the world, but the market has been slow to return to soy proteins despite the development of identity preservation programs where nongenetically modified soy protein products are produced under tight documented controls from the farm and throughout the remainder of the product chain to the end user.

Wheat proteins are used in emulsified meat systems in several countries throughout the world, including Canada, Mexico, Australia, and New Zealand; however,

their use worldwide is not widespread. There are various types of wheat protein products that are used. This would include wheat flour, wheat flour and vital wheat gluten blends, vital wheat gluten, and modified and/or soluble wheat proteins. Wheat flour and vital wheat gluten blends can range from 12% to 50% protein with the primary remaining component being the starch fraction. The modified/soluble wheat proteins typically have good fat emulsification properties but tend to provide little structural integrity to emulsified meat systems, while the other protein products from wheat help provide structural integrity but have limited fat emulsification properties. Wheat is typically added to an emulsion in the form of wheat flour, where processors make use of the protein content from the gluten and the starch portion to help improve water binding and stability.

Injection and Marination Applications

Injection has been used for many years as a means to distribute a brine solution, containing at least water and salt, into whole muscle meat products. In the case of cured meat products, the brine solution would normally include water, salt, sugar, phosphate, nitrite, and erythorbate or ascorbate. Hams, roast beef, pastrami, corned beef, roast pork, fish fillets, turkey breasts, and other whole muscle deli meats are a few of the meat products that can be produced through the use of injection technologies. Plant proteins can be introduced into these brine solutions to increase water binding and reduce purge, to enhance the textural and slicing properties of the product, to decrease product shrinkage (i.e., increase final yield), and to extend the whole muscle or chunked and/or ground meat raw materials. Meat can also be marinated with solutions containing plant proteins to improve the tenderness and/or succulence of meat products, deliver unique flavors to the meat, and maintain and/or increase the holding properties of processed meats in high abuse circumstances, such as when products are held for extended periods on steam tables. Marinade solutions typically contain similar ingredients to those of injectable brine solutions but may also contain particulate seasoning, spices, or other particulate materials that enhance the eating quality and appearance of the finished product (particulate ingredients that can not typically be injected). Whole muscle meats such as chicken breasts, chops, steaks, shrimp, stew meats, and fajita meat pieces are a few of the meats where marinades are used.

In whole muscle and chunked and formed injected meat systems, brine solutions are typically formulated to contain a combination of salt, phosphate, sugars, starches and/or plant protein, and/or food gums (e.g., carrageenan), depending on level of injection/extension. The solutions are injected into whole muscle or muscle pieces, which are then tumbled or massaged to distribute the solution throughout the muscle and to extract salt-soluble muscle proteins. These whole muscle or muscle pieces (muscle pieces being stuffed into casings) are then typically cooked to produce the finished product. Plant proteins used in injection and marination applications have similar characteristics to soy proteins B, C, and E in Table 5.1. Wheat

glutens may be modified by increasing their solubility enough to form dispersed solutions that can be injected into meat products; however, this practice is not widely used in the processed meat industry, as there are better protein alternatives for this application. Wheat protein products have been developed in Australia and Europe to replace soy proteins where ingredients from genetically modified plants are not accepted. As in other processed meat applications, proper hydration in the absence of salt is critical to achieve the desired functional water holding properties and structural integrity from these proteins.

Injection is probably most widely used in the production of boneless ham products around the world. The injection/extension level in these products can range from as little as 10% above the green weight (original weight of meat prior to injection) of the ham raw material to levels over 200%. The level of injection or extension of boneless ham products varies widely from country to country and exact regulations should be determined prior to the development of any product. Within this wide range of injection/extension levels, the ingredient combinations and concentrations vary significantly. In products that are injected 10–25%, the functional properties of the soluble muscle proteins are typically sufficient to provide the necessary functional properties in these products. The use of salt and phosphate alone (see Table 5.2) in low-level injected products is sufficient to provide the functional properties required to produce acceptable finished product. Salt (sodium chloride) is used to solubilize the muscle proteins and to develop flavor in whole muscle and chunked and formed meat systems, while alkaline phosphates are used for their ability to enhance water binding capacity of the meat and reduce shrinkage of the injected ham product through subsequent processing. As injection levels increase between 25% and 50%, it is necessary to incorporate other water binding ingredients into the injected meat system in order to have sufficient water binding capacity to hold the additional formulation water. At the 25–50% injection level, plant protein, carrageenan, or starch (typically modified), alone or in combination at lower concentrations (Table 5.2), can be used to achieve the required water binding and textural properties. Many of the ham and water products produced within the USA would fall in the 50–100% injection level. These products take additional care in formulating and manufacturing in order to obtain the water binding and textural properties required to produce an acceptable product that will retain the added water throughout the shelf life of the finished ham product. Table 5.2 gives recommendations regarding the ingredient levels that are required to produce a quality finished product. Combinations of plant proteins, carrageenan, and/or starch are used in formulation of ham products in the 50–100% injection level.

The final category of ham products are those that are extended to levels greater than 100% above the meat green weight. These ham products are produced in several countries and typically are chunked/ground and formed products. Because of the increased extension level, these products require both careful product formulation and very highly controlled manufacturing processes that take full advantage of lean meat raw material functionality as well as the functionality of nonmeat ingredients (see Table 5.2). These products are highly economical and work well as sliced meat for sandwiches or other applications where the product is eaten chilled.

Table 5.2 Typical non-meat ingredient addition levels used in the manufacture of boneless hams

Infection Level (%)[a]	Typical non-meat ingredient addition in finished meat product (%)						
	Salt	Phosphate	Protein		Carrageenan		Starch
10–25	1–2	0.2–0.5					
25–50	1.5–2.5	0.3–0.5	1.5–2.5	or	0.2–0.3	or	2.0–3.5
50–100	1.5–2.5	0.35–0.50	2.0–3.5	and	0.2–0.6	or	3.0–5.0
>100	2.0–3.5	0.4–0.5	2.0–3.5	and	0.5–1.0	and	5.0+

[a]Level of injection above the green weight (original meat weight) of the boneless ham meat.

Coarse Ground Meat Products

Coarse ground meat products include products such as patties, nuggets, meatballs, meatloaf, pizza toppings, sausages, and restructured fish products (cakes and sticks). Textured plant proteins are the most widely used plant proteins in coarse ground meat systems. The primary textured products used in these products are textured soy flour (TVP®) and textured SPCs; however, textured wheat proteins and pea proteins are available in some markets. There are also textured products manufactured for meat and meat analog applications that use a combination of soy protein and wheat protein. These textured proteins tend to have a firmer meat-like texture in comparison to textured soy or pea proteins. The textured wheat proteins are the most elastic and resilient of the textured protein products and are most commonly used in meat analog applications. Textured plant proteins absorb water and when fully hydrated provide texture similar to coarse ground meat. These textured products are often used in conjunction with soluble plant protein ingredients that have the ability to bind water and fat, such as an FSPC.

In the processing of coarse ground products with plant proteins, the textured plant proteins are first hydrated to the appropriate level using chilled water. Textured protein hydration can take 5–10 min for flaked materials and 20–30 min for small granular material (3–7 mm). Hydrated textured plant proteins are commonly used at a level of 10–30% to replace part of the lean meat component of the product formulation, while the dry functional protein powders are used at a level of 1–2%. Typically, the lean and fat meat raw materials are coarse ground and the raw materials are placed in a mixer with the hydrated textured protein and allowed to mix sufficiently long to obtain a uniform mixture. The functional plant protein is then added and allowed to disperse over the mixture followed by the remaining nonmeat ingredients, including seasoning. The product is allowed to mix sufficiently long enough to achieve the desired extraction of soluble meat protein prior to final grinding and further processing.

Dry and Semidry Fermented Sausages and Other Dry Meat Products

Dry and semidry fermented sausages are chopped or ground meat products having a pH lower than 5.3, as a result of bacterial fermentation or direct acidification with

organic acids (Chap. 11). These products then undergo a drying process to remove 15–50% of the moisture, depending on the specifications of the final product. Dry/semidry fermented sausages include products such as salami, pepperoni, and summer sausage; other dried meat products would include products such as jerky, dried beef, and snack sticks. As with most meat products, the incorporation of plant proteins into dry and/or fermented meat products is used for cost control measures. Plant proteins can be used to replace lean muscle protein for cost reduction measures but can also be used to replace fat for the production of reduced fat products. Application of the plant proteins in these applications is typically accomplished through the production of protein gels that are then reduced in particle size to simulate ground fat and/or lean meat. These granular products can be produced from ISP alone or in combination with SPC or through the use of plant proteins in combination with an animal protein material through several methods as described in the patent literature (McMindes & Richert, 1995; Mott & Johnson, 1993; Parks & Greatting, 1992; Payne & Egbert, 1997; Payne & Egbert, 1999). Proteins used for making these granular products would have similar functional properties to soy proteins A and D in Table 5.1. Granules and/or crumbles produced for the replacement of lean meat are usually colored to produce protein granules/crumbles that resemble the color characteristics of the cured red meat being replaced. One of the major benefits of this process is that meat-like texture and particle definition (typical appearance) can be maintained in reduced fat products as well as in products where the granules/crumbles have been used to replace a portion of the lean meat component. Dry plant protein powders can also be added directly to the dried/fermented product during the manufacturing process. Addition of the dry plant protein increases the protein content of the meat product and decreases the moisture to protein ratio, which can shorten the product's overall drying time and potentially increase production throughput. Plant proteins used for dry addition to dry fermented sausages usually have functional characteristics and pH in the moderate to low range, similar to soy proteins B, F, or G (Table 5.1), where high gel strength, emulsification, water binding, and solubility are not typically desired. Plant proteins with these lower functional characteristics tend to allow for quicker drying under traditional drying conditions.

Summary

The availability and use of plant proteins will need to continue to grow into the future as the world population grows and as this population becomes more prosperous and their meat consumption patterns increase. With this increase in population and an ever-increasing demand for animal protein, there will be a greater need for plant proteins to fill the gap between world animal protein production capabilities and the world demand for protein-based food products. The functional properties of the current plant proteins will need to continue to improve over time and new plant proteins will need to be developed in order to meet the opportunities for future growth in the protein-based food marketplace. Meat processors should begin to

work closely with the manufacturers of plant-based proteins to ensure that their future needs are met from a functional protein standpoint as well as from a technological knowledge base with regard to the use of plant proteins in their processed meat systems.

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Chapter 6
Dairy Proteins

Youling L. Xiong

Introduction

Dairy proteins are generally divided into two groups: caseins and whey proteins, which are distinctly different in their structural, physical, chemical, and functional properties. In fresh milk, caseins exist in micelles (130–160 nm in diameter), which are formed by the association of individual submicelles (~10 nm) through amorphous calcium phosphate bridges (McMahon & Brown, 1984). The micelles exhibit hydrocolloidal characteristics and are readily suspended in the aqueous phase of milk.

In bovine milk, there are four casein proteins, i.e., α_{s1}-, α_{s2}-, β- and κ-caseins, which represent approximately 38%, 10%, 36%, and 12% of whole casein, respectively (Table 6.1). Caseins are phosphorylated as monoesters of serine, but the degree of esterification differs among caseins. The α_{s1}-, α_{s2}-, and β-caseins contain multiple phosphorous groups, which can form complexes with calcium leading to precipitation. κ-Casein, on the other hand, contains only one phosphorous group and, therefore, is less sensitive to calcium (Fox & Kelly, 2004). κ-Casein is also glycosylated, making it more hydrophilic than other proteins in the casein family. Caseins are hydrophobic proteins. The preponderance of proline residues (an imino acid), which are uniformly distributed throughout the molecules, yields caseins an open structure, and thus, high surface hydrophobicity.

Because caseins have low levels of secondary (e.g., α-helix) and tertiary structure, they do not exhibit thermal transitions during heating (Swaisgood, 2003). However, the hydrophobic and polar residues are not uniformly distributed along the sequence. They tend to form clusters and patches, thus giving casein molecules amphiphilic structures with a high surface activity and explaining why caseins are excellent emulsifiers. Their relatively open structure also makes caseins highly susceptible to enzymes, including proteases and transglutaminase (Kuraishi et al., 1997).

Whey proteins in fresh milk are present as individual noncomplex proteins and are highly hydrophilic. Together, they represent approximately 20% of total protein content in milk. β-Lactoglobulin is the most abundant protein in whey, followed by α-lactalbumin, immunoglobulins, serum albumin, and the proeoytic products from cheese making, collectively referred to as proteose-peptone (Table 6.1). When the pH

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Table 6.1 Physicochemical properties of milk proteins

Protein	Total milk protein (%)	Molecular weight	Isoelectric pH	Main functionality
Caseins	80.0			
α_{s1}-Casein	31.4	23,555	4.95	Emulsification
α_{s2}-Casein	8.2	25,238	5.29	Emulsification
β-Casein	29.1	24,028	5.32	Emulsification
κ-Casein	10.3	19,038	5.53	Emulsification
Whey proteins	20.0			
β-Lactoglobulin	10.0	18,362	5.2	Gelation, foaming
α-Lactalbumin	3.8	14,174	4.7	Emulsification
Serum albumin	1.2	66,267	4.8	Gelation
Immunoglobulins	2.2	150,000–1,000,000	5.5–8.3	Gelation
Proteose-peptone	2.5	4,100–40,800		Water binding

Data compiled from Walstra and Jenness (1984) and Modler (2000).

of fresh milk (pH 6.7) is lowered to about 4.7–5.0, the calcium phosphate linkages between casein submicelles are destabilized, leading to hydrophobic association and subsequent precipitation of casein. The proteins that remain soluble are whey proteins. The preponderance of nonionizable polar side chain groups in whey proteins renders them soluble even at their isoelectric pH. The high solubility of whey proteins is responsible for their excellent gelling and water-binding properties.

Milk Protein Products

Various protein products have been prepared from milk for use in meat product formulations, some being rather crude and some relatively pure (Table 6.2). Nonfat milk solid (NFMS) is prepared directly from skim milk by condensing and spray drying milk. The dried product is a mixture of proteins, sugar (lactose), and minerals. Milk protein concentrate (MPC) is made from skim milk by a combination of ultrafiltration and diafiltration. The caseins are in a micellar form, which can be separated from whey proteins by microfiltration. MPC is relatively high in mineral content. Once separated, caseins can be dried and processed into fine powders. The aqueous fraction is used to prepare different whey protein products, such as whey protein concentrate (WPC) and whey protein isolate (WPI).

Separated caseins are of low solubility in aqueous solutions. The solubility is improved by treatment with alkaline agents. Thus, sodium caseinate, a common ingredient used in meat products, is usually produced by reaction of casein slurries with dilute sodium hydroxide at elevated temperatures. Sodium bicarbonate and sodium phosphate may also be used but the reagents are more expensive. After reaction, sodium caseinate is spray- or drum-dried. Potassium caseinate is also prepared from caseins and it is used in dietetic foods. Alternatively, caseins are subjected to limited hydrolysis to modify their structure, solubility, and functional performance in muscle foods.

Whey and whey proteins used in processed meats include sweet whey, reduced-lactose whey, demineralized whey, WPC, and WPI. Commercial WPCs are pre-

Table 6.2 Dairy proteins and applications in meat products

Protein ingredient	Principal functional property	Application	Example
Nonfat milk solid	Texturization, flavor, emulsification	All meat products	Breakfast sausage, frankfurters, meat-based gravies
Sodium caseinate	Emulsification, texturization, meat-binding	Sausage, emulsion-type meats	Uncured pork sausage, frankfurters, turkey rolls
Partially hydrolyzed caseinate	Emulsification, texturization, water binding	Sausage, emulsion-type meats, gravies	Fish balls, pork balls, beef patties, chicken nuggets
WPC or WPI	Water binding, gelation, inhibiting pink color	Injected meats	Fresh sausage, turkey patties, boneless ham
Preheated WPC or WPI	Gelation, texturization	Sausage, emulsion-type meats	Fresh or cured sausage, boneless ham
Texturized WPC or WPI	Texturization, emulsification	Sausage, emulsion-type meats	Beef sausage, frankfurters
Partially hydrolyzed WPI	Antioxidant	All meat products	Precooked beef and pork patties

pared with different protein contents that range from 35% to 80%, and commercial WPIs typically have a protein content of greater than 90%.

Dairy Proteins as Functional Ingredients

Both caseins and whey proteins have been used in comminuted and emulsified meats (red meat, poultry, and fish products), such as frankfurters and bologna, and in coarse ground products, such as fresh sausage, meat patties, and meatballs. Soluble dairy proteins, including WPC and partially hydrolyzed caseins, are also used in marinated or injected meats. Several commercial meat products that contain functional dairy protein ingredients are displayed in Fig. 6.1. These dairy protein ingredients are used to improve moisture retention, fat-binding, and textural characteristics of cooked meats. When used in restructured products, including a variety of boneless ham and chicken rolls, these exogenous proteins can improve the binding strength, firmness, and sliceability.

Skim Milk Powder

Also referred to as nonfat milk solid, skim milk powder is widely used as a neutral filler with good water-binding properties in comminuted products. NFMS also

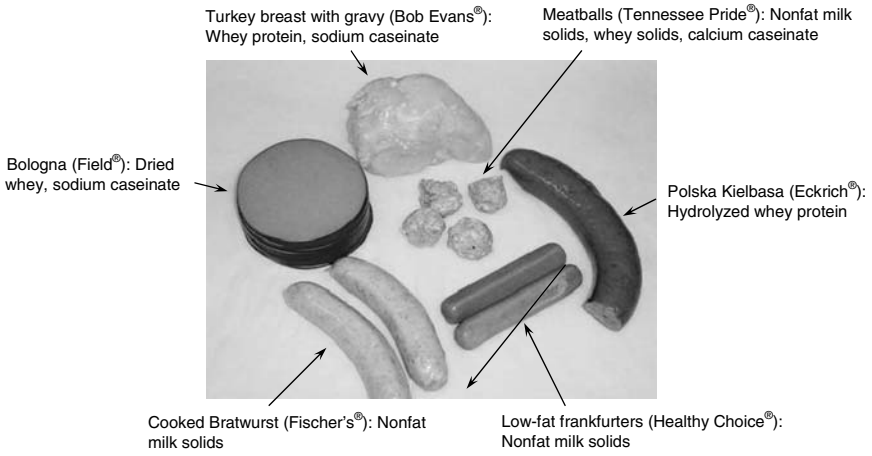

Fig. 6.1 Examples of commercial meat products containing various functional dairy protein ingredients (mention of the product's brand name does not imply author's endorsement of the product).

improves emulsifying capacity and emulsion stability of comminuted turkey and beef muscles (Kurt & Zorba, 2005). When used in meatballs, NFMS improves the product's color, flavor, and sensory acceptability (Hsu & Sun, 2006). Aside from proteins, the relatively high pH is a contributing factor to the good emulsion properties of NFMS. The limitation of the use of NFMS is its high lactose content, which can cause excessive Maillard browning discoloration during heat treatments.

Caseins

The efficacy of caseins and casein-derived dairy products in muscle foods has been extensively investigated. Sodium caseinate (and WPC), which has high solubility, exhibits remarkable swelling ability in meat batters. The increased hydration of meat batters is due to the water binding (thus, swelling) of the nonmeat additives that exist as fillers. However, despite its good solubility, sodium caseinate tends to increase moisture loss in cooked ground meat (Hand, Terrell, & Smith, 1983; Hermansson & Akesson, 1975a, 1975b). Yet, because of its excellent emulsifying capacity, caseinate decreases the loss of fat as free oil.

In dilute emulsion systems, sodium caseinate is a stronger emulsifier than myofibrillar proteins such as myosin. Hence, caseinate can help stabilize myosin-based emulsions probably through hydrophobic interaction with myosin (Imm & Regenstein, 1997, 1998). In emulsified products, casein molecules also complement salt-soluble myofibrillar proteins when forming the stabilizing fat globule membrane during chopping (Atughonu, Zayas, Herald, & Harber, 1998). It is very possible also that, being a strong emulsifier, casein can partition in the

fat globule membrane independent of myosin. Thus, stabilization of fat globules in emulsified meats by caseinate is likely achieved through more or less independent as well as concerted efforts of both dairy and meat proteins. To improve its water-binding ability, caseins are sometimes subjected to limited hydrolysis before addition to meats. Meat batters containing hydrolyzed caseins also exhibit improved textural characteristics. For example, chicken rolls with a sodium caseinate additive had enhanced cohesiveness and, hence, sliceability (Gillett & Carpenter, 1992).

Whey Proteins

Although whey proteins alone form highly viscoelastic gels upon heating, especially under low-ionic strength conditions, they could either improve or impair the textural properties of comminuted meats when used at a level up to 3.5%. For example, the effectiveness of WPC to improve yields and intended texture of pork sausages, beef patties, and turkey rolls differs due to variability in specific composition and processing procedures used to prepare the WPC (Ensor, Mandigo, Calkins, & Quint, 1987; Morr & Foegeding, 1990; Smick & Geist, 1988). It is more common that addition of native whey proteins to comminuted meats lowers the textural properties of cooked products, such as frankfurters (Hung & Zayas, 1992), in contrast to those products that contain sodium caseinate or NFMS as additives. The deleterious effect is attributed, in part, to the incompatibility of native whey proteins with salt-soluble muscle proteins during thermal gelation (McCord, Smyth, & O'Beill, 1998). Hayes, Desmond, Troy, Buckley, and Mehra (2005) compared four β-lactoglobulin-enriched fractions of whey protein for their effects on the physical and sensory properties of frankfurters. The fractions with the lowest calcium level significantly reduced the water-holding capacity, texture, and sensory characteristics of cooked frankfurters, suggesting that a small amount of minerals, particularly calcium, is important to producing an acceptable quality product.

Results from several studies suggested that much of the functionality of WPC in meat products could be traced to the concentration and drying methods that cause various degrees of denaturation or structural unfolding of whey proteins (Beuschel, Culbertson, Partridge, & Smith, 1992; Beuschel, Partridge, & Smith, 1992; Mandigo, Liao, Harper, Morr, & Zadow, 1987). As much as 50% denatured protein can be found in commercial WPC ingredients, and denaturation is usually induced by preheating. In general, those WPC or WPI preparations that contain largely undenatured proteins do not improve, and may even decrease, the textural properties of comminuted products, although the converse is true for water binding. In contrast, partially denatured WPC remarkably enhances the binding and textural properties of sausage and comminuted meat products. Because preheated whey protein can form cold-set gels, it has been used to improve the texture and water binding in minced poultry products (Hongsprabhas & Barbut, 1999).

The texture-enhancing effect of partially denatured WPC can be explained by the lack of interaction of native whey proteins with muscle proteins under normal meat processing conditions. For example, β-lactoglobulin, the principal protein component in WPC, does not unfold until the heating temperature reaches ~78°C (deWit & Klarenbeek, 1984), which is greater than the maximum temperature typically employed for comminuted products. On the other hand, myosin, the main functional muscle protein, undergoes complete structural changes at temperatures less than 65°C. Thus, native WPC or WPI would not undergo significant aggregation or interaction with muscle proteins (Hung & Smith, 1993; Liu, Xiong, & Butterfield, 2000).

Hence, by preheat treatment of the whey protein to destabilize its structure, it may be expected that when a meat batter is cooked to 65–70°C, the reactive groups from unfolded myosin would interact with those from partially denatured β-lactoglobulin. The ensuing hydrophobic aggregation and disulfide cross-linking will lead to a composite gel system of high rigidity and binding strength. This explanation is supported by the experimental evidence that rigidity and hardness of myofibrillar protein gels formed at 65°C increased as WPC solubility decreased at pH 6.0–8.0 (Table 6.3). It is also possible that heat-denatured whey proteins can act as active fillers in comminuted products, i.e., they can interact with surrounding meat proteins to reinforce the gel matrix (Barbut, 2006). It is noteworthy that interactions between whey and muscle proteins can be promoted under oxidizing conditions that may be present during meat processing and product storage (Liu & Xiong, 2000; Liu et al., 2000).

Whey proteins have also been used in "enhanced" meats, i.e., fresh meat that is injected with water with a small amount of NaCl and phosphate compounds (Chap. 1). It has been shown that injection of WPI and β-lactoglobulin solutions (each at a 3.3% protein concentration with 5.5% salt) at a level of 10% of product weight effectively enhanced the moisture content of pork loins, which was largely retained without the use of high-concentration phosphate as a water-binding agent (Hayes, Desmond, Troy, Buckley, & Mehra, 2006). Tenderness, juiciness, and taste of the enhanced meat were rated significantly higher than the noninjected control by consumers.

Table 6.3 Apparent stress at failure (kPa) of mixed chicken salt-soluble myofibrillar protein (SSP) and native or preheated whey protein concentrate (WPC) gels formed in 0.6 M NaCl at 65–70°C and different pH levels

Sample[a]	pH 6.0	pH 7.0	pH 8.0
SSP	32.3	24.0	<4.0
Native WPC	<4.0	<4.0	<4.0
Preheated WPC	<4.0	nr[b]	nr
SSP + native WPC	<4.0	27.1	25.1
SSP + preheated WPC	81.3	90.9	84.1

From Beuschel, Culbertson, et al. (1992); Beuschel, Partdridge, et al. (1992).
[a]Protein solubility: native WPC = 98%; preheated WPC = 41–47%. Protein concentration: SSP-only gels = 4% (w/v); WPC-only gel = 16–20% (w/v).
[b]nr: not reported.

A relatively new technology currently under development is texturization. Texturized whey proteins have been prepared using a similar extrusion process to that long used for the manufacture of texturized soy proteins. Whey proteins can be textured by thermoplastic extrusion under acidic (pH 2–3) or alkaline (pH 11–12) conditions (Onwulata, Isobe, Tomasula, & Cooke, 2006). The alkaline conditions increased whey protein denaturation, resulting in stringy texturized protein products, which may be used as meat extenders. It has been reported that hydrated textured whey proteins can be used to replace up to 40% of the weight of hamburger patties without affecting consumer acceptance of the product (Hale, Carpenter, & Walsh, 2002).

Transglutaminase-Modified Dairy Proteins

Transglutaminase or TGase (glutaminyl-peptide:amine γ-glutamyl-transferase, EC 2.3.2.13), which catalyzes an acyl transfer reaction between carboxyamide groups of glutaminyl residues in proteins (acyl donors) and primary amines (acyl acceptors), is a cross-linking agent that has the potential to improve the functionality of dairy proteins intended for meat processing. Because of its price advantage, microbial TGase is now more acceptable than TGase from other sources to modify food proteins (Zhu, Rinzema, Tramper, & Bol, 1995). Kurt and Rogers (1984) reported that TGase catalyzed cross-linking of myosin to casein. The open structure of casein molecules renders them susceptible to TGase catalysis and hence, superior substrates, when compared with whey proteins and most other food proteins.

The TGase-catalyzed myosin–casein interaction is responsible for the strong bind formed at the junction of meat particles in restructured beef steaks (Motoki & Seguro, 1998) and chicken cubes (Kilic, 2003). The TGase-mediated binding technology can also be used for the production of chicken nuggets, restructured fish fillets, and other muscle foods with enhanced textural properties and sliceability. One particular advantage of TGase is that it has high activity at low ionic strength conditions and at refrigerator temperature. The latter property is particularly attractive for the preparation of fresh restructured meat without the addition of salt and heat (Kuraishi et al., 1997). Cold-set binder formed by TGase-treated caseinate allows the production of restructured pork, chicken, and lamb in the raw, refrigerated state (Carballo, Ayo, & Jiménez-Colmenero, 2006). Hardness and chewiness tend to be higher in cooked samples treated with TGase and caseinate than in control samples (without TGase or casein). It is noteworthy that TGase treatment increased cooking loss, but this may be reduced by adding a small amount of salt. In lieu of salt, other water-binding agents may be used.

Transglutaminase is also capable of enhancing whey protein functionality in comminuted meat products. Although TGase does not promote thermal gelation of mixed whey/meat proteins (Ramírez-Suárez & Xiong, 2002), it stabilizes the emulsions prepared with mixed myofibrillar/whey proteins as emulsifiers (Ramírez-Suárez & Xiong, 2003). Such emulsions are readily converted to semisolid composite gels upon heating, which is reinforced by the cross-linking between fat globule membranes.

Comparison of Different Dairy Proteins

Because of their structural differences, dairy proteins or protein-derived ingredients usually have a different predominant functionality (water binding, gelation, emulsification, etc.). Hence, theoretically, one protein may be suited for a particular type of meat product but not for others. However, in reality, many processed products are a composite system consisting of proteins, fat, minerals, and other substances. A good example of such products is a comminuted meat batter, where fat particles are stabilized by the interfacial membrane as well as the structurally heterogeneous protein gel matrix formed surrounding the fat globules. Thus, ideally, a given protein ingredient that is intended for finely chopped meats should exhibit good emulsification and gelation properties. While many reports claim that one protein is superior to another protein in functionality, a valid comparison can be drawn only if the comparison is made under identical processing and storage conditions.

Barbut and Choy (2007) compared five difference dairy protein ingredients – dry whole milk, skimmed milk, sodium caseinate, regular and modified whey – for use in comminuted chicken breast meat with added water and salt (2.5% NaCl) and cooked to 75°C. At an equal application level (2%, w/w), all dairy ingredients reduced cooking loss, with caseinate showing the best results. However, when used at an equal protein level (2% total protein), the best performing ingredients, in terms of cooking yield, hardness, and fracturability, were whole milk and modified whey. Modified whey was particularly attractive because it was also the most cost-effective ingredient. The modified whey protein was prepared with a proprietary process that modified the protein structure to allow it to be more coagulable and to gel more readily. Entrapment of fat globules in the mixed muscle/dairy protein gel matrix appeared to be a main mechanism for the stabilization by caseinate and modified whey protein (Barbut & Choy, 2007; Su, Bowers, & Zayas, 2000). When the same milk protein ingredients were tested in meat batters prepared from mechanically separated chicken, caseinate and modified whey protein both enhanced textural properties and water-binding potential of cooked meat batters more than did other dairy proteins (Barbut, 2006). The improvement was attributed to the ability of both proteins, especially modified whey proteins, to form distinct protein gel regions within the meat batter (Fig. 6.2).

Ensor et al. (1987) compared WPC (1–3.5%), NFMS (3.5%), and soy protein isolate (SPI, 2%) for use in an emulsion-type sausage. All protein ingredients improved product hardness, but 3.5% WPC surpassed the hardness of both NFMS and SPI treatments. Sodium caseinate is usually found to be a better texture-enhancing protein than other dairy proteins in processed meat although this is not through the gelation mechanism. Hung and Zayas (1992) reported that the addition of 2% caseinate increased the hardness of beef frankfurters compared with the control, while 3.5% regular WPC addition had no effect. However, meat products prepared with caseinate are generally brittle and hence, less acceptable than products containing NFMS or other protein ingredients (Hsu & Sun, 2006). Su et al. (2000) also showed that 2% caseinate added to reduced-fat frankfurters significantly increased

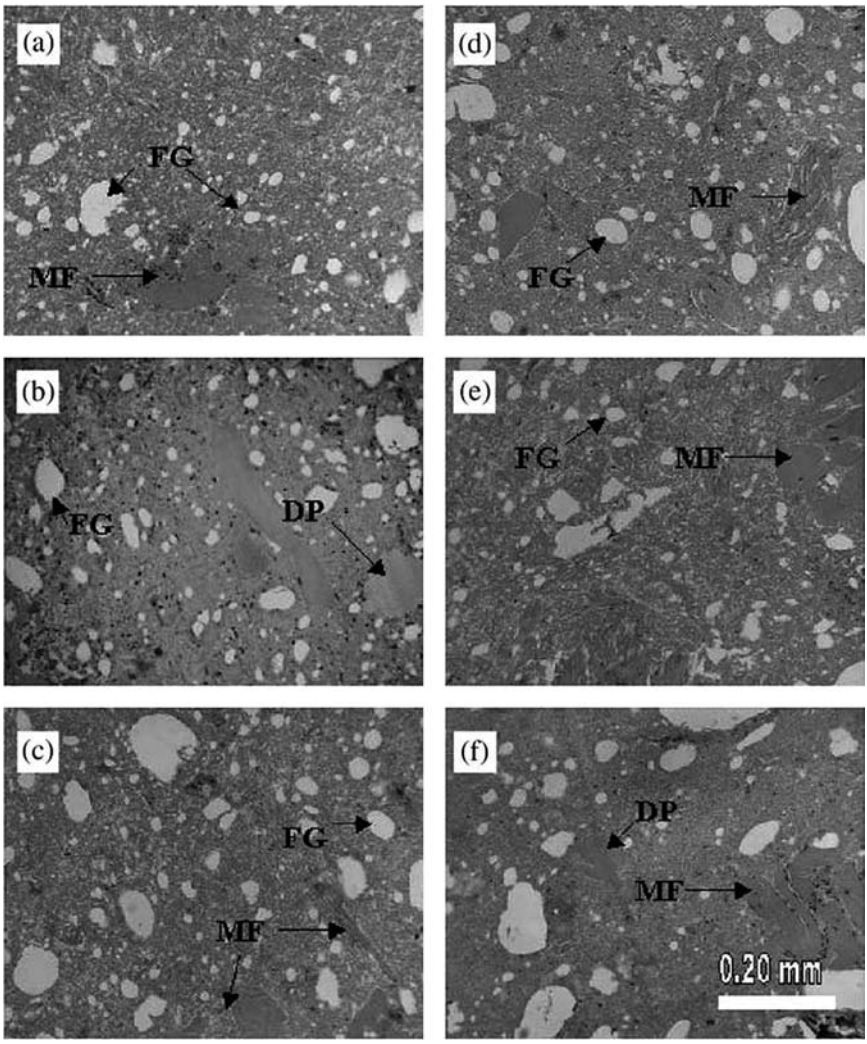

Fig. 6.2 Light micrographs of chicken meat batters: (**a**) control, (**b**) with sodium caseinate, (**c**) with whole milk powder, (**d**) with skim milk powder, (**e**) with regular whey powder, and (**f**) with modified whey powder. DP: dairy protein-filled areas; FG: fat globules; MF: muscle fibers. Bar = 0.2 mm (From Barbut, 2006. Adapted with permission).

the product's hardness. However, it must be recognized that excessive hardness may be undesirable. For example, while the addition of caseinate and soy protein increased the hardness of meat balls, it was NFMS that enabled a texture of intermediate hardness organoleptically more acceptable (Hsu & Sun, 2006). Kerry, Stack, and Buckley (1999) compared three commercial WPCs, sodium caseinate, and soy protein isolate for their contribution to thermal gelation of exudates obtained from massaged, cured pork meat. They noted that both whey and soy proteins increased

the elastic rigidity of the gel, while caseinate hindered the gelation. The finding was consistent with the notion that caseinate is a poor gel-forming protein.

Antioxidant Property of Dairy Proteins

In addition to their texture-related functionality, milk proteins and their enzyme hydrolysates have been shown to exhibit antioxidative activity in model systems (Colbert & Decker, 1991; Peña-Ramos & Xiong, 2001, 2002). Ultrafiltration, ionic exchange, and other membrane techniques are useful methods to separate protein hydrolysates into different peptide fractions. The fractions from hydrolyzed whey proteins that contain strong antioxidant activity were found to have high concentrations of histidine, proline, tyrosine, and lysine residues (Peña-Ramos, Xiong, & Arteaga, 2004).

Cooked pork patties containing 1.5% NaCl and 2% hydrolyzed WPI by Flavourzyme® and Protamex® (Novozymes A/S, Bagsvaerd, Denmark) were found to have significantly improved oxidative stability during refrigerated storage (Peña-Ramos & Xiong, 2003). Intact WPI was also inhibitory of oxidation in cooked pork, but the effectiveness was less than its enzyme hydrolysates. Similarly, caseins and casein peptides were shown to inhibit lipid oxidation in cooked ground beef (Díaz & Decker, 2004). The antioxidant mechanisms have been identified, which include scavenging radicals such as hydroxyl and superoxide radicals chelating prooxidative metal ions, particularly copper, and donating electrons. The overall antioxidant activity of protein hydrolysates is relatively small when compared with phenolic antioxidants, such as vitamin E, rosemary extracts, BHA and BHT. However, the antioxidant activity exhibited by dairy protein peptides is considered an added advantage to the excellent functionality imparted by these common food ingredients in meat products.

Inhibition of Pink Color Defects in Poultry Meat

Pink color development in fully cooked, uncured ground turkey is a defect that gives a false impression that the meat is not cooked or undercooked. This color defect is associated with the binding of heme iron to different ligands, including carbon monoxide and nitric oxide from oven gases and reducing compounds released from muscle cells. However, the conditions that control the pink color formation in ground turkey meat are not well understood, and, in practice, it is difficult to prevent. Schwarz et al. (1997) showed that NFMS could reduce pinking in cooked, uncured ground turkey. Pink color development in ground turkey, induced by nitrite or nicotinamide, was suppressed by the presence of as low as 1.5% NFMS and some WPC samples (Slesinski et al., 2000a, 2000b). Sammel and Claus (2003) also reported that WPC could inhibit pink color development in cooked, uncured

ground turkey. The mechanism appeared to be related to the ability of dairy proteins to compete for binding heme ligands because less nitrosylhemochrome and nicotinamide hemochrome was produced.

Regulatory Issues

In the EU countries as well as countries from most other parts of the world, milk protein, casein, and caseinates are not considered food additives. Therefore, their usage in muscle foods is essentially unregulated. However, in the United States, where dairy proteins intended for meat products are used primarily as binders, extenders and water binders, specific regulations are established to control the level of their application. For example, sodium caseinate used in comminuted meat products is limited to 2% of the finished product weight, and in water-added ham, the maximum level is 1.5% (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 1995). For less concentrated dairy protein ingredients, including NFMS, WPC, dry whey, or reduced-lactose whey, their maximum allowable level in sausage is 3.5% of the finished product weight. It must be noted that when dairy protein ingredients are used in combination with other binders or extenders in comminuted meats, the total amount of additives cannot exceed 3.5% of the product weight. Dairy proteins used as ingredients in meat products must be identified in the product label. Their listing is important for those individual consumers who are immunologically sensitive to milk proteins.

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Chapter 7
Meat-Derived Protein Ingredients

Rodrigo Tarté

Introduction

Meat-derived protein ingredients are a group of high-protein ingredients derived primarily from meat animal by-products and sometimes from meat itself (Table 7.1). In order to adequately understand the status of these ingredients it helps understand how meat and its by-products are defined and regulated.

In a practical sense, *meat* can be defined as "the edible postmortem component originating from live animals," particularly "domesticated cattle, hogs, sheep, goats and poultry, as well as wildlife such as deer, rabbit and fish" (Kauffman, 2001). Although organ meats such as hearts or liver are sometimes considered meat, the term is very often restricted to the edible skeletal muscle tissue of mammalian species (typically bovine and porcine), poultry, and seafood.

From a regulatory perspective definitions of meat are much more specific and restrictive, although they vary somewhat by country. In the United States, regulations define meat as

> The part of the muscle of any cattle, sheep, swine, or goats which is skeletal or which is found in the tongue, diaphragm, heart, or esophagus, with or without the accompanying and overlying fat, and the portions of bone (in bone-in product such as T-bone or porterhouse steak), skin, sinew, nerve, and blood vessels which normally accompany the muscle tissue and that are not separated from it in the process of dressing. (Code of Federal Regulations [CFR], 2007a)

This definition has been amended to include materials derived from advanced meat/bone separation and meat recovery (AMR) systems, provided that AMR machinery does not "grind, crush, or pulverize bones to remove edible meat tissue" and that "bones … emerge essentially intact" (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 2006b). Therefore, mechanically separated meat, which is typically obtained by processes in which bones with attached edible meat are forced under high pressure through sieves or similar devices, is excluded from the definition and, therefore, when used, must be labeled as "mechanically separated (pork, veal, or lamb)" (USDA-FSIS, 2006b). Mechanically separated beef was declared inedible and prohibited from use as

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Table 7.1 Main types of nonmeat ingredients derived from edible animal by-products

Source	Ingredients
Blood	Blood plasma (liquid, frozen, dried) Whole blood (liquid and dried) Red cell protein (decolorized) Plasma transglutaminase
Bone	Gelatin (type B) Edible bone collagen (ossein) Bone collagen hydrolysates (stocks and broths) Edible bone phosphate Edible fat
Pig skin	Gelatin (type A) Stocks and broths
Beef hides	Gelatin (type B)
Poultry skin (chicken, turkey)	Concentrated collagen Stocks and broths Edible fat
Collagen-rich tissues	Concentrated collagen Collagen hydrolysates

human food in early 2004, out of concerns related to bovine spongiform encephalopathy (BSE) (CFR, 2007d; USDA-FSIS, 2004).

In the United States, poultry is regulated separately from *meat*. It is defined as "any domesticated bird (chickens, turkeys, ducks, geese, guineas, ratites, or squabs, also termed young pigeons from one to about thirty days of age), whether live or dead." (CFR, 2007f). A *poultry product* is defined as "any poultry carcass or part thereof; or any product which is made wholly or in part from any poultry carcass or part thereof. … Except where the context requires otherwise... this term is limited to articles capable of use as human food" (CRF, 2007f). Mechanically separated poultry may be used without limit in the formulation of poultry products (CFR, 2007g).

In the European Union, meat is defined as (European Parliament and Council, 2007):

> "[s]keletal muscles of mammalian and bird species recognized as fit for human consumption with naturally included or adherent tissue, where the total fat and connective tissue content does not exceed the values indicated below and where the meat constitutes an ingredient of another foodstuff. The products covered by the Community definition of "mechanically recovered meat" are excluded from this definition.
> Maximum fat and connective tissue contents for ingredients designated by the term '… meat'.

Species	Fat (%)	Connective tissue (1) (%)
Mammals (other than rabbits and porcines) and mixtures of species with mammals predominating	25	25
Porcines	30	25
Birds and rabbits	25	25

(1) The connective tissue content is calculated on the basis of the ratio between collagen content and meat protein content. The collagen content means the hydroxyproline content multiplied by a factor of 8

Note that this regulation, contrary to USDA's, does include poultry meat in the definition of *meat*. On the other hand, however, it excludes mechanically recovered meat, meaning that it must be labeled separately and cannot be counted as part of a product's meat content (European Commission, Health and Consumer Protection Directorate-General, 2001). Also excluded are "[o]ther meat-related ingredients derived from meat protein, fat and connective tissue and which have undergone a treatment such as purification (e.g. gelatine, collagen fibre, refined fats, …), hydrolysation (e.g. protein hydrolysates,…), extraction (e.g. meat extracts, bouillons, …) …" (CLITRAVI, 2002), which includes most of the ingredients discussed in this chapter. It is important to note that in the E.U. the term "animal by-product(s)," or ABP, is explicitly reserved for products of animal origin that are not intended for human consumption (Commission of the European Communities, 2006; European Parliament and Council, 2002; European Union, 2007). The general rules for the manufacturing of meat by-products for human consumption, including definitions, are laid down in Regulation (EC) No. 853/2004 (European Parliament and Council, 2004).

In the United States, meat by-products are in themselves classified by the USDA as "Group 1 Protein-Contributing Ingredients." However, most of the proteinaceous ingredients derived from them fall under the "Group 2 Protein-Contributing Ingredients" classification, because they are generally derived by "hydrolysis, extraction, concentration, or drying" (CFR, 2007c; USDA-FSIS, 1995b). Therefore, the protein they contribute must be considered "nonmeat protein" for formulation purposes, as when calculating *added water* in cooked sausage products (CFR, 2007c) and *protein fat-free* (PFF) in cured pork products (CFR, 2007e).

This chapter approaches the subject in this context and focuses on those meat-derived protein ingredients which have been found to be technically viable for the formulation of processed meat products. The term *meat-derived* – instead of *animal-derived*, for instance – is used to avoid confusion with other protein ingredients of animal origin, such as those derived from dairy, egg, and seafood.

The most commonly used meat-derived protein ingredient over the years has been gelatin, which has been used for many decades. However, there exist a number of other meat-derived protein ingredients with varying properties and degrees of functionality. Although many of these have been available in some form for some time, recent advances in protein and enzyme chemistry, as well as in

extraction and purification technology, have resulted in improvements of their functional properties and continue to push the boundaries of technical and commercial feasibility.

Collagen

Collagen, a family of insoluble fibrous proteins found in all multicellular organisms, is one of the most abundant proteins in nature. It is the most abundant protein in mammals, comprising about 25–30% of total body protein (Bailey & Light, 1989), 1–2% of bovine skeletal muscle, and 4–6% of high-connective tissue muscles (Whiting, 1989). It is a major component of skin, bone, cartilage, tendon, blood vessels, basement membrane (endomysium), and teeth (Bandman, 1987; Stryer, 1988). Collagen is a rod-shaped molecule approximately 300 nm in length and 1.5 nm in diameter. Its basic subunit is tropocollagen (mol wt 300 kDa), which consists of three helical polypeptide α-chains (α1, α2, and α3) coiled around one another into a triple-stranded superhelix stabilized by hydrogen bonds. Variations in the composition of the α-chains of tropocollagen give rise to various collagen phenotypes, 19 of which have been identified (Bailey & Paul, 1998; McCormick & Phillips, 1999). Of these, the most abundant in meat are the fibrous types I, III, and V, and the nonfibrous type IV. Type I collagen predominates in bone, tendon, skin, and the epimysium, types I and III in the perimysium, and types III, IV, and V in the endomysium (Sims & Bailey, 1981). The molecule's C- and N-termini (about 2–3% of the molecule from either end) consist of small, nonhelical regions called telopeptides.

The amino acid composition and sequence of collagen is very unique. It is approximately 33% glycine, 12% proline, 11% hydroxyproline, and 11% alanine, is devoid of tryptophan, and contains the unusual amino acids 3-hydroxyproline, 4-hydroxyproline, and 5-hydroxylysine (Bechtel, 1986). A collagen chain has three amino acid residues per helical turn, with every third amino acid being glycine. This allows glycine, given its small size, to occupy the helice's interior positions.

The collagen molecule is stabilized by intra- and intermolecular covalent cross-links, which are formed when the enzyme lysyl oxidase catalyzes the oxidative deamination of lysine and hydroxylysine to form the aldehydes allysine and hydroxyallysine, respectively. These aldehydes can then form cross-links by reacting with each other (through an aldol condensation), or with a lysine or hydroxylysine residue (to yield a reduced Schiff base product) (Foegeding, Lanier, & Hultin, 1996). The resulting cross-links are reducible and divalent (i.e., they are capable of linking only two collagen molecules together). As an animal ages, these divalent, reducible cross-links gradually convert into more stable nonreducible, trivalent cross-links (Eyre, 1987; Purslow, 1999). The common trivalent cross-link hydroxylysylpyridinoline (HP) may be formed either by the reaction of a divalent ketoamine cross-link with a free hydroxyallysine (McCormick & Phillips, 1999), or by the

interaction of two divalent ketoamine cross-links, which releases a hydroxylysine or lysine residue (Eyre, 1987). Trivalent and multivalent cross-links are very stable (e.g., HP cross-links are heat stable) and possess the ability to link adjacent collagen fibrils; in this way they add strength and rigidity to the intramuscular connective tissue (IMCT) matrix and very likely contribute to the increased toughness of meat from older animals.

Properties and Applications

Collagen sources for use in processed meats include skin, hide, bone, offal, and skeletal muscle (Bailey & Light, 1989). Collagen can be added to meat products either (1) as a constituent of high-collagen meat raw materials (typically high-collagen tissues, such as skeletal muscle connective tissue, beef hides, pork skins, and tripe) or (2) in concentrated form as a direct additive. It has been concentrated from bone (as bone collagen extract), beef hides, pork skins, and skeletal meat connective tissue (Gillett, 1987). Skeletal meat collagen can be concentrated by mechanical desinewing or extracted by low-temperature rendering followed by extrusion, dehydration, grinding, flaking, or milling (Gillett, 1987; Jobling, 1994; Prabhu & Doerscher, 2000; Prabhu & Hull, 2001). In either form, collagen can significantly affect the processability and organoleptic attributes of meat products.

Raw Collagen

Most meat trimmings used in the meat processing industry contain varying amounts of collagen with varying degrees of cross-linking, depending on the age of the animal from which they are harvested. When heated to 60–65°C, the collagen triple helix begins to unravel into single strands, to shrink, and to dissolve (Voutila, Mullen, Ruusunen, Troy, & Puolanne, 2007; Whiting, 1989), leading, in the case of comminuted meat products, to a disruption of the myosin gel, which forms at lower temperatures (Gillett, 1987). This can result in batter instability and breakdown, and lead to increased losses of fluid (i.e., reduced water-holding capacity) and fat (fatting out), as well as the possible appearance of gel pockets and surface gelatin (Bailey & Light, 1989; Whiting, 1989). This effect can be minimized by limiting the use of high-collagen trimmings – a maximum formulated level of 2.5–3% collagen has been suggested, although this may vary on a case-by-case basis (Sadowska, Sikorski, & Dobosz, 1980; Whiting, 1989) – and by processing interventions, such as coarser chopping, increased pH and ionic strength, avoidance of excessive chopping, and gradual, stepwise cooking (Whiting, 1989). High-collagen meat trimmings from older animals (e.g., cull cows and bulls), in particular, can have detrimental effects on product quality and should be used judiciously.

Collagen as an Added Ingredient

The addition of collagen to meat products as a binder has been shown to be advantageous. The functionality of the collagen is dependent on factors such as animal species and age, anatomical source, and extraction conditions. Knowledge of these factors allows for selective manipulation of the collagen's functional properties. The potential use of collagen as a functional additive in meat products dates back to at least the late 1960s (Elias, Komanowsky, Sinnamon, & Aceto, 1970). Since then, much research has focused on its extraction from various sources (species, anatomical locations) and by various means, and on its application in various types of meat products.

The physical extraction and/or concentration of collagen usually involves particle size reduction of collagen – or high-collagen materials – by cutting, grinding, flaking, milling, or a combination of these. Frequently, dehydration and/or freezing steps are incorporated into the process. Some of these approaches have been well documented. A process has been described whereby pretreated hides are cut in strips and progressively reduced in size by cutting with a rotary knife, forcing through a screen with 0.5 to 1.0-in. openings, pumping to a centrifugal cutter with cutting head openings of 0.04–0.20 in. and, finally, pumping to a revolving disc mill to yield collagen fibers (Elias et al., 1970). No actual application of the resulting material was reported. Beef connective tissue (CT) has been modified by sequential grinding, freezing, and flaking, and tested at levels of 0%, 10%, 20%, 30%, and 40% in meat batters formulated to 8%, 16%, and 24% fat (Eilert, Blackemer, Mandigo, & Calkins, 1993). The modified CT increased smokehouse yields, especially in the lower fat batters (peaking at the 20% addition level), but adversely affected emulsion and smokehouse stability.

The development of low-temperature rendering systems has resulted in the commercialization of functional concentrated collagen ingredients. In this process, which may vary slightly by manufacturer, steam and hot water are added to soft materials such as meat lean and fatty trimmings and pig skins. The resulting slurry is then transferred to a decanter centrifuge, which separates it into two streams. The first, liquid, stream contains fat, protein, and water, and is used in the manufacture of meat stocks and broths, as discussed later in this chapter (see section on Stocks and Broths). The second, semisolid, stream is typically dehydrated and further processed by grinding, flaking, milling or granulating, to obtain dry functional collagen ingredients (Jobling, 1994). One commercial pork collagen product (MyoGel® Plus, Proliant Inc., Ankeny, IA; 85% protein, 12% fat), obtained by "low temperature processing of fresh pork trimmings" in a process that involves extrusion and dehydration, followed by drying and milling into a granular form (Prabhu, 2002; Prabhu & Doerscher, 2000), has been found to be capable of binding up to four times its weight in water. Addition of this ingredient to 22–23% fat frankfurters (at levels from 0% to 3.5% in 0.5% increments) and 3% fat restructured ham (at 0%, 1.0%, 2.0%, and 3.0%) was found to help control package purge over 8 weeks of refrigerated storage and to increase cook yields by approx. 1% in frankfurters (at up to 1.0% addition, beyond which no significant increases were observed), but not

in ham (Prabhu, Doerscher, & Hull, 2004). In another study, this same commercial pork collagen product, at a 3% usage level, reduced expressible moisture, but not cooking loss, in boneless cured ham manufactured with up to 100% PSE (pale, soft, exudative) meat. However, when only non-PSE meat was used, a decrease in both cooking loss and expressible moisture was observed with addition of pork collagen (Schilling, Mink, Gochenour, Marriott, & Alvarado, 2003).

In an effort to more clearly elucidate the interaction of concentrated, dehydrated pork collagen (MyoGel® Plus, Proliant Inc.) with myofibrillar proteins, a study evaluated the effects of its addition on thermal and viscoelastic properties of purified porcine myofibrillar protein gels (Doerscher, Briggs, & Lonergan, 2003). It was reported that replacement of 20% or more of the myofibrillar protein with pork collagen resulted in a decreased rate of gel formation, which led the researchers to suggest that perhaps pork collagen interfered with formation of the myofibrillar protein heat-set gel matrix. Of the pork collagen addition levels tested (0%, 10%, 20%, 30%, 40%, and 50%), 10% was found to be the optimum in terms of water-holding capacity, gel firmness, and rate of gel formation. Differential scanning calorimetry (DSC) and oscillatory rheology measurements in this study failed to detect specific protein–protein interactions between pork collagen and myofibrillar proteins.

Similar functional collagen proteins (>70% protein, <28% fat) have also been commercially produced by low-temperature processing of poultry (chicken or turkey) skin, and have been promoted to increase cook yields and decrease formulation costs in various poultry products due to their gelling and water-binding properties (Prabhu, 2002, 2003).

Certain extraction and/or treatment conditions can further modify the functional properties of collagen. Chief among these are heat, chemicals, and enzymes.

Heat-Modified Collagen. The functional properties of collagen can be modified by heating collagen or collagen-rich raw materials under different time/temperature combinations, some of which have been reported in the literature (e.g., 100°C for 30, 60 or 90 min; Sadowska et al.,1980). During processing of most processed meats, native collagen generally melts and becomes gelatin too late in the process (i.e., at temperatures between 75°C and 80°C) to become a part of the batter's gel structure. Precooked collagen, on the other hand, solubilizes early during chopping and is therefore able to provide functionality to the meat batter (Whiting, 1989). This was borne out in a study that evaluated the effect of temperature on the water-binding ability of concentrated pork skin CT gels (Osburn, Mandigo, & Eskridge, 1997). Pork skin CT was first obtained by cutting pork skin into strips, followed by freezing, grinding, refreezing, and flaking. It was then combined with varying amounts of water and heated at 50°C, 60°C, 70°C, or 80°C for 30 min. Under these conditions, it was found that gels produced by heating to at least 70°C had the highest water-binding ability. After cooling, these 70°C gels were tested in reduced-fat (2.0%, 3.5%, 4.3%, 6.8%, and 12.0% fat) bologna, resulting in decreased hardness and increased juiciness.

Enzyme-Modified Collagen. The use of enzyme-modified collagen as an extender or binder for processed meat products is limited by the fact that excessive hydrolysis of collagen can result in high levels of small peptide fragments, which could in turn lead to decreased water-holding and gelling properties. In one study (Satterlee, Zachariah, & Levin, 1973), beef and pork skin collagen were hydrolyzed with either crude pancreatin (a mixture of pancreatic enzymes, such as trypsin, amylase, and lipase), papain or pepsin, and added to sausage emulsions as replacements for nonfat dry milk (NFDM), with mixed results. Shear strength of hydrolysate-containing sausages was lower than for the NFDM-containing control and, although emulsion stability of hydrolysate-containing sausages was slightly higher than for the control, this difference was probably not practically significant. Commercially, the cost of the enzymatic treatment of collagen could, in some instances, make the material too costly in comparison to NFDM and other binders. At present, the enzyme modification of collagen appears to be commercially restricted to the production of flavor-enhancing hydrolysate preparations, as discussed later.

Regulatory Status

Pork collagen is approved in the United States for use in standardized and nonstandardized processed meat and poultry products as a binder and to reduce purge, at a maximum level of 3.5% (USDA-FSIS, 2007).

Gelatin and Gelatin Hydrolysates

Gelatin is the heat-denatured, partially hydrolyzed form of native, insoluble collagen. It is a soluble amorphous mixture made up primarily of three types of free chains: α monomers (mol wt 100 kDa), β dimers (mol wt 200 kDa), and γ trimers (mol wt 300 kDa) (Kijowski, 2001). The denaturation temperature of collagen varies by species and hydroxyproline content. During this process, the molecule's hydrogen bonds are disrupted and its intramolecular (aldol condensation and Schiff base), intermolecular, and main-chain peptide bonds are hydrolyzed, causing the collagen triple helix to unravel (Eyre, 1987). This in turn leads to the disassembly of the collagen fibrils and results in a viscous, colloidal solution of gelatin. This conversion of collagen to gelatin also occurs when meat is cooked; hence the gelatinous material that is sometimes evident after cooked meat is allowed to cool.

Commercial gelatin is obtained primarily from raw materials rich in type I collagen, most importantly pork skin and bones, beef hides and bones and calf skin, through a very controlled stepwise process that involves the chemical

hydrolysis of collagen, followed by heating to denature the molecule to gelatin. Type A gelatins are obtained by the mild acid pretreatment of physiologically young forms of collagen (e.g., pig skins), which have high proportions of acid- and heat-labile cross-links (Bailey & Light, 1989; Eyre, 1987; Stainsby, 1987). Type B gelatins result from the more severe alkali pretreatment of the more highly cross-linked collagen from bone and cattle hides (Pearson & Gillett, 1999; Stainsby, 1987). The isoelectric points of type A gelatins are typically in the pH 6–9 range and those of type B gelatins are at approximately pH 4.8–5.2. Because of this, type A gelatins carry a net positive charge in most food systems, whereas type B gelatins are positively charged in acidic systems and negatively charged in near-neutral systems (Stainsby, 1987). After extraction, gelatin is clarified (by filtration), concentrated (by vacuum evaporation or membrane ultrafiltration), dried, ground, blended, and, sometimes, sterilized (Linden & Lorient, 1999; Stainsby, 1987). Commercial gelatin extracts are mixtures that contain not only α, β, and γ chains, but also other larger (up to mol wt 10^6) and intermediate size molecules as well. The spectrum of molecular species and hence the properties of the resulting extract are affected by changes in the manufacturing process as well as by the nature of the starting raw materials, with type A gelatins being generally higher in lower molecular weight chains than type B (Cole & Roberts, 1996; Stainsby, 1987). As a general rule, the gel strength and viscosity of a gelatin solution decrease with decreasing mean molecular weight (Cole, 2000). While this means that the process must be strictly controlled to ensure consistent quality, it also allows for the functional properties of gelatins to be modified to better suit their intended application.

The properties of gelatin can be further modified by controlled enzymatic hydrolysis of gelatin solutions to reduce the protein's molecular weight to a desired range. The resulting gelatin hydrolysates possess properties similar to gelatin except that, due to their lower molecular weight, they are more easily dispersible in cold water and do not gel at regular processing temperatures.

Properties and Applications

Gelatin is deficient in methionine and completely devoid of tryptophan (Bailey & Light, 1989), both essential amino acids. However, it has excellent functional properties, such as gelling, melting (melts at <35°C), stabilization, film-forming, texturizing, and water-holding properties, which make it a very useful and desirable food ingredient for many applications. Gelatin gels, like those of collagen, are thermoreversible. Dried gelatins are characterized, graded, and commercialized on the basis of their gel strength expressed in Bloom units, defined as the force in grams required to press a 12.5 mm diameter flat-faced, sharp-edged, cylindrical probe 4 mm into 112.5 g of a $6^2/_3$% (w/v) gelatin gel that has been aged 16–18 h at 10°C (Gelatin Manufacturers Institute of America, 2006). Gelatin Bloom values typically range from around 100 Bloom for very weak gels to around 250 Bloom for firm gels (Rosenthal, 1999).

Gelatin is currently used in a wide variety of meat products, such as aspics, canned hams, and canned sausages (Bailey & Light, 1989; Stainsby, 1987). Gelatin and gelatin hydrolysates have also been proposed as external coatings to protect meat against color loss, aroma deterioration, and purge losses (Antoniewski, Barringer, Knipe, & Zerby, 2007; Krochta & De Mulder-Johnson, 1997; Villegas, O'Connor, Kerry, & Buckley, 1999). This protective effect has been attributed to the action of gelatin as a moisture and oxygen barrier.

Regulatory Status

In the United States, gelatin has Generally Recognized As Safe (GRAS) status. It is permitted as a binder and extender in various meat and poultry products at levels sufficient for the intended purpose (CFR, 2007h). Its permitted uses include non-specific products, jellied products (e.g., souse, jellied beef loaf, head cheese), paté-type products (as a covering; product name qualifier required in red meat paté products), canned whole hams (requires product name qualifier), and products where "gelatin" is part of the product name. It may also be used to bind two pieces of meat together. It is not permitted in sausage, luncheon meat, and meat loaves (USDA-FSIS, 2005). Hydrolyzed gelatin is also recognized as a binder rather than a flavoring and is permitted in frankfurters and similar products, as well as poultry franks, at a level of less than 2% (USDA-FSIS, 2005).

In the European Union, gelatin is classified as a food, not a food additive (European Parliament and Council, 2006), and is therefore not subject to food additive legislation. It is not considered meat and must, therefore, be declared separately.

Stocks and Broths

Meat stocks are high-protein (up to 94%) products derived from the liquid stream of the low-temperature rendering of soft materials such as meat lean and fatty trimmings, pig skins, and poultry skins (as previously described in the Collagen section), or the high-temperature rendering of soft materials or hard materials such as edible bones (Campbell & Kenney, 1994). After separation of the fat from the liquid stream, the resulting protein-containing "stick" water is concentrated and spray-dried (Jobling, 1994; Prabhu & Hull, 2001). Meat stock proteins are collagenous in nature.

Applications

Meat stocks are marketed as bases for meat and reaction flavor manufacturing, and as inexpensive protein sources and flavor enhancers for processed meat products. They can be used in sausage products to increase the protein content. In some

instances they can act as stabilizers, emulsifiers, and binders, although that is not usually their primary purpose when added to processed meat products.

Regulatory Status

In the United States, meat-derived stocks and broths fall under current USDA regulations [9 CFR 317.8(b)(7)(ii)] that mandate that "ingredients of livestock and poultry origin must be designated by names that include the species and livestock and poultry tissues from which the ingredients are derived" (CFR, 2007b). Therefore, ingredients such as dried stocks, dried broths, and meat extracts must be designated as "dried (species) stock," "dried (species) broth," and "(species) extract" (e.g., "dried chicken stock," "dried beef broth," "pork extract," etc.) (USDA-FSIS, n.d., 2006a).

Hydrolysates and Flavors

Meat protein hydrolysates can be obtained from meat by-products such as bone residues, mechanically separated meat (MSM), bone cakes from mechanical separation, trimmings (Fonkwe & Singh, 1996; Webster, Ledward, & Lawrie, 1982), blood plasma (Wanasundara, Amarowicz, Pegg, & Shand, 2002), and red blood cells (Shahidi, Naczk, Rubin, & Diosaday, 1984; Synowiecki, Jagiełka, & Shahidi, 1996), among others, as well as from the proteins of meat itself (e.g., myosin and collagen). In fact, since all proteins can be hydrolyzed, by different methods and to varying degrees, the possibilities in this area are seemingly endless. Therefore, although several types of hydrolysates have already been discussed (i.e., hydrolyzed collagen, gelatin, and gelatin hydrolysates), this topic is worth expanding and discussing further.

Although hydrolysis can be achieved by treatment with enzymes, acids, or alkali (Lahl & Braun, 1994), for many applications the enzymatic process is preferred due to its faster reaction rates, mild conditions, and high specificity (Hamada, 1992), and because it allows for better and more precise control of the degree of hydrolysis (DH) and, consequently, of the peptide profile of the hydrolysates obtained (Lahl & Braun, 1994). DH is a measure of the extent of hydrolytic degradation of proteins, and is usually expressed as the ratio of amino nitrogen to total nitrogen (AN/TN), or percent of peptide bonds cleaved (Mahmoud, 1994). It is both a most practical and effective way of monitoring and controlling the hydrolysis process, as well as a major determinant of a hydrolysate's functional properties (e.g., solubility, gelation, water holding, emulsification, flavor, etc.), along with other factors, such as the specificity of the hydrolytic enzymes used, the physicochemical nature of the intact parent protein, and processing conditions (Mahmoud, 1994). Generally, other factors being constant, properties such as emulsion stability, viscosity, and gel-forming ability decrease as DH increases.

This has been related both to the smaller molecular weight and to the increased net charge that results from hydrolysis (Mahmoud, 1994). On the other hand, as DH increases, the flavor contribution of protein hydrolysates increases, primarily due to the presence of low-molecular weight flavor components (e.g., amines, amino acids, and small peptides) and flavor precursors (e.g., nucleotides and organic acids). Therefore, hydrolysis can be carried out to such an extent that it will eventually result in products of such low functional quality (from the standpoint of emulsification, gelation, etc.) that they become strictly limited to use as flavors and flavor enhancers, and for protein supplementation (Synowiecki et al., 1996). In many cases, this is a desirable outcome. Meat flavors notes can also be obtained from meat broths, either by enzymatic hydrolysis or by reacting them with certain Maillard reactants (e.g., reducing sugars). Following hydrolysis, meat protein hydrolysates can be concentrated and used as added ingredients in liquid or powder form (Piette, 1999).

Meat hydrolysates can be either primary (partially hydrolyzed) or secondary (extensively hydrolyzed). Primary hydrolysates result from hydrolysis by one or more endopeptidases of animal (e.g., pepsin, trypsin, chymotrypsin), vegetable (e.g., papain, bromelain), bacterial (e.g., subtilisin from *Bacillus subtilis*, *Bacillus amyloliquefaciens*, or *Bacillus licheniformis*), or fungal (endoprotease from *Aspergillus oryzae*) origin (Piette, 1999; Pinto E Silva, Mazzilli, & Cusin, 1999). A secondary hydrolysis with exopeptidases may be desirable, in order to break down bitter peptides that may form as a result of partial hydrolysis (Pedersen, 1994). These exopeptidases can also be of animal, bacterial (e.g., *Bacillus* spp.), or fungal (e.g., aminopeptidases from *Aspergillus* spp.) origin, and are generally more effective after endopeptidases have reduced the average peptide size. In some instances both endo- and exopeptidases can be used at the same time, thus eliminating the need for a second step.

Knowledge of the extent and type of these hydrolytic reactions allows the process to be manipulated and controlled to yield products with specific functional attributes. The choice of meat protein hydrolysate is thus dictated by the specific functional properties desired for each particular application and may also be limited by commercial availability.

Regulatory Status

In the United States, the Food and Drug Administration (FDA) requires that "[t]he common or usual name of a protein hydrolysate shall be specific to the ingredient and shall include the identity of the food source from which the protein was derived" [21 CFR 102.22] (CFR, 2007i). For meat protein hydrolysates, specifically, this requirement is also mandated by the labeling regulation that applies to all ingredients of livestock and poultry origin [9 CFR 317.8(b)(7)(ii)] (CFR, 2007b), as discussed previously (see Regulatory Status under Stocks and Broths). In line with these regulations, the USDA specifically requires that "hydrolyzed protein of

slaughtered animal species and tissue of origin, other than gelatin, must be indicated, e.g., 'hydrolyzed beef plasma,' 'hydrolyzed pork stock,' and 'hydrolyzed pork skin'" (USDA-FSIS, 1995a). Therefore, even when used solely for flavor, all meat-derived protein hydrolysates and flavors are required to be designated in meat and poultry product labels by their common or usual names and to have their species of origin identified.

Another important distinction that affects the labeling of these ingredients has to do with the extent of hydrolysis of the material. According to the FDA, proteins with AN/TN ratios greater than 0.62 are "highly" hydrolyzed and must be declared as "hydrolyzed (source protein)." Those with AN/TN < 0.62 may be declared as "partially," "mildly," or "lightly" hydrolyzed (e.g., "partially hydrolyzed [source protein]") (USDA-FSIS, 1995a).

In terms of specific usage limits, partially hydrolyzed proteins are permitted in the United States in various meat and poultry products at a maximum level of 3.5% (USDA-FSIS, 2007).

In the European Union, protein hydrolysates and their salts are not classified as food additives (European Parliament and Council, 2006) and are, therefore, not subject to food additive legislation. They are not considered meat and must, therefore, be declared separately.

Blood-Derived Protein Ingredients

Blood makes up approximately 7% of the body weight of mammals (Judge, Aberle, Forrest, Hedrick, & Merkel, 1989), although typical recovery of blood during slaughter of cattle and hogs is about 3–4% of the animals' live weight (Liu & Ockerman, 2001). Whole blood has been used as an ingredient in meat products for generations, to manufacture products such as blood sausage and blood pudding, among others. Bovine or porcine blood has also been used as a raw material for a fairly wide range of highly functional ingredients. These ingredients, as well as whole blood, are used in many countries, but most of them have yet to find widespread acceptance in the United States.

Typically, blood is collected during slaughter and separated into two fractions: plasma (60–80%) and cells (20–40%) (mostly red cells, with smaller amounts of white cells and platelets) (Liu & Ockerman, 2001). Collection must be done in a prompt manner, generally within 20 min. (Halliday, 1973), taking care to minimize hemolysis of the red cells, as the resulting release of hemoglobin could make it impossible to separate the plasma (Knipe, 1988). Anticoagulants (typically citric acid or sodium citrate) are usually added during this stage. Separation of the two fractions is commonly accomplished by continuous high-speed centrifugation or separation, after which the plasma fraction is either frozen, or concentrated and spray-dried.

The composition of whole blood and of its constituent fractions is shown in Table 7.2. Protein levels in blood are known to fluctuate with age and animal

Table 7.2 Composition of blood and of its major fractions

Blood fraction	Protein (%)	Moisture (%)
Whole blood	17–18	75–82
Plasma	6–8	90–92
Cells	34–38	60–62
Dried plasma	70–95	5–10

Table 7.3 Basic properties of the major plasma proteins

Protein	Plasma protein (%)	pI	Molecular weight (kDa)
Serum albumin	56	4.8–4.9	69
α_1-Globulins	5.3	2.7–4.4	44–435
α_2-Globulins	8.4	3.6–5.6	41–20,000
β-Globulins	11.5	3.6–5.9	80–3,200
γ-Globulins	15	5.8–7.3	100–160
Fibrinogen	0.6	–	340

Adapted from: Gorbatov (1988); Howell (1992).
Protein levels vary with animal species and age.

species (Gorbatov, 1988). Nutritionally, blood proteins are deficient in the essential amino acids methionine and isoleucine (Penteado, Lajolo, & Pereira Dos Santos, 1979; Satterlee, 1975; Tybor, Dill, & Landmann, 1975; Wismer-Pedersen, 1979). Due to their dark color and unpalatability, whole blood and red blood cells (discussed later) have traditionally found only limited application as food ingredients (Piot, Guillochon, & Thomas, 1986; Wismer-Pedersen, 1979). The plasma fraction, on the other hand, offers more desirable color and functional attributes, and has therefore garnered more interest over the years (Knipe, 1988).

Blood Plasma Proteins

There are over 100 proteins in plasma. The major ones are the serum proteins albumin, α-globulins, β-globulins, and γ-globulins; and fibrinogen (Table 7.3). Of these, the most abundant, and perhaps the most important commercially, is serum albumin. Dried blood plasma protein (BPP) is an off-white powder almost entirely devoid of pigmentation.

Properties and Applications

Research on the use of BPP as a water-holding and binding agent in meats dates back to at least the late 1970s (Dill & Landmann, 1988). The highly functional properties of BPP can be attributed primarily to its albumin, globulin, and fibrino-

gen content (Chen & Lin, 2002; Dàvila, Parés, Cuvelier, & Relkin, 2007; Foegeding, Allen, & Dayton, 1986). It has also been suggested that BPP contains protease inhibitors, as suggested by the fact that as little as 0.5% has been observed to reduce myosin degradation in surimi (Lou, Wang, Xiong, Wang, & Mims, 2000; Wang, Wang, Mims, & Xiong, 2000).

Plasma protein possesses three functional properties which make them particularly useful as binders in processed meat products: gelation, emulsification, and solubility.

Gelation. BPP has excellent gelling properties. In suspension, it forms a strong, irreversible gel at protein concentrations of 4–5% (Hermansson, 1978) when heated to at least 70°C. Gelation of BPP is dependent on several factors, mainly temperature, pH, heating time, and protein concentration. Although gelation of BPP begins at around 70°C, increasing the temperature to 90–92°C will result in an even firmer gel, as reported for gels made from bovine BPP isolates diluted to 5.2%, 6.9%, 8.7%, and 10.4% protein (Harper, Suter, Dill, & Jones, 1978) and mixed bovine/porcine blood plasma diluted to 5% protein (Hermansson & Lucisano, 1982). It has been suggested that continued heating of bovine serum albumin gels beyond 95°C will yield increasingly stronger gels (Foegeding, Allen, & Dayton, 1986). This property makes BPP especially useful in products that are subjected to very high temperatures, such as canned items and certain restructured (sectioned and formed) products (Terrell, Crenwelge, Dutson, & Smith, 1982; Xiong, 2004). Increases in cooking time and protein concentration will also increase gel firmness (Harper et al., 1978; Hermansson, 1978, 1982), as will the addition of sodium chloride (Hermansson, 1978, 1982), with a level of at least 1.5% being necessary to attain an acceptably firm gel (Harper et al., 1978; Knipe, 1988). The gelation temperature of porcine blood plasma has been observed to be pH-dependent: 67.5°C at pH 4.5, 72.8°C at pH 6.0, and 76.3°C at pH 7.5 (Dàvila et al., 2007). In the same study, as pH increased, so did gel hardness, elasticity, cohesiveness, and water-holding capacity. Water binding in mixed bovine/porcine gels has also been reported to be higher at pH 9.0 than at pH 7.0 (Hermansson & Lucisano, 1982).

Emulsification. Although most comminuted meat products are not subjected to temperatures much above 70–75°C, the excellent emulsifying properties of BPP (Tornberg & Jönsson, 1981) make it ideal for use in these products (Ockerman & Hansen, 2000; Prabhu, 2002; Terrell et al., 1979). BPP has been shown to improve emulsion stability, texture, flavor, juiciness, and peelability of comminuted meat products (Prabhu, 2002).

Solubility. Plasma protein is very soluble over the pH range 5.0–8.0 (Hermansson, 1978; King, de Pablo, & Montes de Oca, 1989). Since most meat products fall in this pH range, BPP is an ideal binder for use in meat processing situations where solubility is critical, such as brine systems. In addition to the products already mentioned, BPP has also been used successfully as a binding agent in sausage (Caldironi & Ockerman, 1982) and ground beef patties (Suter, Sustek, Dill, Marshall, & Carpenter, 1976).

Recommended usage levels of BPP in processed meats are 0.5–2%, depending on desired finished product attributes.

Plasma Protein Fractions

Plasma can be further fractionated into its major constituents – albumin, globulins, and fibrinogen. Precipitation and removal of fibrinogen leaves behind serum, which can then be further fractionated into albumin and globulins.

The functional properties of these individual fractions have been researched for some time. In one series of studies, the gelation and other functional properties of albumin and fibrinogen were investigated in efforts to elucidate the functional mechanisms of plasma protein (Foegeding, Allen, et al., 1986; Foegeding, Dayton, & Allen, 1986). Other studies have focused more specifically on the potential utilization of specific plasma fractions as food ingredients (Chen & Lin, 2002; Dàvila et al., 2007; Penteado et al., 1979). The emulsification capacity of plasma fractions has been evaluated by several studies. In one study (Penteado et al., 1979), the oil emulsification capacity of 1% solutions of bovine BPPs was observed to follow the following pattern: plasma > albumin > globulins (the pH values at which emulsification was measured were not reported). Another study (Ramos-Clamont, Fernández-Michel, Carrillo-Vargas, Martínez-Calderón, & Vázquez-Moreno, 2003) found that oil-in-water emulsions were more stable when made with serum than with albumin (for both beef and pork blood-derived fractions), up to 14 days of storage at 25°C. Results from these two studies suggest a synergistic effect between the different fractions of plasma. In terms of gelation, in an aforementioned recent study of the effects of pH on the heat-induced gelation of porcine albumin, serum, and plasma (Dàvila et al., 2007), 5% gels made from each of these three fractions became progressively weaker as pH decreased from 7.5 to 4.5. Serum gels were weaker than plasma gels at pH 7.5. However, although both gels became weaker at pH 6.0 and 4.5, serum gels were now stronger than plasma gels. This effect was attributed to the presence of fibrinogen in plasma, and suggests fibrinogen may have deleterious effects on the functionality of the proteins in low-pH products, such as fermented sausages. Albumin gels followed a similar pH-dependent trend but, despite albumin being plasma's most abundant component, they were much weaker than serum and plasma gels at all pH levels tested, indicating, as other studies have, that there is a strong synergistic effect between all protein fractions of plasma (Howell & Lawrie, 1984).

Given this possible synergy between plasma protein fractions, it may not be technically or economically advantageous to pursue their use individually. Before this issue is fully settled, however, more research needs to be done on the effects and implications of the use of plasma protein fractions in meat products specifically.

Regulatory Status

The use of blood plasma in meat products is permitted in the United States. Given its animal origin, current regulations require that it be identified on the product label by its common or usual name and that its species of origin be identified (e.g., "dried beef plasma") (USDA-FSIS, 2006a).

In the European Union, blood plasma is not considered a food additive (European Parliament and Council, 2006), and is therefore not subject to food additive legislation. It is not considered meat and must, therefore, be declared separately.

Plasma Transglutaminase

Transglutaminases (TGases; EC 2.3.2.13) are thiol enzymes that catalyze acyl transfer reactions in which γ-carboxamide groups of peptide-bound glutaminyl residues act as acyl donors and primary amines act as acyl acceptors. When the acyl acceptors are the ε-amino groups of lysine residues, inter- and intra-molecular ε-(γ-glutamyl)lysyl covalent cross-links are formed (Fig. 7.1) (de Jong & Koppelman, 2002; Folk, 1980; Griffin, Casadio, & Bergamini, 2002; Motoki & Seguro, 1998). TGases have been found in plants, bacteria, fish, mammals, birds, and amphibians. To date, only TGases obtained from bacteria (Zhu, Rinzema, Tramper, & Bol, 1995) and mammalian plasma (blood clotting Factor XIIIa) have been successfully applied commercially, as they are the only ones that can be produced in large enough quantities and demonstrate adequate cross-linking activity of native proteins (Table 7.4).

Properties and Applications

TGases effectively cross-link casein, whey proteins, soy proteins, wheat proteins, myosin, actomyosin, gelatin, and collagen (Piette, 1999), although activity and substrate specificity are dependent on the origin of the enzyme and the state of the substrate protein chain (Table 7.4), as well as on reaction conditions such as temperature and pH (Kurth & Rogers, 1984). Plasma and erythrocyte TGases require Ca^{2+} as a cofactor, whereas bacterial TGase is calcium-independent (de Jong & Koppelman, 2002). Bacterial TGases have similar activity to plasma TGase and are discussed in detail in Chap. 8.

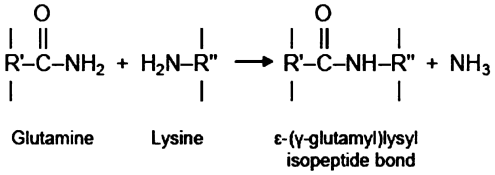

Fig. 7.1 Transglutaminase-catalyzed cross-linking reaction between peptide-bound glutamine and lysine

Table 7.4 Food protein substrate specificity of transglutaminases of different origin

	Degree of cross-linking [a,b]					
	Pig erythrocyte TGase		Bovine plasma TGase		Bacterial TGase	
	− DTT	+ DTT	− DTT	+ DTT	− DTT	+ DTT
α-Lactalbumin	−	±	−	±	+	++
β-Lactoglobulin	−	−	−	±	−	++
Bovine serum albumin	−	+	−	+	−	++
Casein	−	++	++	++	++	++
Hemoglobin	−	−	±	±	±	±
Myosin	−	−	++	++	++	++
Glycinin	−	++	−	−	++	++

From de Jong, Wijngaards, Boumans, Koppelman, & Hessing (2001).
Experimental conditions: 37°C; pH 7.5.
[a]Symbols: (−) no cross-linking; (±) slow cross-linking; (+) moderate cross-linking; (++) fast cross-linking.
[b]DTT: dithiothreitol; promotes unfolding of the protein chain by reducing disulfide bridges.

Plasma TGase can be used to bind uncooked meat pieces together. This enables processors to increase the economic value of low-value meat pieces and trimmings by converting them into restructured products of uniform portion size, shape, and texture (Flores, Boyle, & Kastner, 2007; Nielsen, Petersen, & Møller, 1995; Paardekooper & Wijngaards, G., 1986). In processed meats, TGase can be used to improve the texture of sausages, alone (via cross-linking of meat proteins) (Muguruma et al., 1999) or in combination with other nonmeat proteins added to the system, such as soy protein or casein (Kurth & Rogers, 1984). In addition, plasma TGase appears to offer a technically viable way to help address the current challenges of lowering sodium in meat products (Tseng, Liu, & Chen, 2000) and replacing synthetic food additives such as phosphates (Muguruma et al., 2003).

To date, the only commercially available system that takes advantage (albeit indirectly) of plasma TGase is Fibrimex® (Sonac B.V., Loenen, Netherlands), a system that combines fibrinogen with the enzyme thrombin to bind and restructure meat products (Paardekooper & Wijngaards, 1989). It is currently available as a frozen liquid and as a powder. The liquid version consists of two components: (1) a preparation of beef or pork blood plasma (which naturally contains zymogen Factor XIII, or fibrin-stabilizing factor) to which partially purified fibrinogen has been added and (2) a calcium chloride ($CaCl_2$)-containing solution of the enzyme thrombin (coagulation Factor II; EC 3.4.21.5), also extracted from beef or pork blood plasma. These two components are mixed in a specified ratio of 20:1, respectively,

immediately prior to addition to meat. The dry powder can be added directly or after premixing with water to ensure better dispersion. Once Fibrimex® in either form has been incorporated, the meat mixture must be held at 0–4°C for at least 6 h for optimal binding (Sonac B.V., 2007a). Upon addition and mixing, thrombin catalyzes (1) the breakdown of fibrinogen to fibrin monomers, which polymerize and form a gel and (2) the proteolytic activation of Factor XIII into its active transglutaminase form, Factor XIIIa, which, due to the presence of Ca^{2+} , forms covalent cross-links between individual fibrin molecules, as well as between fibrin and fibronectin, fibrin and collagen (Piette, 1999), fibrin and actin, myosin and actin, and myosin and fibronectin (Kahn & Cohen, 1981). Of these types of cross-links, fibrin–fibrin, fibrin–fibronectin, and fibrin–collagen appear to be the most important in meat applications (Piette, 1999). The combined effect of these cross-links and of fibrin gelation results in a strong bond between meat pieces.

At present, however, the widespread commercial use of TGase (either plasma or microbial) is restricted by the relatively high cost of the material, which makes its use prohibitive in many (although certainly not all) applications.

Regulatory Status

In the United States "beef fibrin," defined as "a component mixture of beef fibrinogen and beef thrombin plasma protein used to bind pieces of meat or poultry together," is permitted at up to 10%, provided it is labeled as required, where the words "Formed with Beef Fibrinogen and Thrombin" must appear either in the product name (at usage levels of 7–10%) or in the product name qualifier (at usage levels of less than 7%) (USDA-FSIS, 2005).

In the European Union, the "use of an enzyme preparation based on thrombin:fibrinogen derived from cattle and/or pigs as a food additive for reconstituting food" is permitted under the regulations for "stabilizers" (European Parliament and Council, 2006). In 2005, at the request of the European Commission, the European Food Safety Authority's Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food concluded that "such enzyme preparation is not of concern from the safety point of view" (European Food Safety Authority, 2005).

Hemoglobin and Red Blood Cells

Approximately 70% of total blood protein is in the form of hemoglobin, which is found in red blood cells (erythrocytes). Therefore, when the plasma and cell fractions of blood are separated, most of the protein actually remains with the cellular, or corpuscular, fraction.

Properties and Applications

As previously mentioned, the use of hemoglobin and hemoglobin-rich materials as nonmeat ingredients in meat products has been limited, due primarily to the dark color and off-flavors they impart. Hemoglobin can be decolorized by treating it with hydrogen peroxide (H_2O_2) (Oord & Wesdorp, 1979) (the resulting verdomethemoglobin has been reported to be insoluble in water and to exhibit inert behavior [Ockerman & Hansen, 2000]), acid-acetone solution (Antonini & Brunori, 1971), carboxymethylcellulose chromatography (Sato, Hayakawa, & Hayakawa, 1981), enzymatic hydrolysis (Stachowicz, Eriksson, & Tjelle, 1977), and aluminum oxide (Piot et al., 1986). Another attempt at overcoming the disadvantages of hemoglobin involves removal of the heme group (Tybor, Dill, & Landmann, 1973, 1975). The resulting globin has good water-holding capacity but does not form a gel when heated, thus limiting its effectiveness.

Hemoglobin and red blood cells have found use in meat products as color enhancers in some countries. Stabilized hemoglobin products, in both liquid and powder form (Sonac B.V., 2007b), as well as spray-dried red blood cells (Proliant, Inc., 2008), are or have been commercially available for this purpose. Another development involves the treatment of red blood cells – or a hemin intermediate isolated from them – with a nitrosating agent (typically nitric oxide) in the presence of a reductant (Pegg & Shahidi, 2000). The resulting so-called Cooked Cured-Meat Pigment (CCMP) is a mononitrosyl derivative of reduced hemin and has been proposed as a coloring agent in composite nitrite-free processed meat systems. As of this writing, this product has not been commercialized.

Regulatory Status

In the United States, blood is permitted in products such as blood sausage, blood pudding, blood soup, and in beef patties, as long as a qualified product name is used (e.g., “Beef and Blood Patties” or “Beef Patties with Blood”). A coating of beef blood is permitted on cured products (e.g., ham, hamette, etc.) if the product name is prominently qualified to reflect the coating (USDA-FSIS, 2005). In all products in which blood is permitted, the term ‘‘blood’’ and the specific kind of blood shall be declared in the ingredient statement (e.g., “beef blood” or “sheep blood”) [9 CFR 317.8(b)(31)] (CFR, 2007b).

Summary

Meat-derived protein ingredients represent another addition to the portfolio of nonmeat ingredients available to meat processors today. As is always the case when deciding on the best ingredients to use for a particular application, the final decision always comes down to a thorough cost vs. benefit analysis. Therefore, careful

design of the desired quality attributes (texture, color, flavor, shelf-stability, label, etc.) and cost structure of the finished product will, in the end, determine which ingredients are most suitable to use. In order to arrive at this end, competing ingredients must be carefully tested and selected, based on their functional attributes and price.

In addition to the functional properties discussed in this chapter, many of the unique benefits of meat-derived protein ingredients stem from the fact that they are derived from meat animals. This makes some of them more consumer-friendly and label-compatible than many other ingredients, especially as processed meat manufacturers today seek "simpler" and "cleaner" ingredient declarations. Meat-derived proteins are nonallergenic, which makes them good potential options for the replacement of commonly used allergenic proteins, such as dairy and soy.

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Chapter 8
Enzymes

C. Tony Payne

Enzymes in Meat Systems

Enzymes themselves are proteins or groups of proteins that act as biochemical catalysts for specific reactions. They generally speed a reaction between substances without themselves being consumed in the process. Enzymes are used in food processing systems in many capacities. Within meat systems two actions predominate: anabolic, building larger molecules from smaller components, and catabolic, degrading larger molecules into smaller molecules. This catabolic group includes classes of enzymes that contain calpains, cathepsin B, and some caspases, enzymes that are naturally found in muscle and are present as a part of the normal control mechanisms associated with muscle growth, development, repair, and turnover, and have been shown to influence tenderness *postmortem*. The anabolic forces would include the various transglutaminase activities, an example being the action of transglutaminase within the blood clotting mechanism. To mimic these natural catabolic and anabolic processes within meat systems we have discovered, isolated, and developed enzymes from plants, animals, bacteria, and fungi to help manipulate these processes in food products.

Because many of the uses of these enzymes require that the enzyme be left in the active state, the U.S. Food and Drug Administration (FDA) and the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA-FSIS) have been fairly diligent about the approval process. The enzymes that have been the most quickly approved have been those that have a history in food and have not been shown to have negative side effects over long-term use. Enzymes from plant sources have been somewhat less restrictive since many come from plants or fruits that have been consumed for centuries. The plant-based enzymes also tend to be naturally free of unwanted side enzyme activities such as amylase. Because of the nature of enzyme isolation and purification, many commercial enzyme preparations approved for use in meat products contain some unwanted side activities or compounds. The FDA has also been very cautious about using microorganisms that are known to have pathogenic effects in humans. All strains approved thus far are pure cultures from nonpathogenic and nontoxigenic organisms and are considered GRAS (Generally Recognized As Safe; FDA Center for Food Safety and Applied Nutrition [FDA-CFSAN], 2007).

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Within meat systems we typically think of four general categories of action based on what the enzymes are used for: (1) tenderizing, (2) cross-linking (adhesion, binding, texturizing), (3) flavor production, and (4) by-product utilization (tanning, etc.). The primary focus of this chapter will be the use of exogenous sources of enzymes in tenderizing and cross-linking applications.

Tenderizing Enzymes

Tenderizing enzymes generally come from one of three sources: plant, fungal, or bacterial. Plant sources have the longest history, with fungal and bacterial being more recent developments. Several of the tenderizing enzymes have been around for a number of years and have been used in basic marinades for their ability to make tough meat more palatable, especially in the case of middle meat cuts from older, cull, or dairy animals. These proteases are available from different suppliers under different trade names, some containing combinations of enzymes, which can make it difficult to determine the actual enzyme activity.

The USDA has approved the following enzymes for use in tenderizing meat products: papain, bromelain, ficin, protease preparations from *Aspergillus oryzae* and *Aspergillus niger*, protease preparations derived from *Bacillus subtilis* and protease produced from *Bacillus subtilis* var. *amyloliquefaciens* (Table 8.1; USDA-FSIS, 2007). This chapter will concentrate on those currently approved by the USDA for inclusion in meat products and mention those that have potential for future use.

Tenderizing Enzymes from Plants

The primary, exogenous enzymes that are of economic importance in meat processing include papain (from papaya), bromelain (from pineapple), and ficin (from figs). Most were accidentally discovered to have tenderizing properties and all are isolated from plants. Papain, bromelain, and ficin all belong to a group of enzymes termed thiol, cysteine, or sulfhydryl proteases because they contain a cysteine residue in their active site. This class of enzymes is similar to those naturally found in meat (calpain, cathepsin B, and some caspases), and it is logical that they would be appropriately used for tenderizing purposes. Other plant enzymes have been more recently studied and will be discussed briefly.

Papain

Papain (EC 3.4.22.2) is a protease derived from the latex portion of the papaya plant (*Carica papaya*), and is gathered by scoring the surface of the mature, unripe fruit, after which the latex is collected and dried (Schwimmer, 1981). Drying can occur

Table 8.1 Tenderizing enzymes approved for use in meat products in the United States

Source	Substance	Use/purpose	Products	Regulations	Meat regulation reference	GRAS[a] reference	Labeling requirements
Plant	Papain (papaya)	To soften tissue	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat shall not result in a gain of 3% above green weight	9 CFR[b] 424.21	21CFR 184.1585	Listed by common or usual name in the ingredients statement
Plant	Bromelain (pineapple)	To soften tissue	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat shall not result in a gain of 3% above green weight	9 CFR 424.21	60 FR[c] 32904 – Final Rule	Listed by common or usual name in the ingredients statement
Plant	Ficin (Ficus)	To soften tissue	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat shall not result in a gain of 3% above green weight	9 CFR 424.21	60 FR 32904 – Final Rule	Listed by common or usual name in the ingredients statement
Fungi	Protease produced from *Aspergillus oryzae*	To soften tissue	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	At a level not to exceed 3% of the weight of the untreated product	9 CFR 424.21	GRAS Notice No. 000090 accompanying letter	Listed by common or usual name in the ingredients statement
Fungi	Protease produced from *Aspergillus flavus oryzae* group	To soften tissue	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat shall not result in a gain of 3% above green weight	9 CFR 424.21	GRAS Notice No. 000090 accompanying letter	Listed by common or usual name in the ingredients statement

(continued)

Table 8.1 (continued)

Source	Substance	Use/purpose	Products	Regulations	Meat regulation reference	GRAS[a] reference	Labeling requirements
Bacteria	Protease preparation derived from *Bacillus subtilis*	Tenderizing agent	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat shall not result in a gain of 3% above green weight	FSIS Directive 7120.1 Amend 13	64 FR 19887–Final Rule	Listed by common or usual name in the ingredients statement
Bacteria	Protease produced from *Bacillus subtilis* var. *amyloliquefaciens*	Tenderizing agent	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat shall not result in a gain of 3% above green weight	FSIS Directive 7120.1 Amend 13	64 FR 19887 – Final Rule	Listed by common or usual name in the ingredients statement
Fungi	Protease produced from *Aspergillus niger*	Tenderizing agent	Raw poultry muscle tissue of hen, cock, mature turkey, mature duck, mature goose, mature guinea, and raw meat cuts	Solutions applied or injected into raw meat or poultry tissue shall not result in a gain of 3% above green weight	FSIS Directive 7120.1 Amend 13	GRAS Notice No. 000089 accompanying letter	Listed by common or usual name in the ingredients statement

Sources: CFR (2007e); FDA-CFSAN (2007); USDA-FSIS (2007).

[a]GRAS: Generally Recognized As Safe.

[b]CFR: Code of Federal Regulations.

[c]FR: Federal Register.

by one of several methods: sun, oven, or spray drying; end products having the highest activity are obtained by spray drying. Papain has broad-spectrum enzyme activity over a wide pH and temperature range (Table 8.2), and it cleaves at basic amino acids or those having large hydrophobic side chains. Resulting peptides having terminal hydrophobic amino acids can result in a product with a bitter aftertaste. For this reason, papain is usually combined with other enzymes in the production of flavorings. Because papain is a thiol protease, oxidation of its cysteine residue under certain conditions can reduce the enzyme's activity or potency. For this reason papain preparations often contain reducing agents such as sodium metabisulfite. Papain is economically available commercially in both liquid and powder forms. It has the longest history of use in meat systems and is the basis for some of the popular tenderizing sprinkle-on products that can be purchased by home consumers (Enzyme Development Corporation, 1999).

Bromelain

Bromelain (EC 3.4.22.32) is a protease that is derived primarily from the stems of the pineapple plant (*Ananas comosus* or *Ananas bracteatus*), although it is found throughout the plant. Following fruit collection the stump is also harvested, peeled, crushed, and the liquid collected. Soluble bromelain is purified from the liquid via ultrafiltration followed by freeze drying or solvent precipitation and sold in powder form. Bromelain powder is actually a collection of individual proteases with similar activities. Although lower than papain, bromelain still has a fairly high temperature of inactivation and broad pH activity range (Table 8.2). Bromelain has been found to be more active against collagen proteins than papain or ficin (Fogle, Plimpton, Ockerman, Jarenback, & Persson, 1982; Kang & Rice, 1970) and effective at improving meat tenderness.

Ficin

Ficin (EC 3.4.22.3) is a protease that is isolated from the latex of species of fig (*Ficus glabrata*) and is composed of several different endopeptidases (Jones & Glazer, 1970). The latex is collected and the enzyme separated following stabilization, degumming, and dialyzing (Englund, King, Craig, & Walti, 1968). The active enzymes preferentially cleave proteins at tyrosine and phenylalanine residues. Because of this preferential cleavage, ficin tends to produce hydrolysates with low bitterness, as well as more controlled tenderization than bromelain or papain. It also has the lowest inactivation temperature of the three primary plant proteases (~70°C), depending on conditions (Table 8.2). A major drawback of ficin is its inconsistent supply, which leads to price fluctuations.

Table 8.2 Properties of plant tenderizing enzymes

Enzyme	EC number	Class	Source	Mol wt kDa	Temperature,°C		pH	Comments
					Optimum	Denaturation		
Papain	3.4.22.2	Cysteine Protease	Papaya	23.4	65	80–90	5–7	More active on myofibrillar proteins
Bromelain (stem)	3.4.22.32	Cysteine Protease	Pineapple stem	20–33.2	50	70–75	5–9	More active on collagen type proteins
Bromelain (fruit)	3.4.22.33	Cysteine Protease	Pineapple fruit	20–33.2	50	70–75	5–9	More active on collagen type proteins
Ficin	3.4.22.3	Cysteine Protease	Ficus latex	25–26	65	70	5–7	Reaction is milder and easier to control
Actinidin	3.4.22.14	Cysteine Protease	Kiwi fruit	23–26	58–62	60	5–7	Collagen activity

Sources: Altman & Dittmer (1974); Lewis & Luh (1988b); Macrae, Robinson, & Sadler (1993); Rao, Tanksale, Ghatge, & Deshpande (1998); Robbins (1930); Schwimmer (1981); Smith & Hong-Shum (2003); Sumantha, Larroche, & Pandey (2006).

Other Plant Enzymes

Actinidin (EC 3.4.22.14) is a sulfhydryl protease derived from the fruit and peel of the kiwi fruit (*Actinidai chinesis*; Arcus, 1959). Actinidin is extracted from kiwi fruit and, because it is found in greater quantities with higher activity in ripe kiwi (Lewis and Luh, 1988b), it represents a potential use for over-ripe or discarded fruit. Actinidin has been studied in meat systems and shown to be capable of milder tenderizing reactions, although at higher enzyme concentrations (Lewis and Luh, 1988a). It also has a lower temperature of inactivation than ficin, bromelain, or papain (Table 8.2) which makes it easier to control the tenderizing reaction without overcooking. Currently, actinidin is not approved in the United States for meat tenderization.

Ginger rhizome protease has also been studied for its ability to degrade meat proteins (Lee, Sehnert, & Ashmore, 1986; Naveena, & Mendiratta, 2004; Thompson, Wolf, & Allen, 1973). It has an optimum temperature of about 60°C and a denaturation temperature of about 70°C. Like bromelain, ginger protease degrades collagen proteins more than myofibrillar proteins. Although potential exists for this protease, cost, regulatory approval, and flavor contribution are its main prohibitive factors.

Fungal and Bacterial Proteases

The fungal and bacterial sources have been developed because of the need for targeting hydrolysis of specific connective tissues at lower inactivation temperatures. In this way, connective tissues such as elastin or highly cross-linked collagen from older animals may be tenderized effectively without excessive cooking. These enzymes are produced via fermentation and the enzyme isolated from the growing culture. Fungal and bacterial enzymes are generally more expensive to produce than those that can simply be isolated from plants, which is one of the reasons that they have not found as widespread use as the plant proteases.

Fungal and bacterial proteases approved for use in meat products are listed in Table 8.1. Many of these products exist commercially as mixtures, so the following discussion may be variable depending on the enzyme supplier. It is always best to consult the supplier for recommended dose and specific enzyme preparation for a particular application. Properties and dose information of some commercial enzyme products have been summarized in a recent paper by Calkins and Sullivan (2007).

Fungal Enzymes

Proteases from *Aspergillus oryzae* have good proteolytic action against both collagen and elastin. This multiple action on different proteins is due to the enzyme preparation having a variety of protease activities, including: alkaline proteinase (EC

3.4.21.14), aspartic proteinase (EC3.4.23.6), and neutral proteinase (EC 3.4.24.4) (GRAS notice 000090; FDA-CFSAN, 2007). Because the products contain a collection of enzymes that are active over a very wide pH range and have a moderate temperature of denaturation (70°C), proteases from *A. oryzae* make it easy to control the extent of meat tenderization. The primary protease originating from *A. niger* is an aspartic proteinase (EC 3.4.23.6). Proteases from some *Aspergillus* spp. can exhibit some degree of amylase side activity, which can pose a problem in products that contain starch. Because of this side activity and high production costs, these enzymes have not gained widespread use as tenderizing agents in the meat industry. However, some of the neutral proteases from *A. oryzae* have found use as debittering agents (Sumantha, Larroche, & Pandey, 2006). Ashie, Sorensen, and Nielsen (2002) studied a specific aspartic protease and reported that it improved meat tenderness by 25–30%. The enzyme had a relatively low temperature of inactivation (60°C), was not adversely affected by normal meat pH and ingredient combinations, and tenderizing activity was expressed primarily during cooking and not during storage.

Bacterial Enzymes

Proteases from *Bacillus* include enzymes of two main types: subtilisin (EC3.4.21.62) and neutral proteinase (EC3.4.24.28). Protease preparations from *Bacillus subtilis* and *Bacillus subtilis* var. *amyloliquefacien*s have been reclassified together as the same enzyme. Both have found fairly recent approval in meat products (Food and Drug Administration [FDA], 1999) and behave much like ficin because of their lower inactivation temperatures. They have also been generally easier to control and the neutral protease is less expensive to produce than ficin.

Other Microbially Derived Proteases

Other researchers have studied alternate sources for tenderizing enzymes, including collagenases (Miller, Strange, & Whiting, 1989; Tunick, 1988) and elastases (Qihea, Guoqinga, Yingchunb, & Hui, 2006). Collagenases have been demonstrated to have promising activities, but because they are generally found in bacteria that have some health significance they have not been commercially approved or accepted for use in food products. Similarly, other strains of fungi have been studied, including *Rhizomucor miehei* (Ashie, Sorensen, & Nielsen, 2005) and *Aspergillus sojae* in the form of raw soy sauce (Tsuji, Hamano, Koshiyama, & Fukushima, 1987; Tsuji & Takahashi, 1989). These studies indicate that both sources have potential for use in tenderizing meat products. Specifically, soy sauce production uses a species of *A. sojae* and one might expect that proteases responsible for the characteristic flavor of soy sauce might also have tenderizing action on meat proteins, especially since they come from related organisms. This method of addition might also make labeling more consumer-friendly.

Labeling of Tenderizing Enzymes in Meat Products

Meat products that have been tenderized using enzymes are regulated by the following rules (USDA-FSIS, 2005): (1) they must be labeled as "tenderized with [approved enzyme]"; (2) the tenderizing solution is limited to 3% water (9 CFR 424.21; Code of Federal Regulations [CFR], 2007e), (3) combinations of tenderizing and flavoring solutions are permitted but limited to a total of 10% of the green weight of the meat, and (4) if used in combination, separate flavoring solutions are limited to 7% and statements for both additions must appear on the product label (e.g. "Tenderized with Papain" and "Marinated with up to a 7% solution of …"), in no particular order.

Although not common today, the practice of antemortem injection of tenderizing enzymes is regulated by 9 CFR 317.8(b)(25) (CFR, 2007a) and 9 CFR 381.120 (CFR, 2007d). In these regulations carcasses of animals treated with papain by antemortem injection shall be roller branded "tenderized with papain." Parts not so marked shall be labeled as "tenderized with papain" (USDA-FSIS, 2005). Trimmings from this method may be used in fresh meat products up to 25% of the formula, provided the finished product is immediately frozen and that distribution is limited to institutional use only. The labeling record should state the conditions and means of inspection control. Meat from this method may be used in cooked ground beef products up to 25% of the formula without showing the ingredients of the solution (USDA-FSIS, 2005).

Use of Tenderizing Enzymes in Meat Products

The proper use of enzymes in meat tenderization effectively involves understanding their strength and specificity, combined with the method of delivery and the total time they will be active in the particular product. Note that worker safety is also a very important aspect of using these products.

Enzyme Strength and Specificity

Most plant tenderizing enzymes are nonspecific when they start to degrade a protein. It is important to note that because of differences in potency or activity, the effectiveness of plant enzymes can vary from manufacturer to manufacturer. Each tends to standardize the enzymes to different levels. In many cases, the analysis method can also be different so it becomes very difficult to compare enzymes. It is very important to understand the strength of the particular protease to be used. The plant enzymes are typically analyzed for their ability to clot milk (Balls & Hoover, 1937) and are standardized based on an activity measured as a Milk Clot Unit (MCU). The assay involves mixing a known amount of milk powder with water and adding a known amount of enzyme. The amount of time that is required to clot the system is used to

gauge the strength of the product and is reported in MCU. In this way, the strength of the enzyme can be standardized so that the reaction is a bit more targeted and less likely to become over-active. Typical use levels of botanical proteases for meat tenderization require 1,000 to 5,000 MCU per pound of treated meat. The amount of MCUs per pound of meat will vary with hold time and cooking conditions.

Bacterial and fungal enzymes are typically more specific in their targets and the degree of reaction is easier to control. Their methods of standardization typically differ from the plant enzymes and use different methods and associated activity units to determine the strength of the powdered product. This might involve their ability to degrade one of several different proteins which may or may not contain meat proteins. Because all of these enzymes have particular reactivity sites, these analyses may not indicate how they will react in a meat system. With most of the current standardization methods there is an inherent flaw in applying this information to meat, as pointed out by Fawcett and McDowell (1986); the substrates used to access the enzyme activity are not meat components and therefore do not predict the ability and the extent of tenderization very accurately. However, these are at least somewhat indicative of the basic activity of these crude enzyme preparations and can be used as a guide for dosage.

For these reasons, it is impossible to compare the activity of a fungal or bacterial enzyme with bromelain, ficin, or papain. There is no correlation between the assay methods. It may be possible to correlate a specific blend of plant protease with a specific blend of a microbial protease based on the final tenderizing of the meat. However, that comparison is only valid for those two enzyme systems used under the conditions of the specific factory. It cannot be used as a "rule of thumb" comparison at different plants with different meat handling systems.

As with most biological systems, a time/temperature relationship exists for enzyme reactions. Dose levels in combination with reactions times and temperatures can generally be used to control the reactions. In general, at a given concentration, enzymes follow a reaction rate such that the rate of the reaction doubles for every 10°C increase in temperature, up to optimum reaction rate (Moodie, 2005). Dosage can be adjusted based on the time the protease will have to react before being denatured by cooking or freezing, or the length and temperature of cooking. Fogle et al. (1982) demonstrated the differences in dose required when cooking large beef cuts by a long and slow method vs. a fast method. The differences in enzyme level to achieve the same level of tenderness were quite different, with faster cooking times and higher temperatures requiring higher enzyme doses than cooking at low temperature for longer times. This would be expected, since the enzymes would be in their optimum temperature conditions for a longer period with the slower cooking method and would not be denatured as quickly by high temperature.

Method of Delivery

The tenderizing enzymes have traditionally been added by several different methods. Sprinkle applications have been the most widespread and have resulted in the

availability of low-strength preparations for retail use. The enzyme is generally mixed with a suitable carrier like salt, flavorings, or other components and the consumer simply sprinkles the mixture on the surface of the meat. Time for the enzyme to react may or may not be allowed for prior to cooking. Within the meat industry, tenderizing enzymes are typically incorporated by any of the following methods: (1) inclusion in marinade or injection brine, (2) topical application immediately before freezing, or (3) injection into the animal prior to slaughter. All of these methods involve some sort of dose level determination based on the reaction/storage temperature, length of storage, method of cookery, and other factors. It is important that the enzymes be applied evenly so that localized over-tenderizing does not cause inconsistent texture. Injection and tumbling will give more even distribution than tumble marination, depending on the thickness of the items being processed, with thicker items having more variability in tenderness because of slower diffusion of enzyme.

It must be kept in mind that tenderization is a time/temperature relationship, so if the steaks are to be marinated, frozen and shipped frozen, a different dose would be used than if they were to be shipped and sold fresh, or precooked prior to shipping. The use of proteases that have high temperatures of inactivation (papain, bromelain and, to a lesser extent, ficin) in precooked products is not ideal. For example, in beef steaks, where a lesser degree of doneness is usually desired, the product must be cooked to a well-done degree of doneness to inactivate the enzyme. While the cuts are more tender, this over cooking usually causes these products to become dry and less palatable. However, the bacterial enzymes lend themselves well to tenderizing precooked products. This is typically done by injecting the bacterial protease into muscle followed by bagging and cooking of the meat. Lower internal temperatures favor higher quality finished products because bacterial tenderizers are easier to inactivate at lower temperatures than those from plants.

Antemortem injection of tenderizing enzymes has been commercially used to distribute approximately 2–5 ppm of enzyme to all parts of the carcass using the animal's circulatory system. This method was originally developed in beef and patented by Swift and Company as the Proten™ system (Beuk, Savich, Goeser, & Hogan, 1959). Rhodes and Dransfield (1973) have also studied this method in sheep and determined that it also increases tenderness of older animals. The major drawback of this system was that muscles of the round or leg, and organs like the liver, received higher doses due to their more extensive vascular systems. This resulted in over-tenderization of these tissues. This method was used commercially for a short time, but has limited use today.

Worker Safety

It is important to remember that enzymes are proteins and, as such, they can eventually result in sensitivities in workers who handle them frequently (Dransfield, 1994). Over exposure can result in workers developing allergies or

other health issues. Particular care must be taken to avoid protease inhalation and skin contact through the use of personal protective equipment, including dust masks, gloves, and clothing that covers exposed skin. It is worth reviewing the safe handling practices for enzymes of the Enzyme Technical Association (ETA, 2008).

Cross-Linking Enzymes

Much time and energy has been devoted to finding methods to restructure meat products and maintain the eating characteristics of whole muscle. Numerous non-meat ingredients have been studied in cold binding systems (Payne, 2000; Payne 2001) including calcium alginate systems (Chap. 3), plasma binding systems (Chap. 7), chemical systems involving alkaline compounds, and other cross-linking agents found as components of liquid smoke. The enzyme systems are no exception, and the primary focus has been with a family of enzymes known as transglutaminases (TGase).

The use of transglutaminase in food systems has been well documented in several summaries (Kuraishi, Yamazaki, & Susa, 2001; Kurth & Rogers, 1984; Nielsen, 1995; Yokoyama, Nio, & Kikuchi, 2004; Zhu, Rinzema, Tramper, & Bol, 1995). Because of its characteristic reaction, TGase has the ability to affect texture, binding, and yield parameters of many protein-containing food products (Kurth & Rogers, 1984; Motoki & Nio, 1983; Motoki & Seguro, 1998). There are two basic systems in meat processing that use transglutaminase: (1) the enzyme derived from a microbial source and commercialized in Japan and (2) an animal blood-based system where the blood is separated into clotting factors that are later recombined (Chap. 8). This blood-based system contains Factor XIII, a component known to have transglutaminase activity (Chung, Lewis & Folk, 1974). The transglutaminase systems will be covered in detail here.

Transglutaminase

Sources

Various forms of TGase are present throughout nature in everything from microorganisms (Ando et al., 1989) and crustaceans (Kumazawa et al., 1997), to plants (Icekson & Apelbaum, 1987; Lilley, Skill, Griffin, & Bonner,1998; Margosiak et al., 1990) and vertebrates (Kumazawa, Nakanishi, Yasueda, & Motoki, 1996; Kumazawa, Sakamoto, Kawajiri, Seguro, & Motoki, 1996) including humans (Chung et al., 1974). It is thought that almost every living organism has some form of TGase involved with its metabolism. Mammalian sources of TGase are some-

what difficult and expensive to isolate in sufficient quantities to be economically feasible. Plant forms have not been developed to any extent, although work is under way (Carvajal-Vallejos et al., 2007). Microbial forms of transglutaminase (mTGase) are the only ones currently feasible for food applications due to cost and availability.

Enzyme Reaction

Transglutaminase (γ-glutamyl-transferase, EC 2.3.2.13) is an enzyme that, simply stated, cross-links proteins. It does this through the acyl transfer between a primary amine and a γ- carboxyamide of a peptide or protein-bound glutamine, resulting in the formation of a ε-(-glutamyl)lysine cross-link. This primary reaction generally results in a covalent cross-link formed between glutamine and lysine present in the protein molecules. Because of this action, TGase has the ability to act as an adhesive or a texturizing agent in meat and other food systems.

By far, the majority of food research has been conducted using a microbial form of the calcium-independent enzyme produced from the bacterium *Streptoverticillium mobaraense*. Additionally, several patents exist using this enzyme in combination with various protein sources to produce specific food products. This particular TGase is produced by the nongenetically modified microorganism characterized by Ando et al. (1989). TGase has an activity temperature range of 0–65°C, with optimum reactivity at approximately 55°C. TGase begins to denature at temperatures above 65°C and is generally completely denatured by about 70–75°C. It is active over a fairly wide pH range of 4–9, with an optimal range of pH 6–7. The active site of the enzyme involves a cysteine residue and is therefore subject to oxidation under certain conditions. There are also patents (Soeda, Hondo, & Kuhara, 2000) for oxygen stable mTGase, and this stabilized product also has self-affirmed GRAS status (GRAS notice 95).

Approved Meat Uses for TGase

TGase was one of the first groups of food ingredients to undergo self-affirmed GRAS approval (GRAS notices 4, 29 & 55; FDA-CFSAN, 2007). Table 8.3 summarizes the approvals and the references for the standardized products where TGase is allowed. Within these regulations, the enzyme must be listed as one of the following within the ingredient statement: (1) enzyme, (2) TG enzyme, or (3) TGP enzyme. In many cases, TGase is sold as a part of a preparation, which contains proteins and other ingredients. Regulations governing the "other components" must also be considered when labeling products containing these preparations.

Table 8.3 U.S. regulatory approval categories of microbial transglutaminase

Product category	Use in product	Level permitted	Comments	Regulation reference
Nonstandardized products				
Texturizing agent in meat and poultry food products where texturizing agents and binders are permitted	Binder or texturizing agent	Levels not to exceed 65 ppm of the product formulation	Classified as a binder with a 65 ppm limit	FSIS Directive 7120.1 Amend 13
Modified Meat and Poultry Products as per Policy Memos 121B and 123	Modified products	Levels not to exceed 65 ppm	Health modified products. Label per recommendations in Policy Memos 121B and 123	FSIS Directive 7120.1 Amend 13
Standardized products				
Fabricated meat cuts	Binder or texturizing agent	Levels not to exceed 65 ppm of the product formulation	Required use of "formed" or "reformed" in product name	9 CFR 319.15(d)
Fabricated poultry cuts	Binder or texturizing agent	Levels not to exceed 65 ppm of the product formulation	Required use of "formed" or "reformed" in product name	9 CFR 381.129
Poultry rolls	Binder or texturizing agent	Levels not to exceed 65 ppm	Binder regulations apply	9 CFR 381.159
Uncooked, restructured poultry breast products	Binder or texturizing agent	Levels not to exceed 100 ppm	Required use of "formed" or "reformed" in product name	FSIS Directive 7120.1 Amend 13
Cooked sausage products	Binder or texturizing agent	Levels not to exceed 65 ppm	Binder regulations apply	9 CFR 319.140
Certain cured pork products	Binder or texturizing agent	Levels not to exceed 65 ppm	Binders are only permitted in certain cured pork products, such as "Ham Water Added," "Ham and Water Product-X% of Weight is Added Ingredients," and "Ham with Natural Juices'	9 CFR 319.104
Roast beef, parboiled, and steam cooked	Binder or texturizing agent	Levels not to exceed 65 ppm	Binder regulations apply	9 CFR 319.81

Consult the USDA-FSIS or CFR for current regulations (CFR [2007b]; CFR [2007c]; CFR [2007e]; USDA-FSIS [2007]).

Substrate Protein Sources

When a substrate protein for transglutaminase is considered, all proteins are not created equal. TGase reacts to varying degrees with many proteins under different conditions (Kim, Carpenter, Lanier, & Wicker 1993; Kuraishi et al., 1997; Motoki & Nio, 1983; Sakamoto, Kumazawa, & Motoki, 1994). The extent of the reaction is largely determined by the availability of glutamine and lysine within the protein and the actual reaction conditions. TGase reacts very well with sodium caseinate, gelatin, soy proteins, and myosin. It reacts moderately well with the wheat, collagen, and egg yolk proteins. Reactions with whey proteins, egg albumin, and myoglobin are dependent on the conditions of the reaction and of the proteins themselves. TGase has little or no reaction with the muscle protein actin. It is important to note that processing conditions can be used to improve the reactivity of many proteins by exposing glutamine and lysine, thus allowing greater reactivity with TGase. Therefore, several sources should be considered and evaluated when selecting a dry food protein for reaction with TGase. Although a laborious process, food products can be analyzed for the extent of a TGase reaction by digesting the protein to the actual ε-(γ-glutamyl)lysine cross-link bond and then measuring the number of bonds (Sakamoto, Kumazawa, Kawajiri, & Motoki, 1995).

Application Areas

In order for transglutaminase to function in a food system, the food system must contain a protein that has reactive sites available for the enzyme, as well as pH, temperature, and reaction conditions that allow activity. For this reason, TGase preparations (enzymes + other ingredients) are formulated to contain the proper ratio of enzyme to substrate protein or other carrier. The level of TGase used in a particular product varies by application and method of addition. Table 8.4 lists general guidelines for many popular applications in meat, poultry, and seafood products. These recommended levels may change as new preparations and sources for TGase are developed.

In general, the available preparations for meat systems are for two uses: (1) texture modification (TM), where the enzyme is standardized on an inert carrier, usually maltodextrin, or (2) binding applications (BA), where the enzyme is combined with proteins (sodium caseinate, gelatin, etc.) or other functional ingredients (phosphate, etc.). In addition, because of susceptibility to oxidation, these preparations have traditionally been packaged in high barrier foil-lined pouches that contain oxygen scavengers. Care must be taken to reseal and refrigerate or freeze any unused portion of the dry enzyme between applications, as the enzyme activity will diminish over time with exposure to oxygen at room temperatures. The

TM-type preparations are designed to go into systems where ample protein is available to act as a substrate for the enzyme, while the BA preparations are used in systems where either the protein is not available for reaction or is present in insufficient quantities to allow TGase to properly bind the system.

Texture Applications

Texture in Emulsion or Finely Ground Systems For TM applications, TGase is usually added via TM preparations that contain only the standardized enzyme, although there are instances where either type of preparation may be used (Table 8.4). For emulsion applications, the enzyme may be added to the product along with the salt. Typical meat emulsion systems are formed through extraction of protein via salt solubilization. This soluble protein in the presence of energy acts to emulsify and stabilize fat in the system. Through covalent bonding, mTGase functions in this system to increase cross-linking of the solubilized protein by forming an even stronger matrix, providing further stabilization of the emulsion (Ramírez-Suarez & Xiong, 2003).

Sausage Applications. TGase can act to greatly enhance the texture and bite characteristics of franks. During frank manufacture, TM-type preparations are added during the chopping process to distribute and hydrate the enzyme. Following comminution and linking, the product is allowed to stand for a short time prior to cooking, or a "speed reaction" is programmed into the cooking cycle, to allow the enzyme to react at an elevated temperature (~55°C) for a short time. TGase strengthens the product texture by cross-linking and reinforcing the protein matrix before and during the heating process (until it is denatured by heat), resulting in much higher gel strength/bite characteristics and greater yield (Ruiz-Carrascal & Regenstein, 2002). Kuraishi et al. (1997) have also shown that mTGase can improve the texture characteristics of lower salt meat products to the point they are similar to their higher salt controls without TGase.

Surimi-Based Products. Surimi products (washed fish protein) contain a naturally occurring transglutaminase (Lee & Lanier, 1995; Seki et al., 1990) that is responsible for the texture/gel strength and bite characteristic of kamaboko (fish sausage) products that have gone through a "Suwari" setting process. The Suwari process usually involves holding a chopped fish paste at an elevated temperature (25–40°C) for a specified time (~1–6 h) that varies greatly between processors. During this process, the kamaboko mixture develops cross-links via the naturally occurring TGase present in the product, followed by steam cooking. Microbial TGase has been successfully used to improve the texture or further reduce the cost of set-type kamaboko products. In this latter case, the exogenous TGase is added during the kamaboko chopping processes and the molded product subjected to the "Suwari" process. In this manner, kamaboko products can be formulated with reduced surimi raw block but retain the same finished product texture (Seguro, Kumazawa, Ohtsuka, Toiguchi, & Motoki, 1995). Therefore, a small supplemental

Table 8.4 Applications and typical use rates of general transglutaminase preparations used in meat applications

Application	Preparation type/strength Amount of preparation normally used for application[a]		Application methods generally used[b]			Main purpose of using TG	Potential issues
	Texture appl.(TA): enzyme only (100U/g powder)	Binding appl. (BA): enzyme + protein (50U/g powder)	Dry sprinkled onto product/ into mixer	Slurry with water	Mixer/tumbler dry or slurry addition		
Red meat and poultry							
Emulsion products	0.025–0.30%	Possible to use	X			Improve texture	Short reaction time is necessary; low salt product requires more enzyme
Sausage products	0.05–0.30%	Possible to use	X			Improve texture	Holding time before stuffing
Cooked meat (ham or bacon trims)		0.75–1.30%		X		Novel product; value addition	May need additional protein in system
Chunked and formed products		0.25–1.30%	X	X	X	Improve texture; portion control	Issues with excess marinades or brines
Restructured poultry – light meat		0.75–2.00%			X	Portion control; value added trimmings	Issues with excess marinades or brines
Restructured poultry – dark meat		0.30–1.30%			X	Portion control; value added trimmings	Issues with excess marinades or brines
Restructured meat logs (beef, pork; pieces >1″)		0.25–1.00%		X	X	Portion control; eating characteristics	Issues with excess marinades or brines

(continued)

Table 8.4 (continued)

Application	Preparation type/strength Amount of preparation normally used for application[a]		Application methods generally used[b]			Main purpose of using TG	Potential issues
	Texture appl.(TA): enzyme only (100U/g powder)	Binding appl. (BA): enzyme + protein (50U/g powder)	Dry sprinkled onto product/ into mixer	Slurry with water	Mixer/tumbler dry or slurry addition		
Hams – macerated and tumbled extracted protein	0.05–0.20%		X			Stronger muscle to muscle bonding	Must stuff immediately after mixing into product
Injection applications: whole muscle	0.025–0.20%					Improve texture/bite character	Issues with proteins in brine, over-reaction
Seafood							
Shrimp-binding		1.00–1.30%		X	X	Binding; portion control; novel product	Shrimp variety
Shrimp, fish, eggs (soaking solution) – texture	Varies					Firm texture	Varies
Scallops		1.00–1.30%		X	X	Portion control; novel product	Scallop condition
Fish fillets		0.25–1.30%	X	X		Portion control; firm texture; close gape	Age of fish

[a]Particular applications may require more or less. Use as a guideline only.[b]X = typical method of addition.

dose can be added during the chopping procedure, giving the enzyme the time and temperature necessary to make it cost effective to add to this system.

Generally, the high speed imitation crab stick lines have not found widespread use of TGase because of the very short reaction time possible in this process. In this process the mixture of surimi, salt, phosphate, starches, flavorings, and cryoprotectants is chopped to a fine paste. The mixture is then pumped into a ribbon shape that then passes over a series of heating units (flame or radiant heat) where it is in the form of a cooked roll within ~2 min postextrusion. Generally, the level of mTGase necessary to cause a texture change in this short reaction period is cost-prohibitive, although some equipment modifications have been shown to make the addition cost effective.

Texture in Injection Systems. TGase can also be used in injection systems to modify texture. TM-type preparations are generally used, although more specialized compositions are commercially available. When adding the enzyme in brine systems, it should be added to the brine just prior to injection. If the brines contain protein, care must be taken not to add it too early and to keep the brine cold to slow the enzyme reaction and prevent the brine from becoming excessively viscous. Specific TGase preparations have been developed and patented to prevent the enzyme from acting before it is injected into the meat (Susa, Nakagoshi, & Sakaguchi, 2004; Susa & Numazawa, 2001). Other patents demonstrate the use of TGase to improve the quality and processing characteristics of PSE pork and turkey by firming, binding and improving cooking yields in processed pork and turkey breast products (Milkowski & Sosnicki, 1999).

Whole Muscle Products. TGase may also be used in certain applications to change the bite or slicing characteristics of whole muscle products. In this case very low levels of the enzyme (0.025%–0.1 of a TM-type preparation in finished product) are added to the injection brine. The resulting product has a texture that is more fibrous and meat-like than the same formulation without the enzyme. It must be noted that excessive doses can have a negative effect on product yield.

Binding Applications

Binding applications usually involve the topical coating of the surfaces to be joined with a BA-type preparation that contains a combination of protein and TGase. Sodium caseinate and gelatin are two proteins that possess the characteristics necessary for them to function effectively with mTGase in binding systems. Their linear nature seems to make them better suited for the application than globular proteins. For example, mixtures of mTGase, sodium caseinate, and water at the proper ratios can form a gel within a few minutes, while mixtures containing only water and sodium caseinate will not gel at room temperature. Although mTGase acts to increase the gel strength of a number of food proteins, not all act as a contact adhesive for binding. For example, the gel strength of a soy protein isolate can be increased 50% to 100+% using TGase (dose-dependent), but it is generally not effective as a topical adhesive in binding applications.

Binding preparations may be added by either dry or wet application (Table 8.4). Dry addition involves evenly coating the surfaces with a dry dusting of the preparation. Wet addition involves making slurries using the dry preparation and the appropriate amount of water, and applying this solution by manually painting, mixing, or tumbling the products to coat the surfaces. Care must be taken to coat only so much product as can be formed within a given time period (15–30 min for maximum bond strength) since, once wetted, the reaction begins. Preparations have also been recently developed and patented (Ishida & Nakagoshi, 2007) that are stable as liquids for much longer periods (hours). These preparations make use of pH adjustment to suspend the activity of the enzyme until it is applied to the meat surface. Once applied, the meat buffers the solution pH into the proper range for enzyme activity and the reaction begins. This product is marketed as Activa™ TG-GS (Ajinomoto Co., Tokyo, Japan) in the United States and contains a combination of transglutaminase, gelatin, and phosphate. Once applied, this preparation must also be formed within 15–30 min to maximize binding strength. Once formed the products are allowed to react from 6 to 18 h for the binding to occur. If used in a chunked and formed binding application, typically either the dry or wet methods will work so long as the moisture level is controlled.

Scallops. One of the first major uses of TGase in seafood was for binding of small species of scallops into larger scallop-sized portions. There were generally some problems binding these items because of soft texture and excess moisture associated. In general, the process required about 65–100 ppm of enzyme in combination with higher levels of proteins to help manage the water and form the matrix that binds these products together. Gentle mixing to incorporate the enzyme maintains the proper scallop structure and a 24–36-h reaction time produces products with enough strength to be sliced in the raw state. Researchers have studied the mTGase system with other cold binding systems and have shown that the TGase system produces acceptable restructured scallops (Beltrán-Lugo, Maeda-Martínez, Pacheco-Aguilar, Nolasco-Soria, & Ocaño-Higuera, 2005; Suklim et al., 2004).

Chunked and Formed Portions. Raw-bonded, chunked, and formed meat products using meat raw materials of varying size in combination with BA-type mTGase preparations can be easily produced using a mixer and stuffing system. In most systems, between 5% and 30% marinade can be used to extend products that are to be bonded, as long as the meat raw material has the ability to absorb the marinade to a tacky consistency. Care must be used to ensure the meat absorbs as much marinade as possible before the preparation is added. Vacuum mixing and stuffing improves binding by removing trapped air in the finished product. As previously mentioned, care must be taken to control batch size so all product can be formed within a short period based on the preparation and application method. The amount of preparation necessary is dependent on the portioning method. If products are cut frozen, much less enzyme is required than if they are portioned from a fresh state.

TG Used to Bond Larger Pieces of Muscle. Binding of larger cuts is generally done by dry-sprinkling or slurry-coating the surfaces to be joined. The products are then held together in some manner (casing, vacuum-packaging, etc.) to allow reaction (6–18 h). One of the primary applications of mTGase is in the bonding of beef tenderloins. Because of their wedge-shape, beef tenderloins tend to have excessive waste when they are traditionally portioned into filet mignons. Using raw bonding technology, two tenderloins may be joined head to tail, stuffed into a casing or form and allowed to react. This cylinder of tenderloins can then later be portioned into precise portion controlled entrees, eliminating the expensive trimmings formed by traditional portioning methods.

The importance of bonding technology is even more apparent with recent studies on muscle profiling of beef (von Seggern, Calkins, Johnson, Brickler, & Gwartney, 2005) which look at alternate ways to merchandize muscles from a beef carcass based on modified fabrication methods and muscle characteristics. Several of these muscles have portioning issues. Some muscles such as the petit tender (*teres major*) have a size and shape that is difficult to portion effectively. Others, like the Flat Iron (*Infraspinatus*), once denuded, also have pieces with the wrong fiber orientation, shape, and thickness to effectively portion. In both cases, these issues can be eliminated if they are bound into a larger portion. This can be done by layering thin muscles to align fiber orientation such that highly desirable portions can be fabricated. Farouk, Zhang, and Cummings (2005) investigated fiber orientation (parallel vs. perpendicular vs. mixed) of beef cuts treated with binding agents and found no difference in the orientation strength but positive advantages in raw visual appeal for those cuts joined with parallel fiber orientation. TGase-restructured beef items generally have good raw color, but some researchers (Farouk, Hall, Wieliczko, & Swan, 2005) have found them tougher and less palatable than products manufactured using other binding methods. However, this may have resulted from the study's use of high levels of enzyme and extended reaction time (7 days). This technology has also been studied in binding fish fillets into sizes that are more easily portioned or processed (Ramírez, Del Angel, Velásquez, & Vázquez, 2006; Ramírez, Uresti, Téllez, & Vázquez, 2002).

Factors Affecting Reactions. The recommended inclusion rate for the enzyme varies based on factors such as protein source, protein content, availability of the proper amino acids to participate in the cross-linking reaction, reaction time, reaction temperature, food production process, and other formula components. In general, muscle proteins have different reactivity rates with TGase. Pork, beef, and chicken dark meat (leg & thigh) tend to react very well. Chicken light meat (breast and wing), scallops, and shrimp require a longer reaction time and/or greater enzyme dose to achieve the same bond strength. In poultry, this is thought to be caused by increased levels of the peptides anserine and carnosine in chicken light meat (Kumazawa, Numazawa, Seguro, & Motoki, 1995). Other polyamines are known to influence the mTGase reactivity in different systems (Sato, Ohtake, Kohno, Abe, & Ohkubo, 2003). To achieve the same level of binding, it typically takes 1.5 to 2 times more TGase to bond light poultry meat than dark meat. This can be achieved by a

combination of reaction time, enzyme concentration, and reaction temperature. In shrimp the higher doses are needed because of the contortion and shrink that shrimp muscle undergoes during cooking. Extra bond strength is necessary to prevent separation. Salt and phosphate addition have positive effects on bind strength due to their ability to solubilize proteins at the surface of the muscle. It must also be noted that tenderizing enzymes generally negate the effects of mTGase in meat systems unless they are applied topically after the TGase bonding is complete.

As with all enzymes, it is possible to use too much TGase in a given application. Overdose can result in reduced cook yields and dry products that are very tough. For most every application there is an optimal level that will give the desired sensory and processing properties. Because of differences in procedures, those levels must be individualized for a given system and raw material.

Summary

Enzymes allow for the transformation of underutilized products or by-products into products with much higher value. It is extremely important to understand the dose and conditions necessary to optimize a particular enzyme for its given application. Harnessing the power of enzymes will allow product developers to think outside of the box when it comes to developing new and different meat products. This includes developing better ways to merchandize the various muscles, with their inherent flavor, texture and eating characteristics, to maximize the overall value and consistency of these products.

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Chapter 9
Spices, Seasonings, and Flavors

Peter M. Brown

A Brief History

An entire text could be devoted to the history of spices. When caveman placed the first piece of meat over fire, the concept of flavor was created.

In ancient times, spices were status symbols in Europe and throughout the Mediterranean for the wealthy who ate them (Uhl, 2000). Spices had enormous trade value, not only as flavoring for food, but as medicines, preservatives, and perfumes (Uhl, 2000). As global travel developed, the spice trade expanded, resulting in the exchange and demand for spices not common to particular populations. India, Asia, and China introduced anise, basil, cardamom, cinnamon, clove, garlic, ginger, mace, mustard, nutmeg, onion, tamarind, and turmeric. The Middle East and Mediterranean countries exposed bay leaf, coriander, cumin, dill, fennel, fenugreek, rosemary, sage, sesame, and thyme. North America and the Latin American countries provided allspice, annatto, chile peppers, chocolate, and sassafras.

Based on archaeological excavations, chile peppers have been documented in Mexico back to 7000 B.C., ground saffron in Asia to around 3200 B.C., and from the Bible, King Solomon counted spices among the valuables in his treasury (Uhl, 2000). By land or sea, spices were some of the first commodities instrumental in promoting trade from the beginning of time.

Blended seasonings first appeared with the centralization of the meat industry at the turn of the twentieth century. Up until the end of World War II, many blended seasonings, primarily for fresh sausage and cures for hams, bellies, picnics, and jowls were peddled by traveling salesmen right to the farm and sold in small quantities to suffice the fall slaughter. As meat plants grew in numbers and old world recipes were passed on, the varieties of blended seasonings began to flourish.

Introduction

The consuming public has probably never pondered that the hundreds of meat products available to them on a daily basis are actually processed from only three protein species: beef, pork, and poultry. Food technologists and meat processors

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have done a tremendous job of providing variety by manipulating these proteins with combinations of flavor, thermal processing, portion size, and packaging. In today's market, global relocation of ethnic groups and the demand for variety and convenience continue to fuel the development of new meat products. In addition, the health conscious consumer is demanding cleaner labels, reduced fat, lower sodium, exciting flavors, and eye-appealing appearance that can be table-ready in a short period of time. The aging baby boomers are somewhat reinventing the wheel in their desire for natural and organic meat products made with meat from free range animals, as they remember from childhood. This is a truly desirable concept indeed, but a very significant challenge for the food industry.

It is these challenges that encourage the meat processor to focus on having a working knowledge of the functional ingredients available to optimize every aspect of production. Spices, flavorings, and seasonings are major contributors of the building blocks of successful processed meat products. Their use in combination with the other ingredients discussed in this book allows meat processors to improve and extend their current production capabilities.

Definitions

Spices: The root (ginger, garlic, onion), fruit (chili peppers, bell pepper), bark (cinnamon, cassia), or berry (black pepper, juniper) of herbaceous and deciduous plants. Natural spices are available fresh, dried, whole, or ground.

Herbs: Primarily the leafy portion of herbaceous plants. Examples of herbs include sage, oregano, basil, and thyme. These ingredients are also available fresh, dried, whole, or ground.

Resins: Concentrated, liquid versions of spices and herbs that are manufactured through steam, chemical, or mechanical extraction. These extractions can be specific for aroma, flavor, or color, or any combination of these attributes.

Flavorings: A large group of flavor components made up of spray-dried ingredients, including protein broths, dehydrated fruit bits and powders, Worcestershire powder, a variety of vinegar powders, smoke flavors, and soy sauce powder. Unlike spice resins, which are concentrated, most spray-dried flavors do not possess the same concentration of flavor as their original counterparts. Virtually, any liquid flavor is available in a dry form that can be incorporated into a blended seasoning. Flavorings can also be created from the reaction or fermentation of amino acids to include savory flavors or flavor enhancers such as hydrolyzed plant proteins, autolyzed yeasts, and monosodium glutamate.

Seasonings: The blended combination of two or more ingredients to provide a complete and balanced functional addition for further processing of meat and food products.

Properties

An important decision a processor must make is how to optimize ingredient selection for the target product. Will the product be fresh or fully cooked? What will be the method of display, retail or frozen for food service? What is the targeted price point? All of these factors play a part in spice and flavor selection. A basic knowledge of the advantages and disadvantages of available spice forms will aid in this selection (Table 9.1).

Spices are made up of carbohydrates (including sugars, fiber, and gum), fat, protein, ash, and a complex variety of chemical compounds that includes volatile and nonvolatile oils. These oils can be further broken down into aldehydes, esters, alcohols, sugars, alkaloids, and phenols. The volatile oils represent primarily the aromatic aspect of spices, but also contribute greatly to their flavor. The nonvolatile oils (oleoresins) are flavor compounds that give spices their pungent, hot, sweet, or bitter notes. Table 9.2 lists the compounds primarily responsible for the flavor notes of some of the more commonly used spices.

Spices are grown throughout the world. Oregano is available from Mexico and the Mediterranean. Sage can be of Albanian or Balkan origin. Paprika can be Spanish, Hungarian, or from several Middle Eastern countries. Mustard is primarily a crop of Canadian origin. Egyptian fennel and marjoram are highly prized. The United States is known for aromatics such as bell peppers and a variety of hot peppers. Conditions for growing, harvesting, and processing can vary greatly between regions. Extrinsic environmental factors such as drought, unseasonable cold, or an overabundance of rain can impact the quality, availability, and price of these commodities. Quality spice and seasoning companies should have critical specifications in place for minimal microbial counts, color attributes, mesh size, and volatile oil content to insure consistency to the processor.

Table 9.1 Advantages and disadvantages of various spice forms

Spice Form	Advantages	Disadvantages
Fresh whole	Fresh flavor	Flavor and color intensity
	Natural appearance	Variation
	Label friendly	Potential for high microbial counts
	Limited shelf life	Availability
Dried ground	Process friendly	Decreased aroma, flavor, and color
	Increased shelf life	Dust prone
	Better availability	
Extractives	Uniform color, flavor, and appearance	Too concentrated for untrained handling
	Low usage	Flavor and aroma not typical of natural spice
	Lower cost	
	Readily available	Color and flavor depreciates over time
	Easily stored	
	Water soluble if needed	

Lewis (1984); Tainter and Grenis (1993); Uhl (2000).

Table 9.2 Primary flavor components of common spices

Spice	Primary flavor components
Red pepper	Capsaicin
Black/white pepper	Piperine, chavicine
Mustard	Allyl isothiocyanate
Ginger	Gingerol
Garlic/onion	Diallysulfide
Sage	Borneol
Clove	Eugenol

Lewis (1984); Tainter & Grenis (1993); Uhl (2000).

Spices key on the consumer's sensory perception of sweet, sour, bitter, and salty. In recent years, the culinary industry has introduced a new taste sensation, *umami*, with claims that it must be added to the four listed above. Umami is not new; it was discovered in 1908 by Kikune Ikeda, a Japanese scientist. Umami, which means "savory or delicious," utilizes the consumer's perception of the glutamic amino acid occurring naturally in meats, cheeses, and protein foods (Maga, 1998). This concept helps to explain why foods containing MSG or other glutamate salts, such as hydrolyzed plant proteins and several yeasts, promote a richer or heartier flavor. Ingredients of glutamic origin have received negative consumer acceptance over the last decade and, although not considered an allergen, monosodium glutamate has been categorized as a sensitive ingredient, along with sulfites, lactose, and artificial food colors (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 2005b).

Recent advances in ingredient technology have resulted in a very diverse offering of food ingredients that meat processors can use. With the diversity and advancement of food processing ingredients, it is advantageous for the meat processor to identify with ingredient professionals to establish their product goals and work together to create functional blended ingredients to optimize their value-added products.

Function

Let us now investigate how blended seasonings are formulated for a particular end user and what the variables can be in regard to usage levels of ingredients. The combination of two or more ingredients results in a blended seasoning. A mixture of salt and sodium phosphates for vacuum tumbling or injection sounds simple enough, but depending on the target percent pick up and desired level of salt, both ingredients must be calculated to optimize the finished product. Spices and flavors are also unique in that they are self-limiting; too high levels can adversely impact the product's flavor profile balance.

A complete blended seasoning can contain ingredients from all chapters in this text. Regulations in reference to spices and flavorings only have a few restrictions, which are mostly related to color. There are several traditional processed meats that do have a standard of identity which call for particular product to contain certain specific spices. A good example is fresh Italian sausage. In addition to being limited to 35% fat, as opposed to 50% in other fresh sausages, this product must contain at least three spices from a list that includes fennel, basil, oregano, paprika, black pepper, and garlic. In addition, fresh Italian sausage is one of only a few that is allowed to contain paprika, along with Fresh Chorizo and Fresh Longaniza (USDA-FSIS, 2005a).

Fresh sausages are generally formulated with salt levels between 1.5% and 2.1%. Chicken sausages are typically formulated with higher salt levels. A sweetener may or may not be included and the balance of ingredients will be spices, spice extractives, and possibly a microbial inhibitor.

Modifications to this formulation would be based on the anticipated end-use and shelf life of the finished product. Product made with this formulation would be a traditional, mild sausage. To maximize fresh retail display, a portion of the natural ground spices could be replaced with spice extractives. If the product were to be frozen, a synthetic antioxidant, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), or propyl gallate (with a synergist such as citric acid to enhance their function), or a natural antioxidant, such as extractive of rosemary or oregano, would be incorporated to retard oxidative rancidity. In the United States, permitted amounts of antioxidants are calculated based on fat content, as described in the regulations (Code of Federal Regulations [CFR], 2007a) and in Chap. 13. For BHA and BHT in fresh pork sausage, permitted levels are 0.01% when used alone and 0.02% when used in combination with other antioxidants. Microbial inhibitors such as lactates and/or acetates are now allowed to preserve freshness and reduce the incidence of *Listeria monocytogenes* and other organisms. Lactates can be used at up to 4.80% and the acetates/diacetates at up to 0.25% (Chap. 14).

The blend in Table 9.3 can be adjusted for a hot (spicy) fresh Italian sausage by the addition of 2 oz of crushed and/or ground red pepper (30,000 heat units [hu]). Oleoresin of capsicum is also an option, with 12–15 ml being sufficient. To modify the blend for a fresh sweet Italian sausage, an additional 12 oz of sugar is all that is necessary. It must be kept in mind that these are starting points and can be further adjusted for different target levels of heat or sweetness.

Spices are one of the easiest and most convenient methods to expand standard and traditional processed meats. The incorporation of one or more additional spices to an existing product such as smoked sausage or bratwurst can lead to the creation of a new meat product. For example, the addition of 8 oz of dehydrated diced jalapeños and 2 oz of ground jalapeños to a 100 lb batch of smoked sausage results in a new product offering. This product can be further recreated with the addition of 5–7 lb of shredded high-temperature melt cheddar or American cheese. As previously stated, crushed, ground or extractives of red pepper can make any product "hot and spicy." Cracked black pepper always assures a second look at a traditional Polish or Bratwurst. A meat processor can continue to utilize the original blended

Table 9.3 Sample blended seasoning for fresh Italian sausage

Ingredients	lb	oz
Pork trimmings (max. 35% fat)	100	
Water	3	
Salt		24
Dextrose and/or sugar (increase dextrose for additional browning)		4
Fennel, whole and/or cracked		4
Paprika (140 ASTA)		4
Fennel, ground		2
Black pepper (butcher grind, 14, 18, or 24 mesh)		3
Garlic powder		0.5
Basil, ground		0.5

Based on a 100 lb meat block.

seasoning and keep on hand bulk spices to add when wanting to alter the original flavor. It is traditional in the upper eastern seaboard to add marjoram at Easter time to several traditional sausage products.

A more recent trend in upscale retail processing establishments is the incorporation of fresh or blanched vegetables, such as spinach, basil, or sun dried tomatoes, to create consumer interest. However, unless the products are fully cooked, the incorporation of such ingredients can compromise the shelf life. A meat processor is only limited by the imagination when using spices alone or in combination to create new products.

Seasoning blends are formulated for flavor, functionality, and appearance. Depending on a processor's manufacturing protocol, blends can be totally inclusive (i.e., containing other nonflavoring functional ingredients) or contain only the flavoring portion of the product. Some meat processors prefer to add salt and cure first for protein extraction and color development, whereas others find it more convenient to have a complete blend and implement grinding and mixing accordingly.

Table 9.4 lists the basic ingredients that are typically found in the major categories of processed meat products. For vacuum-tumbled and pumped whole-muscle products a two-step process may be required. The first step would involve an internal flavor system that is soluble, including the ingredients listed in Table 9.4. Cured products would also include several functional ingredients such as sodium phosphates, sodium erythorbate/ascorbate, and curing salt. Once the internal flavors and other functional ingredients are incorporated through static marinade, vacuum tumbling, or injection, a topical rub may be applied to create additional flavor and eye appeal. Topical rubs are formulated to include salt, sweeteners, spices, and a functional ingredient such as modified or native starch (Chap. 2) to provide additional water pick-up and adherence of the spices. These meat products can then be packaged uncooked or thermally processed for convenient consumer preParation.

Formulation of blended seasonings will often be dependent on regional and national flavor preferences.

Table 9.4 Basic ingredients commonly used in processed meat products

Product category	Ingredients
Fresh sausage	Salt, sweeteners, spices, spice extractives, natural flavors, antioxidants, microbial inhibitors
Cured, fully cooked sausage	Salt, sweeteners, sodium phosphates, spices, spice extractives, microbial inhibitors, sodium erythorbate/ascorbate, curing salt
Vacuum-tumbled and pumped whole-muscle valued added products	Salt, sweeteners, sodium phosphates, starches/gums, spice extractives, and flavorings

Characteristic Spice Profiles of Processed Meat Products

The characteristic spice profiles of some popular sausage products are presented in Table 9.5

The following comments for each product category are offered to supplement that information. Once again, these profiles can vary depending on region or intended end user.

1. *Fresh sausage products*. With adjustments in salt and water, and the incorporation of additional functional ingredients addressed in other chapters, any of the products listed in Table 9.5 can be made into cured and fully cooked versions.
2. *Cured, smoked, and fully cooked products*. Variations of these products can be obtained by the addition of large particle size spices (e.g., cracked black pepper) or natural smoke flavor (hickory, mesquite, or the more trendy applewood, cherry, or pecan), changes in meat particle sizes, or a different choice of casing, any of the products listed in Table 9.5 can be made into cured and fully cooked versions.
3. *Whole-muscle and value-added products*. Occasionally a base pump or marinade will be used to impart internal flavor notes that can enhance several different topical rubs. In addition to several other functional ingredients, such as sodium phosphates (Chap. 1), gums (Chap. 3), and microbial inhibitors (Chap. 14), these pumps will include salt, dextrose, onion salt, garlic salt, and extractives of black and red pepper.

The formulation of topical rubs is only limited by the imagination. Flavor profiles can include: “Garlic Herb and Sundried Tomato,” “Tuscany,” “Spicy Thai,” “Pesto,” “Honey Barbecue,” along with the standard “Lemon Pepper,” “Cajun,” “Hot and Spicy,” “Teriyaki,” and “Savory.” It is in these topical rubs that flavoring ingredients such as soy sauce powder, Worcestershire powder, natural and artificial fruit and vegetable powders and dehydrated bits, vinegar powder, honey powder, and butter flavors are typically used.

Table 9.5 Characteristic spice profiles of sausage products

Category/product	Ingredients
Fresh sausage	
Fresh pork sausage (Southern U.S. profile)	Salt, sugar, dextrose, sage, black pepper (32 mesh), ground red pepper (30,000 hu), crushed red pepper (can also add these spices in extractive form to boost the natural spice notes)
Fresh pork sausage (Midwestern U.S. profile)	Salt, dextrose, black pepper (42 mesh), ground red pepper (10,000 hu), nutmeg, mace, cinnamon, ginger, and their extractive counterparts
Fresh pork sausage (Northeastern U.S. profile)	Salt, dextrose, black pepper (42 or 80 mesh), red pepper (10,000–30,000 hu), coriander, nutmeg, and their extractive counterparts
Fresh pork sausage (maple-flavored)	Any of the above profiles, plus sufficient sugar or brown sugar (for desired sweetness), maple oil (typically composed of natural and artificially ingredients)
Fresh bratwurst (Wisconsin-style)	Salt, dextrose, sugar, nutmeg, white pepper, mace, ginger, black pepper (42 mesh), extractives of nutmeg and mace
Fresh bratwurst (Bavarian)	Salt, sugar, onion (powder, granulated, minced, and/or fresh), black pepper (32 mesh), sage, extractives of black pepper
Fresh Polish sausage	Salt, sugar, garlic powder, white pepper, and coriander
Fresh Cajun sausage	Salt, chili pepper, red pepper (30,000 hu), black pepper (24 mesh), white pepper, garlic powder, onion powder, cumin, savory, and oregano
Fresh chorizo	Salt, chili pepper, cumin, garlic powder, black pepper (32 mesh), red pepper (30,000 hu), clove, paprika, oregano, and extractives
Cured, smoked and fully cooked sausage	
Bologna and frankfurters	Salt, corn syrup solids, dextrose, onion powder, garlic powder, coriander, white pepper, clove, extractives of cinnamon, red pepper, clove, and pimento leaf oil
Summer sausage	Salt, dextrose, garlic powder, white pepper (32 mesh), and coriander
Cooked salami	Salt, corn syrup solids, garlic powder, coriander, white pepper, and whole black peppercorns
Knockwurst	Salt, dextrose, nonfat dry milk, mace, white pepper, allspice, coriander, paprika, and garlic powder
Mettwurst	Salt, dextrose, nutmeg, white pepper, celery, allspice, marjoram, ground caraway seed, coriander, and whole mustard seed
Longaniza	Salt, dextrose, garlic (granulated), vinegar, paprika, black pepper, marjoram, and non fat dry milk
Braunschweiger	Nonfat dry milk, salt, dextrose, onion powder, white pepper, clove, allspice, sage, marjoram, nutmeg, and ginger

Regulations

In the United States, blended seasonings fall under U.S. Food and Drug Administration (FDA) regulations, although in meat processing the blends must also conform to USDA guidelines. Both agencies require that ingredients in a meat product be listed in order of predominance on the label. The FDA requires the inclusion of all ingredients, including sublistings. The USDA allows for the omission of certain incidental ingredients such as anticaking and flow control agents.

These include sodium aluminosilicate, tricalcium phosphate, silicon dioxide, and vegetable oils, at levels not to exceed 2%. Sweeteners used as carriers for plating extractives can also be considered incidental at low levels. Another relatively recent change in regulations is that the labeling of any hydrolyzed protein ingredient must specify the source of the protein (i.e. soy, corn, wheat, etc.) (CFR, 2007b; USDA-FSIS, 1995).

According to the Food Allergen Labeling and Consumer Protection Act of 2004 (Congress of the United States, 2004), the food source of any ingredient that falls under one of the eight recognized categories of allergens must also be identified, either by naming it in parenthesis following the name of the ingredient (e.g., "lecithin [soy]), or by qualifying statement next to or immediately after the ingredient statement (i.e., "contains soy, milk, etc.") (United States Food and Drug Administration, Center for Food Safety and Applied Nutrition [FDA-CFSAN], 2008).

As stated earlier, the flavor components of blended seasonings are self-limiting. Too much of a certain spice will throw off the balance of an intended flavor profile. Therefore, most regulations in regard to spices are targeted to their coloring attributes. All spices used primarily for color are allowed in fully cooked meat products. Their limitations or seParate listings pertain to fresh comminuted or whole-muscle products. Among these, as mentioned earlier, paprika is limited to certain fresh sausages and can also be used in any whole-muscle poultry product. The coloring spices include paprika, annatto, and turmeric. These cannot be included within spices and must be labeled seParately in both their dried and extractive versions.

In the United States, there exist standards of identity for certain flavor profiles. A good example is "Cajun." A Cajun product must contain red pepper, black pepper, white pepper, garlic, and onion (USDA-FSIS, 2005a). With the emergence of ethnic flavors, standards of identity have also been developed for other specific flavor profiles.

Blended seasonings usually make up only a small portion of a recipe. Traditional processed meats may include seasonings at levels of up to 3%, and even high-end value-added products that include both internal and external functional ingredients, flavor, and visual spices will rarely exceed 10%. The point is that seasonings and other functional ingredients only contribute to a fraction of the total cost. Many functional ingredients will actually lower product cost when used as meat replacements or through increased yields.

Summary and Current Trends

The food industry is the largest private sector industry in the United States. Meat fabrication and processing account for 25% of this industry. According to the USDA, meat consumption in the United States in 2002 was 195 lb (boneless trimmed weight equivalent) per person (USDA, 2003).

Per capita, Americans consumed 7 lb more red meat, 46 lb more poultry, and 4 lb more fish and shellfish than 50 years ago. It is noteworthy that this 50 year time frame correlates with industrialization from off the farm to centralized production of meat and food products. Quality and convenience for the consumer has been a positive marketing issue for much longer than recently recognized. Rising consumer incomes, value-added meat products, name brands, and the evolving food service industry have been primarily responsible for this trend.

In 2002, 32% of meals were consumed away from home (USDA, 2003). The meat industry has met this challenge by providing the food service industry with meat products of quality equivalent to home preParation. Meat-based soups, stews, chowders, and chili are formulated, prepared and cooked at the processor level and only require reheating at the restaurant or institution. Military and hospital foods are premanufactured and distributed in the heat-and-eat manner as well. Today's consumer places many demands on the meat processor.

Quality, convenience, and cost are three of the most important consumer concerns. More recently, there has been a trend towards "clean label," "all natural," and even "100% organic." These categories of food products place challenging demands on ingredient suppliers as well as processors. Clean label products may, in some cases, be the less difficult of these challenges to meet. In this regard, the elimination of allergenic ingredients and the addition of certain useful but unappreciated functional ingredients work to satisfy both processor and customer. These meat products do result in limitations in terms of variety and flavor impact, as well as lower yields that can substantially reduce juiciness and overall palatability. Replacement alternatives also can significantly increase the cost of the finished product.

The USDA regulations for "natural" (USDA-FSIS, 2005a) date back to 1982, although, as of this writing, they are in the process of being revised (USDA-FSIS, 2006). The interpretation of "natural" has expanded significantly since the early 1980s. In many cases, large grocery and restaurant chains have their own interpretations of the term and have established their own guidelines for what ingredients qualify as natural. This category of products might include ingredients such as sea salt, raw sugar (turbinado, evaporated cane juice), natural spices, and minimally processed garlic, onion, tomato powder, smoke flavor, and other natural flavors.

Organic guidelines (National Organic Program [NPO]; CFR, 2008) were implemented into policy in 1990 and to date are straightforward and precise in terms of the requirements to qualify foods for organic status. Growers and processors must meet specific criteria and be inspected to maintain organic status. The NPO also includes a list of ingredients (National List of Allowed and Prohibited Substances) that are allowed to be used in certified organic products. Organic regulations permit food products to contain varying percentages of certified organic ingredients. Products meeting the criteria for one of two categories, "100% organic" and "95% organic," qualify to use the USDA organic seal on their packages. Products made with at least 70% organic ingredients fall under a third category, and may be labeled "made with organic ingredients" but may not use the USDA organic seal.

The use of spices, flavors, and blended seasonings will continue to play a major role in the evolution of meat processing. Ethnic flavors are booming. The consumer's desire for clean labels and that "homemade flavor" will continue to challenge the meat processor to provide quality and convenience in processed meats, yet within the Parameters to meet plant production capabilities.

Traditional processed meats will prevail, but new trends are once again shifting to convenient, leaner, sodium-reduced, nutritious, full flavor and eye-appealing meat products for the future. Meat is already an excellent source of B vitamins, zinc, and iron. However, future trends may include nutrient fortification (Chap. 4), a common modification in other segments of the food industry, but not presently allowed in meat products in the United States.

Research and development in processed meat products must embrace the demands of an ever-increasing, ethnically diverse, society. This challenge should be regarded as exciting from the standpoint of limitless flavor combinations and highly technical processing methods that will result in new age products with a spin on traditional and old world processing methods.

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Chapter 10
Smoke Flavor

Jeffrey J. Rozum

Introduction

Fire! Since man first learned to tame it, fire has been used for food preparation and preservation. Fire provided a cooked product with a different aroma, flavor, and color than what early man was used to. It also prevented the food from spoiling as fast to allow for food without having to hunt everyday. It will never be known for sure if learning to cook food was a well thought-out process or an accidental discovery, but it was one of the biggest advancements of the time.

Today fire and the smoke produced from it are still very important factors in food taste and preservation. Man no longer needs to cook over an open fire everyday for his dinner, but the flavor and attributes from this cooking method live on in our daily lives. Almost every culture has at least one important cooking method that involves smoking their foods, from hibachi in Japan, to street grilling in Thailand, to the use of chulas in India, to kebobs in the Middle East, to churrasco in Brazil, to barbequing in the USA. There are two main ways food manufacturers can add a smoke flavor to meats, the old way of cooking with a live fire and by using smoke condensates.

During the burning of wood, numerous compounds are formed and their interaction with foodstuffs can cause infinite reactions and flavors. Not all of these reactions and flavors are good, but many are. The controlled burning, or pyrolysis, of wood, can affect the compounds formed and thus the flavor and quality of the outcome. Over the course of the past 40 years, a great deal of research has been done to identify what happens when wood is burned and what products are formed during this reaction. This chapter will try to summarize some of this research and give the reader a better understanding of the complexity of smoking foods.

Pyrolysis of Wood Components

Wood contains three major components that are broken down in the burning process to form smoke. This burning process is called pyrolysis, which is simply defined as the chemical decomposition by heat. The major wood components are

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cellulose, hemicellulose, and lignin. From these the major smoke components are formed; acids and aldehydes from cellulose and hemicellulose, and phenols and tars from lignin. The ratio of these smoke products depends on several factors, including the burning temperature, the type of wood, the consistency of the wood, the moisture content of the wood, and the amount of oxygen present during the burning (Maga, 1988). Each of these three major wood components has its own function in food preservation and flavor development.

Pyrolysis of Cellulose

Cellulose comprises most of the mass of most wood species. It is a linear polymer glucan composed of 9,000–15,000 glucose units bound by β-(1,4) linkages. From this component a large number of pyrolysis products are formed, but the most important are aliphatic acids and aldehydes. It is the acids which provide smoke with its tartness and some of its antimicrobial activity. They are also very important in the development of skin formation in sausages as well as in the acceleration of the nitrite curing reaction in cured meats. The aldehydes are responsible for the surface color development of processed meats and other foods. Figure 10.1 demonstrates a series of pathways proposed by Byrne, Gardener, and Holmes (1966) for the breakdown of cellulose to lower molecular weight carbonyl compounds.

Pyrolysis of Hemicellulose

Twenty to thirty-five percent of wood mass is hemicellulose. Hemicellulose is a heterogeneous group of polysaccharides containing 15–200 pentose and hexose sugars joined by β-(1,4) linkages. The greatest number of these units are xyloses, thus they are also know as xylans. These structures are the first components of wood to undergo thermal decomposition during pyrolysis. From this decomposition, the ring structures break down, forming the aldehydes which give smoke its browning capability. Figure 10.2 shows a mechanism for formation of carbonyl compounds. Other products formed are carboxylic acids and furans, which provide some of smoke's overall flavor.

Pyrolisis of Lignin

Mature hardwood trees, those most likely to be used for smoking, contain 20–25% lignin, a three-dimensional polymer composed of phenyl propane units linked together through aliphatic three-carbon side chains. The thermal destruction of lignin produces mainly phenolic compounds, with the incomplete breakdown form-

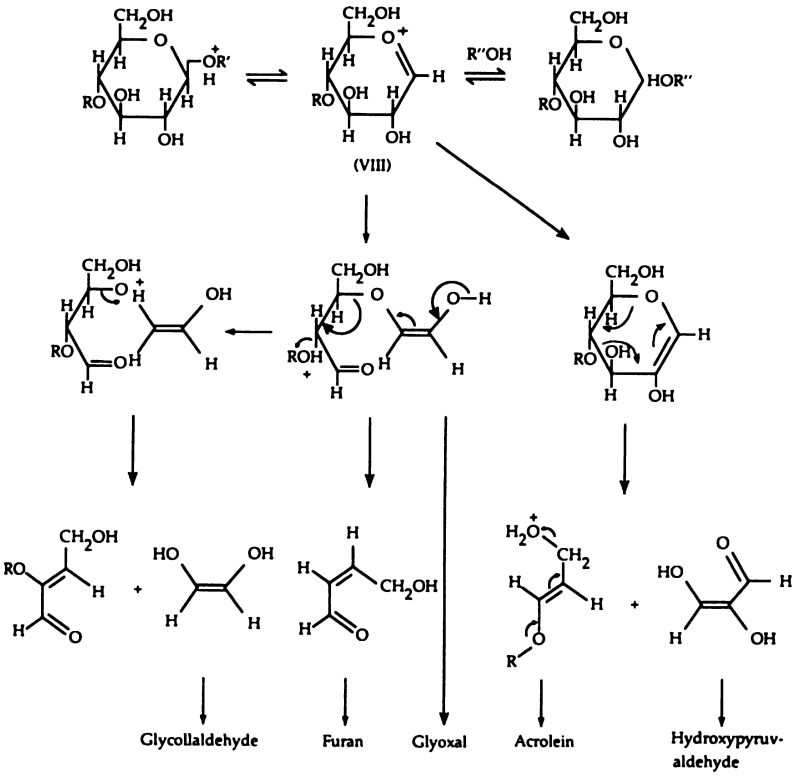

Fig. 10.1 Mechanisms for the formation of carbonyls (From Byrne et al., 1966 Copyright Society of Chemical Industry. Permission granted by John Wiley & Sons Ltd on behalf of the SCI).

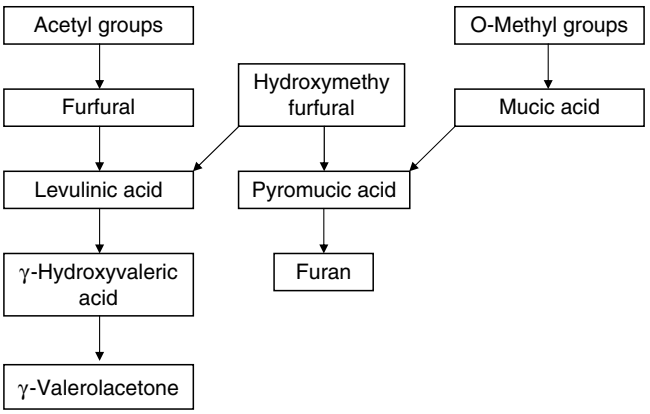

Fig. 10.2 Thermal degradation products from hemicellulose.

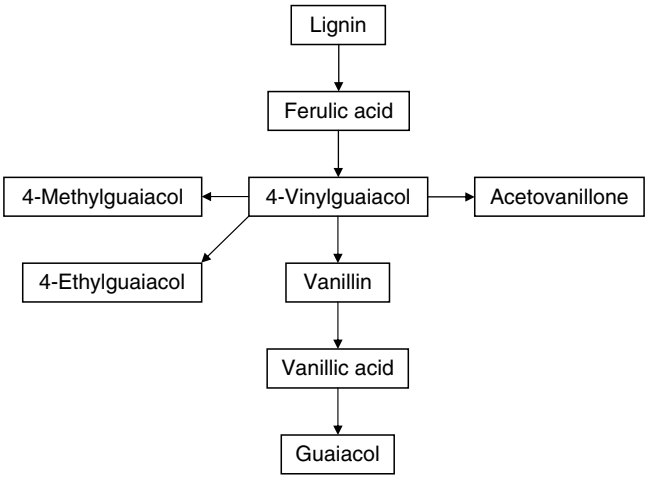

Fig. 10.3 Thermal degradation products of lignin.

ing the tars in smoke. Figure 10.3 shows the thermal degradation pathway from lignin pyrolysis (Gilbert and Knowles, 1975). The main phenol found in smoke formed in this process is 2,6-dimethoxyphenol, or syringol. Syringol and the other phenolics account for most of the flavor of smoked foods. The other major phenolic components of smoke condensates are eugenol, isoeugenol, guaiacol, phenol, and cresols (Table 10.1). Flavor produced by smoking foods is a combination of unreacted smoke components, which are mainly phenols, and reacted smoke-protein components.

Burning various hardwood types can develop different flavor profiles. Softer hardwoods burn faster and at a lower temperature. This leads to more of the smaller phenolics which give a lighter smokiness. Extremely hard hardwoods, such as mesquite, burn much slower and at a much higher temperature. This causes the smoke to contain larger phenolic compounds, which are much more robust. By knowing which phenolic flavors are produced from various wood sources, it is possible to vary the flavor provided by the smoke. Phenolics are also responsible for most of smoke's antimicrobial and antifungal activity.

Table 10.1 Phenol descriptors

Compound	Odor description	Flavor description
Phenol	Sweet, oily	Pungent
Guaiacol	Phenolic, oily, smoky, sweet	Phenolic, sharp, smoked sausage, sweet
Syringol	Smoky, spicy, aromatic, smoked sausage, phenolic, sweet	Phenolic, smoky, freshly charred wood, dry, sharp
o-Cresol	Phenolic, sweet-fruity, aromatic, smoked sausage	Sweet, sharp, unpleasant smoky, burning
Isoeugenol	Sweet-fruity, vanilla, phenolic	Sweet-fruity, mild smoke, dry, sharp
Eugenol	Smoky, nutmeg, clove	Sweet smoke, clove

Browning Reactions of Foods

There are two main types of reactions involved in the browning of foods, one caused by enzymatic reactions and the other by nonenzymatic reactions. Enzymatic reactions cause apples to brown when they are cut. The introduction of oxygen to the compounds in the fruit causes a brown color to form as the fruit flesh breaks down. This is a polyphenoloxidase reaction.

Nonenzymatic browning reactions include caramelization, ascorbic acid reactions, and the carbonyl-amino acid reactions, otherwise know as the Maillard reaction. Caramelization occurs from the thermally induced degradation of sugars. Ascorbic acid reactions are formed most notably in high-ascorbate foods such as oranges. This reaction causes browning and some off flavor formation, such as butterscotch in an orange.

The reaction that is of major significance in smoked foods is the Maillard reaction. This is the reaction of a carbonyl compound (from smoke) and an amino acid (from a food source) which forms brown pigments. These pigments range from tan to black, have a high molecular weight, and are odorless, poorly defined, and dispersible, but not soluble, in water. The color that is formed is related to the temperature, humidity, protein content and source, and time. The reaction will proceed at room temperature, but very slowly. As the temperature increases, the reaction rate increases, thus requiring less time for brown pigments to form. Water is necessary to start the reaction, as water needs to enable the reactants to come together. However, as the reaction progresses, water must be removed for the darker colors to be produced. A humid environment causes more "muddy" or tan colors to form instead of rich browns and dark reds. Table 10.2 describes some pathways of the Maillard reaction, as all of the reactions are not known for certain.

Table 10.2 Stages of the Maillard reaction

Stage	Reactions	Properties
Initial	Condensation, enolization, Amadori rearrangement. With proteins, glucose and free amino groups combine in 1:1 ratio	Reducing power in alkaline solution increases. Storage of colorless 1:1 glucose–protein product produces browning and insolubility
Intermediate (buff yellow; strong absorption in near-ultraviolet range)	Sugar dehydration to 3-deoxyglucosone and its -3, 4-ene, HMF, and 2-(hydroxyacetyl) furan; sugar fragmentation; formation of alpha-dicarbonyl compounds, reductones, pigments	Addition of sulfite decolorizes; reducing power in acidic solution develops; pH decreases; sugars disappear faster than amino acids. With proteins, acid hydrolysis fails to regenerate the sugar(D-glucose). Positive Elson–Morgan test for amino sugars (Amadori compounds)
Final (red-brown and dark-brown color)	Aldol condensations; polymerization; Strecker degradation of alpha amino acids to aldehydes and N-heterocyclics at elevated temperatures. Carbon dioxide evolves	Acidity; caramel-like and roasted aromas develop; colloidal and insoluble melanoidins form; fluorescence; reductone reducing power in acid solution; addition of sulfite does not decolorize

As mentioned earlier, the pyrolysis of cellulose and hemicellulose produces carbonyls. The most prevalent carbonyls formed are glycoaldehyde (hydroxyacetaldehyde), pyruvaldehyde, glyoxal, diacetyl, furfural, acetol, and formaldehyde. Of these, the first two provide the majority of the coloring capability, the next three are minor coloring agents, and the last two have no reaction color at all. The principal carbonyl, glycoaldehyde, is used as an indicator, being the most prevalent and the most important to the browning of smoked meats.

Smoke Creation

There are four major methods utilized in smoking meats to produce a smoke flavor. All four types provide all the necessary components needed to flavor and color meats and other foods. Three are natural vaporous methods, where the smoke is generated in or near the smokehouse. All of the components generated are deposited on the surface of the meat and on the interior of the smokehouse. One of the oldest is the open pit fire. Some smokehouses still use a process where a fire is built in the basement of the smokehouse and the smoke is allowed to come up through the floor and smoke the foods. This requires little equipment, but there is not much capability to control the temperature or the amount of smoke that is actually applied to the food. Another type is the smoldering smoke generator. This is where a smoke generator on the outside of the smokehouse produces smoke by sawdust falling onto a hot plate and smoldering. The amount of airflow over the sawdust is controlled so a fire does not occur, so it just smolders. The resultant smoke is drawn into the smokehouse and circulated for preset lengths of time. A third method is friction generation. In this method, a whole piece of wood is loaded into a generator and forced down onto a rotating plate. This high-speed rotating plate causes friction on the end of the log, thus generating smoke. Smoke is generated at low temperatures without any glowing process. There is no flame generated in this process and the resulting smoke is drawn into the smokehouse and circulated.

The fourth type is smoke condensates. This is where smoke is made under controlled conditions in a smoke condensate factory, stored in a liquid or dry form, and then shipped to the meat factory for use. There are two main smoke condensate production methods. The first is the calciner method, an indirect heat method where a rotating drum is heated. Sawdust is introduced into one end of the drum and is heated by the walls of the drum as it rotates. When it is hot enough, the sawdust begins to pyrolize, releasing its smoke components. These rise back up the drum and into a condensing column. In the condensing column, water, or previously formed smoke, is continuously circulated from the top of the condensing column to the bottom. As it falls, it comes in contact with the rising smoke, causing a countercurrent extraction to occur. In this process, all condensable smoke components are captured in the water phase as a smoke condensate. The ash or charcoal that is formed is removed via the opposite end of the rotating drum and the noncondensable compounds are removed. This process is highly regulated to control the atmos-

phere and to ensure that the product coming out of the condensation column is uniform. In this method, as with the others, there is no direct flame, just a smoldering process. Once the smoke condensate is collected, it is allowed to settle so that the tars fall out, as they are not very water soluble. These can then be collected and used as a fuel source. This process, in some form, is the one utilized to manufacture most of the smoke condensates in the market.

The second smoke condensate production method is Rapid Thermal Processing, or RTP. This method uses a much faster heating process, as described in a patent by Underwood and Graham (1989). High velocity air is heated in a controlled loop. When a certain temperature is obtained, heat transfer media (htm) is added to the loop and circulated with the air. Once the htm gets to the critical temperature, sawdust is introduced into the flow of the circulating superheated htm. On contact with the htm, the sawdust particle is instantaneously pyrolized into a smoke cloud. This smoke is then sent into a condensing column similar to the one described earlier. Decreasing the amount of time the sawdust spends in the reactor greatly affects the component outcome of the smoke. Due to lower residence times, lignin does not have enough time to break down into smaller components; therefore, there are fewer total phenolics in the smoke composition. On the other hand, the short residence time means that the carbonyls formed from the cellulose fraction do not break down in the reactor. Thus carbonyl levels are much higher in smoke condensates from this process.

A final option for smoke application is a combination process where traditional natural vaporous smoke and smoke condensates are both used. This method is done by a short shower of smoke condensate followed by a short traditional natural vaporous smoke application or an atomization step followed by a short traditional step. Production by this method has several benefits for those who wish to continue using traditional smoking methods. Smoking time can be greatly reduced, leading to shorter cook cycles and faster production. Less smoking time means less tar formation in the house, which helps reduce cleanup time and lower emissions.

Cold Smoking

Most people are aware of using a smoking process during a cooking schedule that involves high temperatures to thoroughly cook the food. However, there are also several items that are smoked at temperatures just above room temperature. These are called cold smoked. Cold smoked foods are generally processed at temperatures between 15°C and 25°C, whereas hot smoked foods are processed between 55°C and 80°C. Typical cold smoked products include hams, salami, some bacons, fish, and cheese. Cold smoking schedules are generally quite long, lasting for days, in comparison to hot smoking, which is accomplished in minutes to hours. Since cold smoked foods are not really "cooked," they generally are fermented, salt cured, or dry cured to preserve them. Cheese is cold smoked to prevent the fats from melting out of the product. In general, the same browning reactions take place, except they take a little longer to occur.

Natural Vaporous Smoke Versus Natural Smoke Condensates

Both these methods of smoke generation produce similar products. In natural vaporous smokehouses, the smoke is generated by burning the wood. With smoke condensates it is generated in a smoke factory under controlled conditions. In the process of smoke generation, there are several components that must be taken care of from an environmental standpoint. The light organics that are produced must be, or should be, collected and burned. Charcoal must be disposed of. Tar must be removed from the smokehouse or disposed of. When using natural vaporous smoke, each factory must have its own methods for removal of these substances, which could include an air handling system with an afterburner, landfill costs for the char, and cleaner costs for cleaning tar off smokehouses. In a smoke condensate factory, all the noncondensable vapors are collected and sent to an afterburner, which can be fueled by burning the tar collected from smoke generation. The char produced can be sold for use in charcoal briquette manufacture or burned as a source of fuel.

Another very important difference between natural vaporous and natural smoke condensates is that the latter contain lower levels of polycyclic aromatic hydrocarbons, or PAHs. These are the carcinogenic compounds found in smoke. The largest portion of the PAHs in smoke is in the tar. During traditional smoking, the tar is added to the food product along with the other smoke components. There is nothing to prevent it, but the amount is influenced by processing differences. This can cause dangerous levels of carcinogens to be present on the food product. The EU has a limit of 5 ppb benzo(a)pyrene (BaP), the most common PAH in smoke, on traditionally smoked items. In contrast, the limit is 0.03 ppb BaP on a finished smoked item when smoke condensates are used. This is due to the fact that the content of PAHs in natural smoke condensates can be controlled to provide a healthier product. In the production process, the smoke condensates are allowed to settle for a few days prior to further processing. This allows a large percentage of the tar, and hence the PAHs, to fall out of the product. Further removal can be done with various methods, including cation exchange, as described by Underwood and Rozum (1995). This ensures that smoke condensates themselves will contain less than 10 ppb BaP.

Environmental Impact of Meat Smoking

The environmental impact of smoking with traditional vaporous smoke versus natural smoke condensates is detailed in a study by Fengel and Wegener (1984), which looked at the effects of both types of smoking in the French market. In this study, the French market, with a population of 60 million people, showed a consumption of 26,160 tons of smoked fish and 127,530 tons of smoked meat, for a total of 153,690 tons of smoked muscle foods. In a traditional smoke setting, 20 kg of sawdust is needed for each ton of smoked food, thus requiring 3,073,800 kg of sawdust

for all of the smoked foods. When this amount of sawdust is burned in traditional smoke generators, 491,808 kg of tar and 983,616 kg of charcoal are produced. This is a total of 1,475,424 kg of emissions added to the environment. Figures 10.4 and 10.5 summarize the results of the study. This process would also require 87,745 kg of chemical detergents to clean the smokehouses. If natural smoke condensates are used, we can assume that the same amount of sawdust would be used, but it would all be processed in limited facilities. During this production, a very large portion of the charcoal and tar are recycled in the smoke condensate factory. That leaves only 7,685 kg of ash left as landfill material. Also, since there are no tars left in the

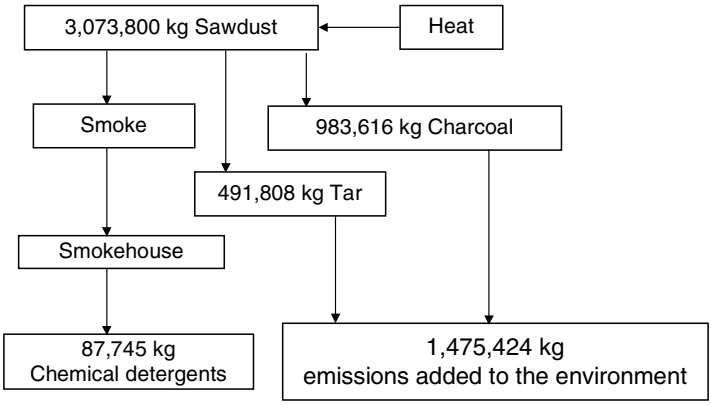

Fig. 10.4 French environmental impact of smoking foods with traditional vaporous smoke (data from Fengel & Wegener, 1984).

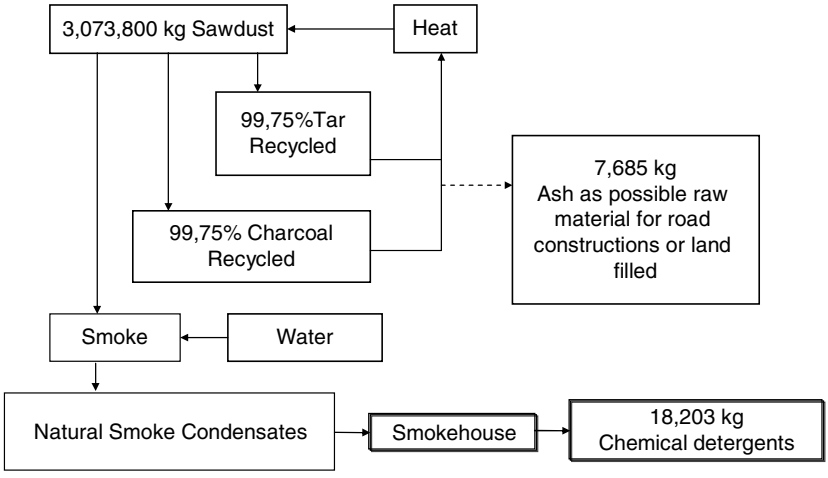

Fig. 10.5 French environmental impact of smoking foods with smoke condensate (data from Fengel & Wegener, 1984).

smoke, the smokehouses are cleaner, requiring only 18,203 kg of detergents, an 80% reduction. There is also a reduction of particulate matter of 85–87% and a 64–81% reduction in volatile organic compounds (VOC), according to a recent private study (Taylor & Lamers, 2003)

Evolution of Smoke Flavor

Traditional vaporous smoke has not changed much in many years. There have been some modifications to the equipment and how it operates, but for the most part it is still the same process developing the same flavor. Smoke flavors can be modified by changing the moisture content of the sawdust (which affects burning rate) and the temperature at which the smoke is generated. Air leaks in the generator can affect several things, among them the browning capability of the smoke, since the introduction of oxygen into the burning area causes the carbonyl content to drop rapidly. Varying the air mixture in the generator will also affect the flavor that is produced.

Natural smoke condensates have progressed greatly in the past 40 years since their inception. In the beginning, there was only one smoke condensate available. Today there are several companies that produce hundreds of finished smoke products. This increase in variety can be attributed to two factors: improvements in smoke generator technology and research into how to use the smoke produced. Initial smoke condensates were made with a simple reactor where sawdust was fed into a heated chamber and allowed to smolder. The new wave of technology was the calciner process. This process allowed producers to manufacture larger quantities of smoke condensates more efficiently than with the original methods. Conditions were more controlled, allowing for various operating parameters to be used in order to obtain different flavor profiles. The newest technology is the RTP process. This broadened the scope even more, allowing for more precise pyrolysis and, hence, for greater product variety. With the capability of having two processes, the smokes can be blended for further flavor modifications.

From the basic condensates formed on the reactors, several more processes can be applied to the condensates to change their flavor and function. Some of these processes include neutralization, dilution, concentration, resin treatment, carbon treatment, extractions, addition of emulsifiers, reaction with other ingredients, spray drying, and distillation, to name a few. Basic smoke condensates are produced to target about 25–30% dissolved solids in the form of acids, carbonyls, phenols, and other smaller groups of organic compounds. The fine balance of the water and nonwater soluble components in smoke condensates makes them a very interesting solution. They are water based, but if more water is added, the organic stability of the solution is disrupted and some components will fall out of the solution. One way to prevent this is by using various emulsifiers, such as polysorbate 80, to allow for clear solutions upon dilution.

These basic condensates will contain anywhere from 5% to 12% acid, as acetic, depending on the production method. Higher levels of acid allow for more organics

to be dissolved in the solution without any precipitate formation. This gives the base smoke condensates a pH of 2–3. However, when smokes are added to sodium nitrite-containing solutions or meat, neutralized smokes are needed in order to prevent nitrous oxide formation. To allow for their safe use in these applications, smokes are made with a pH of 5–5.5, higher than the pKa of acetic acid (4.74). Another interesting aspect of neutralization is the deprotonation of pyrazines. Pyrazines are six-member ring structures that contain two nitrogen molecules. These compounds are very potent aromatically and can dramatically change the flavor of a smoke, especially when the hydrogen molecule is removed. Pyrazines are found in smokes at ppb levels, but they do have a very big impact in flavor.

Resin or carbon treatments can be used to remove certain components from smoke. Generally, the most commonly removed components are phenols, but carbon is not very specific and can remove other components as well. Underwood (1990) describes a method of using two resin treatments to remove phenols and pyrazines from a smoke solution to provide a low-flavor, dilutable product.

There are several ways extractions are used in smoke condensates. The primary method extracts smoke condensates with vegetable oil. In this process oil-soluble phenolics are transferred from the smoke condensate to the vegetable oil, forming a smoked oil which can provide flavor without browning capability or acid content. Other work has been done to extract smokes with solvents to remove various compounds from them, but this is not widely used.

One of the first commercial smoke condensate products available was CharZyme, a product produced by Red Arrow Products Co, LLC. This product is made by reacting barley malt flour with a smoke condensate and then heating to drive a reaction forming a dry product that is used widely in the seasoning and meat industry. The protein in the barley malt flour reacts with the carbonyls in the smoke to develop meaty and savory flavors. Other carriers that can be used for developing flavors are corn flour, yeast, and potato flour. Smoke flavors can also be spray-dried onto various carriers to yield a dry product that can give a flavor similar to the liquid version.

Distillation can be used to form various products by removing or concentrating certain smoke fractions. These solutions can then be used to provide a smoke condensate with a variety of different properties.

Food Preservation with Smoke

Smoke imparts not only flavor, color, and other sensory effects to foods, but it also provides preservation effects. Smoke can be a very effective antimicrobial and antifungal agent. Several studies have been done showing varying degrees of microbial inhibition, some showing better effectiveness against some organisms than others. Wendorff (1981) measured the antibacterial and antifungal activities of a few smoke condensates against some common food bacteria and fungi (Tables 10.3–10.5). It is believed that the phenolic content of smoke is the greatest contributor to its antimicrobial and antifungal effects. However, Rozum (1995) found that when a

Table 10.3 Antibacterial properties of smoke condensates in culture media

	% Inhibition			
Smoke flavoring	*E. coli*	*S. aureus*	*P. aeruginosa*	*L. viridescens*
CharSol C6[a] (0.25% v/v; pH 2.4)	33	72	52	99
CharSol C6[a] (0.25% v/v; pH 5.0)	11	31	51	97
CharSol C6[a] (0.25% v/v; pH 7.0)	3	25	54	15
Acetic Acid, 6.5% (0.25% v/v)	25	52	29	99
CharDex[b] (0.1% w/v)	0	77	46	21
AroSmoke P50[c] (0.25% w/v)	20	60	62	10
CharOil[d] (0.25% v/v)	0	55	52	85

From Wendorff (1981).
[a]Liquid smoke condensate, 6.5% acid, Red Arrow Products Co., LLC, Manitowoc WI.
[b]Dry smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.
[c]Emulsifier-based smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.
[d]Oil-based smoked condensate, Red Arrow Products Co., LLC, Manitowoc WI.

Table 10.4 Antifungal properties of smoke condensates in culture media

	Zone of inhibition (mm^2)		
Smoke flavoring	*Penicillium* spp.	*Aspergillus niger*	*Aspergillus flavus*
CharSol C6[a] (0.25% v/v; pH 2.4)	21	18	14
CharSol C6[a] (0.25% v/v; pH 5.0)	19	16	13
CharSol C6[a] (0.25% v/v; pH 7.0)	17	12	11
Acetic Acid, 6.5% (0.25% v/v)	16	12	13
CharDex[b] (0.1% w/v)	12	9	8
AroSmoke P50[c] (0.25% w/v)	9	9	8

From Wendorff (1981).
[a]Liquid smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.
[b]Dry smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.
[c]Emulsifier-based smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.

Table 10.5 Antioxidant properties of smokes versus commercial antioxidants

	Peroxide value (meq/kg fat)				
Antioxidant	Initial	Week 1	Week 2	Week 4	Week 26
Untreated control	0.8	3.5	8.5	18.4	43.1
CharOil[a] (0.4%)	0.8	1.2	2.0	2.4	3.7
CharOil[a] (0.2%)	0.8	1.3	2.1	3.0	4.6
AroSmoke[b] (0.04%)	0.8	1.2	2.0	2.2	3.4
AroSmoke[b] (0.02%)	0.8	1.4	2.1	2.9	4.9
BHA (0.02%)	0.8	1.4	2.1	3.3	12.5
BHT (0.02%)	0.8	1.3	1.9	2.8	4.8
Propyl gallate (0.02%)	0.8	1.4	2.1	3.0	5.8

From Wendorff (1981).
[a]Oil-based smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.
[b]Emulsifier-based smoke condensate, Red Arrow Products Co., LLC, Manitowoc WI.

mostly phenolic smoke fraction was used in cooked chicken, bacterial growth was not controlled. It is believed that, when faced with multiple organisms, smoke alone is not an effective antimicrobial agent, but can be an effective hurdle when used in combination with other agents.

The phenolics in smoke condensates can also act as strong antioxidants, as demonstrated by Wendorff (1981) in pork fat. Many private research studies and casual observations have shown that smoke-processed meats do not develop rancidity as cured, unsmoked meats.

Application of Smoke Condensates

Smoke condensates can be applied to foods in many ways, including atomization, drenching/showering, internal addition, or topical application. Atomization is a process where compressed air and a smoke condensate meet at a nozzle, forcing the liquid out as a cloud (Fig. 10.6). When calibrated correctly, the cloud should be in small enough particles that it will stay suspended and not coalesce and form droplets that will fall out of the air. This cloud then duplicates the cloud of smoke formed in the smokehouse during traditional smoking. Generally the smoke cloud is allowed to fill the entire house. Once full of smoke, the air is circulated inside the house for a time to move the cloud around the foods in the smokehouse. In this method, the smoking cycle is very similar to a traditional smoke cycle, except that

Fig. 10.6 Atomization cloud in a smokehouse (courtesy of Red Arrow Products Company LLC, Manitowoc, Wisconsin, USA).

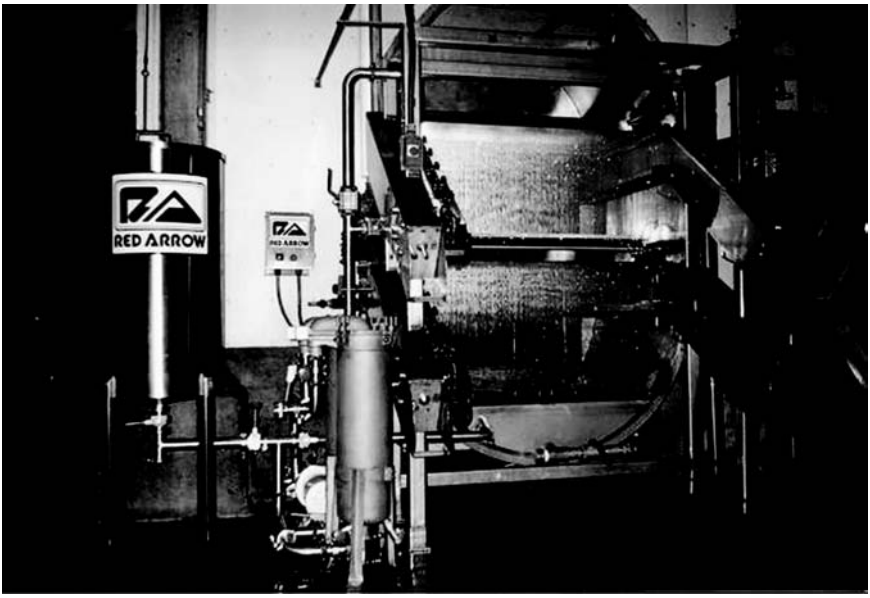

Fig. 10.7 Smoke condensate drench on a continuous frankfurter line (courtesy of Red Arrow Products Company LLC, Manitowoc, Wisconsin, USA).

the smoke is generated by atomization instead of a smoke generator. This method does not necessarily save time, but it does provide advantages over traditional smoking, such as uniformity of product color and flavor, lower cleaning costs, and lower emissions.

The most efficient method for applying smoke condensates is by drenching or showering. In this method, product is showered with a 5–50% smoke condensate solution for 15–90 s. In most cases showering is done before the product is put into the smokehouse. The product may be placed into a separate showering cabinet where the smoke solution is showered over the product and re-circulated, or it can be done in a continuous oven as one of the zones of the oven (Fig. 10.7). The smoke concentration can be maintained by monitoring the titratable acidity of the smoke and adding back smoke and/or water to maintain the desired level of acidity. Acid level can be used, as all smoke components are removed at equal rates by the food products as they are showered. Drenching allows for a shortened cook schedule, as there is no need to have the product go through a smoking cycle. A typical cook schedule versus a traditional schedule is shown in Tables 10.6 and Tables 10.7. Other advantages of showering include very uniform flavor and color, minimal cleanup (as the smokehouse is only used to dry and cook), and very low emissions. and 10.7

Smoke can also be added to food products internally. This can be done to increase the flavor of larger diameter products or to products that may not normally be smoked. The most common item to which smoke is added internally is bacon.

Table 10.6 Traditional smoke schedule for frankfurters in natural casing

Step	Time	Dry bulb	Wet bulb	Relative humidity	Dampers	Smoke
1	5 min	45°C	38°C	68%	Open	Off
2	90 min	50°C	–	0%	Open	Off
3	30 min	55°C	–	0%	Closed	On
4	15 min	65°C	52°C	48%	Closed	On
5	15 min	75°C	60°C	50%	Open	Off
6	To 72°C internal	80°C	74°C	78%	Closed	Off

Table 10.7 Traditional cook schedule for showered frankfurters in natural casing

Step	Time	Dry bulb	Relative humidity (actual)	Dampers
1	20 min	55°C	20–30%	Open
2	20 min	60°C	20–30%	Open
3	To 72°C internal (ca. 20 min)	78°C	100%	Closed

Pork bellies can be injected with brine that can include smoke condensates to provide flavor without external smoking. Another smoke can be added upon cooking to provide color. Other products where internal addition is common are hams, sausages, fermented meats, cook-in-bag items, cheese, and poultry. This can be a very cost-effective way to add a smoke flavor without the need for additional equipment.

External addition of smoke flavors is generally done within a seasoning blend. Dry or oil-based smoke condensates can be added to seasoning blends to develop a desired flavor, often to mimic various cooking styles. These products can be sold fresh, fresh-frozen, or fully cooked. Smoke flavors can also be added to a marinade that may or may not be tumbled into a meat product. External applications can provide flavor, color, and antimicrobial and antioxidant properties to products.

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Chapter 11
Fermentation and Acidification Ingredients

Frédéric Leroy and Luc De Vuyst

Introduction

The distinct sensory qualities and remarkable shelf-life characteristics of fermented sausages, as compared to cooked sausages, are largely due to acidification of the meat batter. Traditionally, acidification of the raw meat is the result of a microbial fermentation process, i.e. lactic acid production by lactic acid bacteria (LAB). Acidification is generally combined with protection from oxygen (stuffing into casings), extensive salting, and curing (Chap. 1), and with an ageing stage for product maturation. The latter stage can be absent, short, or long, depending on the type of product, and leads to drying, resulting in a lower water activity, as well as to a complex and desired flavor formation (Campbell-Platt & Cook, 1995; Lücke, 1998). Sometimes, smoking or heating is applied as a last step in the manufacturing process. Heating is common in the United States, where regulations require a core temperature of 58.3°C before selling the end-product (Lücke, 1998). The almost anaerobic environment and the low pH and water activity values that prevail in the sausage are to be considered as the main hurdles that inhibit undesirable microbial growth and lead to a relatively stable end-product.

In the case of spontaneously fermented sausage or sausage prepared through back-slopping, LAB that cause acidification of the meat and, hence, start the fermentation process, originate from the raw material or production environment. However, LAB can also be added deliberately by the sausage manufacturer as a starter culture to the meat batter (Campbell-Platt & Cook, 1995; Hugas & Monfort, 1997). In contrast to spontaneous fermentation, where the manufacturer relies on the presence of a "house microbiota" (counts of 10^2–10^3 LAB per gram of fresh batter), the addition of a starter culture leads to high initial LAB counts (10^6–10^7 per gram of fresh batter). This enhances acidification, leads to a more standardized and predictable production process, shortens the development of firmness and the overall ripening time, and improves food safety (Lücke, 1998).

As an alternative to the use of LAB starter cultures, some manufacturers prefer to apply chemical acidulants, mainly to shorten the production process. Best results have been obtained with glucono-delta-lactone (GdL) (Barbut, 2006). However, a

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disadvantage of chemical acidulants is that they generally induce rapid and poorly controlled acidification, leading to inhibition of flavor development.

The present chapter focuses on fermentation and acidification ingredients that are applied in sausage technology. In particular, it will discuss starter cultures for sausage fermentation, including both classical and novel, functional starter cultures, as well as chemical acidulants.

Starter Cultures

Classical Starter Cultures

General Aspects

Starter cultures for sausage fermentation are composed of nontoxic, nonpathogenic, and phenotypically and genetically stable microbial strains that possess activities that contribute to the fermentation of the meat, leading to proper acidification, flavor, texture, and color development, and microbial stability of the end-product. Generally, they contain selected LAB as acidifiers, which are often combined with Gram-positive, catalase-positive cocci (GCC) for reasons of color stability and flavor development. The raw meat batter is inoculated with the starter culture, which is frequently produced and commercialized as a food ingredient by specialized starter culture-producing companies. Alternatively, the sausage manufacturer maintains and produces in-house starter cultures. Commercial starter cultures contain LAB and/or GCC and are distributed frozen or freeze-dried on suitable carriers (Lücke, 1998). DVI-type commercial starter cultures enable direct vat inoculation of the meat batter, without preceding growth of the culture(s). In certain types of mold-ripened, nonsmoked sausages, the application of selected molds as surface starter cultures is considered to be advantageous. Also, yeasts may be applied as internal or external starter cultures.

Besides the microbial biomass and the carrier material (e.g., milk powder), starter cultures usually contain other ingredients, such as cryoprotectants (e.g., sodium glutamate, sucrose, and lactose) and manganese (added as growth factor for the lactobacilli involved). The glucose naturally present in the meat is usually too low to allow sufficient acidification, so that fermentable carbohydrates, usually dextrose, are added to the meat batter to obtain an appropriate end-pH and acceptable textural properties (González-Fernández, Santos, Jaime, & Rovira, 2006). Nevertheless, some genuine salamis are still made without carbohydrate addition (Lücke, 1998). Manganese, the active component of spices such as black pepper, is sometimes added to starter culture preparations because of its stimulatory effect on the LAB and the acidification rate (Coventry & Hickey, 1993; Leroy & De Vuyst, 2005; Zaika & Kissinger, 1984).

Lactic Acid Bacteria

LAB are crucial for sausage fermentation because of their acidification capacity based on the conversion of fermentable carbohydrates into lactic acid. Generally, homofermentative lactobacilli, such as strains of *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, and *Lactobacillus pentosus*, and/or pediococci, such as *Pediococcus acidilactici* and *Pediococcus pentosaceus*, are applied (Campbell-Platt & Cook, 1995). In traditional, spontaneously fermented European sausage, *Lb. sakei* and, to a minor extent, *Lb. curvatus*, usually dominate the sausage microbiota (Andrighetto, Zampese, & Lombardi, 2001; Baruzzi, Matarante, Caputo, & Morea, 2006; Comi et al., 2005; Coppola, Giagnacovo, Iorizzo, & Grazia, 1998; Fontan, Lorenzo, Parada, Franco, & Carballo, 2007; Parente, Grieco, & Crudele, 2001; Rantsiou et al., 2005; Rebecchi, Crivori, Sarra, & Cocconcelli, 1998; Santos, González-Fernández, Jaime, & Rovira, 1998). Because of their natural competitiveness in fermented sausage, they are excellent starter cultures for slow-type fermentations at low temperature (20–25°C). At these temperatures, acid formation by pediococci is too slow and the performance of cultures containing lactobacilli is superior (Lücke, 1998). Also, it results in excellent flavor characteristics. In the United States, pediococci are more commonly used, due to historical and technological reasons, resulting in more quickly acidified sausages (Campbell-Platt & Cook, 1995). Although lactobacilli are dominant in fermented sausages, they were originally not very well suited as starter cultures, because they did not readily survive lyophilization. Also, the first commercial starter culture, a strain of *Pediococcus acidilactici*, was marketed in the United States, where higher temperatures are applied (30–40°C). With the advent of frozen culture concentrates and better freeze-drying methods, the use of lactobacilli became more common. Although rapid and strong acidification of the meat batter contributes to a stable fermentation, enhanced food safety, and improved cohesiveness and sliceability of the end-product, flavor development may be reduced due to inhibition of endogeneous and microbial enzyme activities.

Gram-Positive, Catalase-Positive Cocci

For reasons of color stability and flavor development, GCC are frequently used in sausage starter cultures (Campbell-Platt & Cook, 1995). They are nonpathogenic, coagulase-negative staphylococci, and/or kocuriae, such as *Staphylococcus carnosus*, *Staphylococcus xylosus*, and/or *Kocuria varians*. Their main role is to convert the curing agent nitrate into nitrite through nitrate reductase activity. Nitrate displays little technological activity as such, but nitrite actively contributes to food safety and the development of a stable color through the formation of nitric oxide myoglobin (Chap. 1). In addition, GCC produce catalase, which removes the color-affecting hydrogen peroxide that may be produced by nonstarter LAB.

Flavor development by GCC is mainly ascribed due to their metabolic activities related to amino acid conversion and fatty acid oxidation (see below). GCC are

inhibited by a rapid pH decrease, which may have negative effects on flavor development. This occurs mainly in fast-ripened sausages that are fermented at high temperatures (above 25°C) and contain high amounts of fermentable sugar (up to 2%) (Lücke, 1998). If nitrate rather than nitrite is used as a curing agent, requiring nitrate reductase activity, the addition of low amounts of sugars (0.2–0.3%) and at least 10^6 CFU of GCC per gram of sausage is recommended.

Fungi

Most producers of raw, mold-ripened, nonsmoked sausages still rely on the "house microbiota." However, molds are sometimes applied as surface starter cultures, particularly in Southern-European countries (Campbell-Platt & Cook, 1995; Lücke, 1998). They may contribute to the characteristic mature flavor and appearance (e.g., white, furry coating) of such sausages. In Italian-style fermented salami, for instance, molds grow on the surface and reverse the fall in pH due to oxidation of lactic acid, giving a final pH of 6.0–6.2 (Campbell-Platt & Cook, 1995). Moreover, inoculation with a mold or yeast surface starter culture helps prevent the growth of undesired molds that produce mycotoxins or lead to unacceptable product quality (López-Díaz, Santos, García-López, & Otero, 2001). Fungal surface starter cultures are usually applied as freeze-dried spores or liquid spore concentrates that are added to water and sprayed on the casings (Campbell-Platt & Cook, 1995). Alternatively, the starter is applied by dipping the sausages into a suspension of conidia of an appropriate starter mold before ripening (Lücke, 1998). Nontoxinogenic and technologically suitable strains of *Penicillium nalgiovense* and *Penicillium chrysogenum* are frequently used.

Less is known about the role of yeasts in meat fermentation (Cocolin, Urso, Rantsiou, Cantoni, & Comi, 2006). Both flavor and color can be improved by the selection of suitable *Debaryomyces hansenii*, *Yarrowia lipolytica*, or other yeasts as starter cultures (Campbell-Platt & Cook, 1995; Gardini et al., 2001). Yeast may be applied as surface cultures, as well as directly in the meat mix for internal use, leading to color and flavor development and an increase in pH.

Limitations of Classical Starter Cultures

Despite a successful contribution of the currently applied starter cultures to overall product standardization and food safety, several limitations can still be identified. They relate to a lack of distinctive qualities in industrially produced fermented sausages as compared to spontaneously fermented sausages, potential mismatches between the applied commercial starter culture and the sausage technology, persisting microbial hazards, and the perception of a negative health image of fermented meat products (Fig. 11.1).

Industrial fermented sausages are often of inferior gastronomic quality when compared to artisan products prepared by spontaneous fermentation or back-slop-

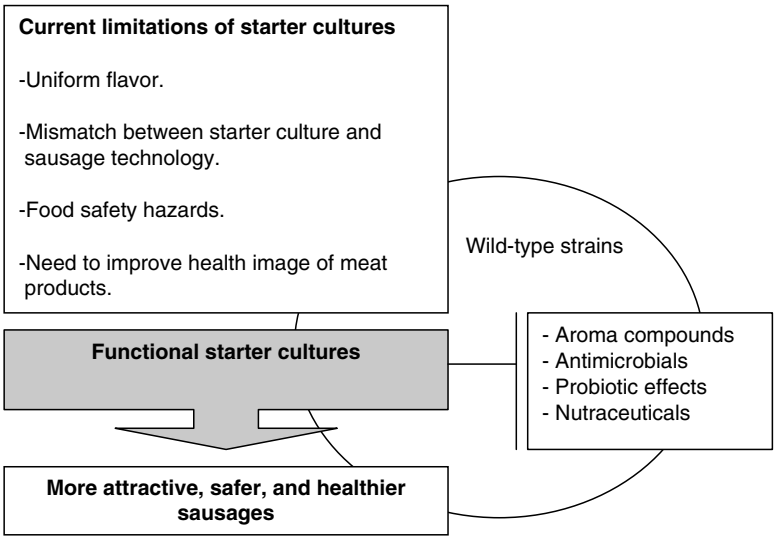

Fig. 11.1 The need for a new generation of starter cultures with functionalities leading to enhanced quality, safety, and healthiness of fermented sausages.

ping (Leroy, Verluyten, & De Vuyst, 2006). The distinctive qualities of artisan products are obviously related to the superior quality of the ingredients used and the elaborate production process, but it is likely that the specific composition of the "house microbiota" also plays a role. In artisan chorizo, for instance, variation in the flavor-affecting metabolic activity of GCC has been related to the manufacturing location (García-Varona, Santos, Jaime, & Rovira, 2000). Part of the "dullness" and uniformity of industrially produced sausages may well be due to the limited diversity of available starter cultures. Indeed, most starter cultures that are industrially applied originate from the same few major starter culture-producing companies.

In some cases, the rationale behind starter culture selection may be questioned. The starter culture applied is not necessarily in tune with the applied fermentation process, so that commercial starter cultures may not always be able to compete well with the "house microbiota" (Leroy et al., 2006). This may, for instance, be the case for European starter cultures mainly produced in Northern Europe, when applied in Southern European meat plants (Samelis, Metaxopoulos, Vlassi, & Pappa, 1998). Cultures should ideally be selected according to the specific formulation of the meat batter and the applied fermentation technology, since the interaction of environmental factors will select a limited number of competitive and dominant strains (Rebecchi et al., 1998). For instance, pediococci, *Lb. pentosus*, and *Lb. plantarum* are poorly competitive in sausage batter when compared to *Lb. sakei* and *Lb. curvatus*, and are therefore less commonly detected in large amounts in spontaneously fermented sausage. Despite the fact that they may lead to a sufficient initial acidification, they are not always able to control the fermentation process, due to

insufficient inhibition of nonstarter LAB (Coventry & Hickey, 1991). In addition, *Lb. plantarum* is a strong acidifier, which may lead to overacidity in mildly fermented Mediterranean-type sausages (Garriga et al., 1996).

With respect to microbial hazards, a well-performed sausage fermentation process will eliminate most pathogenic bacteria. Nevertheless, *Staphylococcus aureus* (Sameshima et al., 1998), *Escherichia coli* (Ammon, Petersen, & Karch, 1999; Normanno et al., 2002; Pichner, Hechelmann, Gareis, & Steinruck, 2006; Sauer, Majkowski, Green, & Eckel, 1997), *Salmonella* (Bremer et al., 2004; Ferreira et al., 2007; Siriken, Pamuk, Ozakin, Gedikoglu, & Eyigor, 2006), and *Listeria monocytogenes* (Colak, Hampikyan, Ulusoy, & Bingol, 2007; Encinas, Sanz, García-López, & Otero, 1999; Farber, Daley, Holley, & Usborne, 1993; Ferreira et al., 2007; Gianfranceschi et al., 2006; Siriken et al., 2006; Thevenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005) are still of concern, especially in short-ripened, semidry, or moist sausage, in the case of hygiene-management deficiencies, or when initial pathogen loads are high (Nightingale, Thippareddi, Phebus, Marsden, & Nutsch, 2006; Pichner et al., 2006).

Besides quality and safety limitations that should be tackled, there is also room to improve the healthiness of the end-product by selecting health-promoting starter cultures, such as probiotic starter cultures and starter cultures that are able to produce health-promoting compounds. This would meet the trend for healthier meat products (Jiménez-Colmenero, Carballo, & Cofrades, 2001).

Functional Starter Cultures

Definition and Aim

To overcome the limitations mentioned above, the use of new, functional starter cultures is being explored. Functional starter cultures offer an additional functionality compared to classical starter cultures and represent a way of improving and optimizing the fermentation process and achieving tastier, safer, and healthier food products (Leroy & De Vuyst, 2004; Leroy et al., 2006).

Functional Starter Cultures to Produce More Attractive Sausages

For a fermented sausage to be attractive to the consumer, it needs to smell, taste, and look good. Based on their metabolic activities, functional starter cultures can be used to further improve sensory quality. The selection of microbial strains should be done carefully since the metabolic capabilities differ between strains and depend on the applied technology and ingredients (Masson, Hinrichsen, Talon, & Montel, 1999; Olesen & Stahnke, 2000, 2003, 2004; Selgas, Casas, Toledo, & García, 1999; Selgas, Trigueros, Casas, Ordóñez, & García, 1995; Stahnke, 1999b; Sunesen & Stahnke, 2003).

Sausage flavor, being the result of taste (salt, lactic acid, peptides, and amino acids) and aroma (bacterial metabolites, lipid autoxidation products, and other volatile compounds), is obviously influenced by the ingredients and processing used, but also by the sausage microbiota (Ansorena, Gimeno, Astiasarán, & Bello, 2001; Beriain, Lizaso, & Chasco, 2000; Claeys, De Smet, Balcaen, Raes, & Demeyer, 2004; Ordóñez, Hierro, Bruna, & de la Hoz, 1999; Stahnke, 1999a, 1999b). The use of selected strains that produce interesting aromas as functional starter cultures could lead to more tasty sausages and to a reduction of the ripening time.

A first approach would be to select for LAB that display flavor-inducing metabolic activities, beyond the production of lactic acid (Leroy et al., 2006; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003). However, lactobacilli and pediococci generally do not possess strong proteolytic or lipolytic capacities (Kenneally, Leuschner, & Arendt, 1998; Zuber & Horvat, 2007) and display low catabolism of branched-chain amino acids necessary for the formation of typical sausage aroma compounds such as 3-methyl butanal (Larrouture, Ardaillon, Pépin, & Montel, 2000). Nevertheless, they may produce volatiles through fermentation of carbohydrates (Molly, Demeyer, Civera, & Verplaetse, 1996) and, for some LAB, the production of lipase appears to be significant under sausage ripening conditions (Lopes, Cunha, Clemente, Carrondo, & Crespo, 1999). The use of LAB other than lactobacilli and pediococci seems more interesting. Enterococci, for instance, may display several metabolic activities that are of importance in traditional fermented sausages (Comi et al., 2005; Hugas, Garriga, & Aymerich, 2003). *Lactococcus lactis* subsp. *cremoris* could be applied to increase free amino acids (Herranz et al., 2004), whereas a strain of *Carnobacterium piscicola* has been suggested because of its aroma production from leucine metabolism (Larrouture-Thiveyrat & Montel, 2003).

Since the major contribution of most meat-associated LAB to flavor seems to be limited to the production of lactic acid, selected strains of GCC, with specific peptide uptake systems and branched-chain amino acid converting and fatty acid oxidizing activities, appear to be more appropriate for the generation of specific aroma compounds (Hugas & Monfort, 1997; Leroy et al., 2006). *Staphylococcus xylosus* and *S. carnosus*, for instance, contribute to sausage aroma through the conversion of amino acids and free fatty acids (Beck, Hansen, & Lauritsen, 2004; Olesen, Meyer, & Stahnke, 2004; Stahnke, Holck, Jensen, Nilsen, & Zanardi, 2002; Tjener, Stahnke, Andersen, & Martinussen, 2004a, 2004b). Compounds such as 3-methyl butanal and 3-methyl butanoic acid, derived from leucine, have been linked with sausage aroma (Montel, Reitz, Talon, Berdagué, & Rousset-Akrim, 1996; Stahnke, 1995). Methyl ketones, originating from incomplete β-oxidation in staphylococci, are also considered as important flavor compounds (Engelvin, Feron, Perrin, Mollet, & Talon, 2000; Fadda, Lebert, Leroy-Sétrin, & Talon, 2002; Montel et al., 1996; Stahnke, 1999a).

Finally, the use of selected atoxigenic molds and yeasts also offers perspectives for flavor improvement. Superficial inoculation of the sausage with molds contributes to sensory quality due to lactate oxidation, proteolysis, degradation of amino acids, lipolysis, lipoxidation, delay of rancidity, and reduced water loss due to

slower evaporation (Benito, Rodríguez, Martín, Aranda, & Córdoba, 2004; Bruna, Fernández, Hierro, Ordóñez, & de la Hoz, 2000; Bruna et al., 2001, 2003; García, Casas, Toledo, & Selgas, 2001; Sunesen & Stahnke, 2003; Sunesen, Trihaas, & Stahnke, 2004). For instance, a characteristic popcorn odor in mold-fermented sausages has been ascribed to 2-acetyl 1-pyrroline production by the molds (Sunesen & Stahnke, 2003). The role of yeasts in sausage flavor formation is less characterized but seems to be related to lipolysis and the production of volatiles, including esters and alcohols derived from branched-chain amino acids (Flores, Durá, Marco, & Toldrá, 2004; Gardini et al., 2001; Olesen & Stahnke, 2000).

Functional starter cultures may also contribute to color formation and stability, for instance when reduced levels of nitrite and nitrate are used. Nitrite and nitrate are required in sausage fermentation technology as curing agents for microbial stability and color formation, but may lead to the formation of nitrosamines that may have negative health implications (Campbell-Platt & Cook, 1995). Microbial strains that generate nitrosylated derivatives of myoglobin, converting brown metmyoglobin into red myoglobin derivatives, could be useful to partially take over the coloration function of curing agents. The latter possibility has been demonstrated with strains of *Lb. fermentum* in smoked sausages (Moller, Jensen, Skibsted, & Knochel, 2003).

Functional Starter Cultures to Produce Safer Sausages

Acidification of the raw meat is the main contribution of the starter culture to the safety of fermented sausages (Lücke, 2000). However, not all sausages are fermented to pH values that are low enough to guarantee the absence of pathogens. Moreover, there is increasing concern about the acid tolerance of *L. monocytogenes* in fermented foods (Gahan, O'Driscoll, & Hill, 1996) and its recovery from fermented sausages (Colak et al., 2007; Encinas et al., 1999; Farber et al., 1993; Ferreira et al., 2007; Gianfranceschi et al., 2006; Siriken et al., 2006; Thevenot et al., 2005). The production of other antimicrobial compounds, in addition to the generation of lactic acid, could therefore lead to safer products.

Most promising are LAB that produce bacteriocins, which are antibacterial peptides or proteins that kill or inhibit the growth of other Gram-positive bacteria (Leroy & De Vuyst, 2000). LAB produce a diversity of bacteriocins that are generally active towards other LAB, contributing to the competitiveness of the producer, but also towards foodborne pathogens such as *L. monocytogenes* (Ennahar, Sonomoto, & Ishizaki, 1999). Therefore, the application of bacteriocin-producing LAB in the meat industry offers a way of natural food preservation (De Martinis, Alves, & Franco, 2002; Hugas, 1998; Hugas & Monfort, 1997; McMullen & Stiles, 1996; Stiles & Hastings, 1991), without risk for human health (Cleveland, Montville, Nes, & Chikindas, 2001). Moreover, in situ bacteriocin production does not lead to organoleptic or flavor imperfections, as has been shown through taste panels for fermented sausages manufactured with different strains (Coffey et al., 1998; Hugas, Garriga, Aymerich, & Monfort, 1995; Urso, Rantsiou,

Cantoni, Comi, & Cocolin, 2006). Evidently, bacteriocins are not meant to be used as sole means of food preservation, but should be appropriately integrated in a multihurdle preservation system, at all times respecting good manufacturing practice.

Strains of several species of lactobacilli that are relevant for meat fermentation have been shown to produce bacteriocins or bacteriocin-like compounds: *Lb. sakei* (Aymerich, Garriga, Monfort, Nes, & Hugas, 2000; De Martinis & Franco, 1998; Garriga, Hugas, Aymerich, & Monfort, 1993; Rosa, Franco, Montville, & Chikindas, 2002; Samelis, Roller, & Metaxopoulos, 1994; Sobrino et al., 1991; Tantillo, Di Pinto, & Novello, 2002; Tichaczek, Nissen-Meyer, Nes, Vogel, & Hammes, 1992), *Lb. curvatus* (Mataragas, Metaxopoulos, & Drosinos, 2002; Sudirman, Mathieu, Michel, & Lefebvre, 1993; Tichaczek et al., 1992; Xiraphi et al., 2006), *Lb. plantarum* (Aymerich et al., 2000; Enan, El Essawy, Uyttendaele, & Debevere, 1996; Garriga et al., 1993; Messi, Bondi, Sabia, Battini, & Manicardi, 2001; Rekhif, Atrih, & Lefebvre, 1995), *Lb. brevis* (Benoit, Mathis, & Lefebvre, 1994), and *Lb. casei* (Vignolo, Suriani, de Ruiz Holgado, & Oliver, 1993). Their use as starter cultures permits a reduction in *Listeria* levels in fermented sausage (Benkerroum et al., 2005; Campanini, Pedrazzoni, Barbuti, & Baldini, 1993; De Martinis & Franco, 1998; Dicks, Mellet, & Hoffman, 2004; Hugas et al., 1995; Hugas, Neumeyer, Pagés, Garriga, & Hammes 1996; Schillinger, Kaya, & Lücke, 1991). As an alternative to lactobacilli, bacteriocin-producing pediococci (Berry, Liewen, Mandigo, & Hutkins, 1990; Foegeding, Thomas, Pilkington, & Klaenhammer, 1992; Lahti, Johansson, Honkanen-Buzalski, Hill, & Nurmi, 2001) or enterococci (Ananou et al., 2005; Callewaert, Hugas, & De Vuyst, 2000; Hugas et al., 2003; Sabia, de Niederhäusern, Messi, Manicardi, & Bondi, 2003) may be used. Also, bacteriocin-producing *Lactococcus lactis* strains have been used as new, functional starters for fermented sausage manufacture, despite the fact that they are not particularly adapted to sausage technology, e.g., displaying sensitivity to nitrite (Benkerroum, Daoudi, & Kamal, 2003; Benkerroum et al., 2005; Coffey et al., 1998; Scannell, Schwarz, Hill, Ross, & Arendt, 2001).

Even though several challenge tests have proven successful and major companies have added bacteriocin-producing starter cultures to their product range, they are not common practice yet as they have found their way to the market only recently and on a limited scale. Technological and industrial implementation appears to be the bottleneck. This may be related to the fact that, in order to be successful, starter strains have to be carefully selected and must be in tune with existing production technologies. Bacteriocin activity in situ is usually lower than may be expected from in vitro experiments (Campanini et al., 1993; Schillinger et al., 1991), which may be due to low in situ production, genetic instability, the inability to uniformly distribute bacteriocin throughout the product, low solubility of the bacteriocin, inactivation by meat proteases, resistance of the target strain, and interference by meat components, in particular adsorption to fat and meat particles (Aasen et al., 2003; Cleveland et al., 2001; Dicks et al., 2004; Ennahar et al., 1999; Knorr, 1998; Leroy & De Vuyst, 2005). For optimal performance, it is recommended to use strains that are well adapted to the sausage environment,

preferably sausage isolates (Leroy, Verluyten, Messens, & De Vuyst, 2002). Also, it is preferable to analyze the kinetics of promising strains before implementation, to avoid mismatch with process technology. Such in vitro kinetic studies in meat simulation media have, for instance, shown that the sausage isolates *Lb. sakei* CTC 494 (Leroy & De Vuyst, 1999a), *Lb. curvatus* LTH 1174 (Messens, Verluyten, Leroy, & De Vuyst, 2003), and *Lb. curvatus* L442 (Mataragas, Metaxopoulos, Galiotou, & Drosinos, 2003) display optimal bacteriocin production under conditions of pH and temperature that prevail during European sausage fermentation. A combined model was set up to simulate the functionality of *Lb. sakei* CTC 494 under sausage fermentation conditions, confirming in situ studies (Leroy & De Vuyst, 2005). Also, in vitro interaction tests under sausage fermentation conditions have shown that bacteriocin production by *Lb. sakei* CTC 494 is able to explain inactivation of *Listeria*, warning, however, of the existence of a bacteriocin-tolerant listerial subpopulation which may be of importance at unusually high listerial loads (Leroy, Lievens, & DeVuyst, 2005a, 2005b). Moreover, the specific ingredients (e.g., spices, nitrate/nitrite, and salt concentrations) and process technology (e.g., fermentation temperature) used can severely interfere with the functionality of the starter culture (Hugas, Garriga, Pascual, Aymerich, & Monfort, 2002; Hugas & Monfort, 1997; Hugas et al., 1996; Leroy & De Vuyst, 1999b; Urso et al., 2006; Verluyten, Leroy, & De Vuyst, 2004; Verluyten, Messens, & De Vuyst, 2003).

Besides bacteriocin-producing LAB, microbial strains that produce other antimicrobial compounds (e.g., lysostaphin, reuterin, reuterocyclin) may be proposed as novel, functional starter cultures (Leroy et al., 2006).

To prevent the growth of *S. aureus*, introduction of the lysostaphin gene of *Staph. simulans* biovar. *staphylolyticus* into meat starter lactobacilli (Cavadini, Hertel, & Hammes, 1996, 1998; Gaier, Vogel, & Hammes, 1992) or *Penicillium nalgiovense* (Geisen, Ständner, & Leistner., 1990) has been considered. Lysostaphin is an endopeptidase that specifically cleaves the glycine–glycine bonds unique to the interpeptide cross-bridge of the cell wall of *S. aureus*.

Lactobacillus reuteri-containing starter cultures that produce reuterin or reutericyclin may also offer interesting antimicrobial effects (Gänzle & Vogel, 2003; Ross, Morgan, & Hill, 2002). Reuterin is a mixture of monomeric, hydrated monomeric, and cyclic dimeric forms of β-hydroxy-propionaldehyde with a broad spectrum of activity, including fungi, protozoa, and a wide range of Gram-positive and Gram-negative bacteria. Reutericyclin is a tetramic acid antibiotic that is active towards Gram-positive bacteria. Application of purified reuterin on the surface of Turkish-style beef sausage was able to inhibit growth of *L. monocytogenes* but not *Salmonella* (Kulea an & Çakmakçi, 2002). The inclusion of reuterin- or reutericyclin-producing *Lb. reuteri* strains in potential meat starter cultures needs further investigation but shows interesting preliminary results, for instance with respect to the inactivation of *E. coli* (Muthukumarasamy & Holley, 2007).

Microbial inhibitory action by meat-associated LAB and GCC is not always ascribed to specific metabolites and remains frequently unspecified (Papamanoli, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002; Papamanoli et al., 2003;

Pidcock, Heard, & Henriksson, 2002; Työppönen, Markkula, Petäjä, Suihko, & Mattila-Sandholm, 2003; Villani et al., 1997). Moreover, new antimicrobials with application possibilities are still being discovered. For instance, strains of *Lb. plantarum* produce a number of interesting compounds, including a mixture of low-molecular-mass molecules that act synergistically with lactic acid (Niku-Paavola, Laitila, Mattila-Sandholm, & Haikara, 1999), 3-hydroxy fatty acids (Sjögren, Magnusson, Broberg, Schnürer, & Kenne, 2003), antifungal cyclic peptides (Ström, Sjögren, Broberg, & Schnürer, 2002), and phenyllactic acid and 4-hydroxyphenyllactic acid (Ström et al., 2002; Valerio, Lavemicocca, Pascale, & Visconti, 2004). Most of these compounds are active towards molds and yeasts, but some of them also towards Gram-positive and Gram-negative bacteria, including *Listeria* and *Samonella*.

Functional Starter Cultures to Produce Healthier Sausages

Although meat is a food with high nutritional value, some consumers perceive meat products as unhealthy (Arihara, 2006). This can be ascribed to the image of meat as such but also to the presence of nitrite, salt, and fat. Adding nutritional assets to meat products could be a strategy to promote them as valuable elements of a high quality diet and to meet the trend for healthier meat products (Arihara, 2006; Jiménez-Colmenero et al., 2001).

The use of probiotic LAB starter cultures, something which is already common in the dairy industry, seems promising (Hammes & Hertel, 1998; Incze, 1998; Kröckel, 2006; Työppönen, Petäjä, & Mattila-Sandholm, 2003). Probiotics are live microorganisms that, when administered in adequate amounts, confer a beneficial health effect on the host (Food and Agriculture Organization/World Health Organization [FAO/WHO], 2001). Although the concept of probiotics in meat is not new, only few manufacturers offer raw fermented sausages with probiotic LAB. This is probably due to the more artisan orientation of sausage manufacturers as compared to the dairy industry, a larger variety of products, as well as a number of uncertainties concerning technological, microbiological, and regulatory aspects (Kröckel, 2006). The application of probiotic LAB must in all cases be based on a careful selection procedure, if any health claims are to be taken into account. A first strategy consists of checking existing commercial starter cultures (Erkkilä & Petäjä, 2000) or sausage isolates (Klingberg, Axelsson, Naterstad, Elsser, & Budde, 2005; Papamanoli et al., 2003; Pennacchia, Vaughan, & Villani, 2006; Pennacchia et al., 2004) for potential probiotic properties, such as survival capacities under simulated gastrointestinal conditions (Erkkilä & Petäjä, 2000; Klingberg, Axelsson, et al., 2005), interaction with Caco-2 cell layers (Klingberg, Axelsson, et al., 2005; Klingberg, Pedersen, Cencic, & Budde, 2005; Pennacchia et al., 2006), and inhibition of pathogens as an important health-promoting property (Klingberg, Axelsson, et al., 2005). Alternatively, it may be investigated if strains with probiotic properties, for instance intestinal isolates, perform well in a meat environment during sausage fermentation. Such strains should be competitive in a fermented sausage

environment to withstand the natural meat microbiota, survive the fermentation process, and be present in the end-product in numbers that are sufficient to cause health-promoting effects (Arihara et al., 1998; Erkkilä, Petäjä et al., 2001; Erkkilä, Suihko, Eerola, Petäjä, & Mattila-Sandholm, 2001; Pidcock et al., 2002; Sameshima et al., 1998). When strains from nonmeat origin are used, it should also be tested if no negative sensory effects occur (Erkkilä, Petäjä, et al., 2001; Erkkilä, Suihko, et al., 2001; Pidcock et al., 2002). Most importantly, human studies should confirm the functionality of such probiotic fermented sausages. Until now, results have been moderately successful. The daily consumption of 50 g of probiotic sausage containing *Lb. paracasei* LTH 2579 by healthy volunteers during several weeks has been shown to modulate host immunity, but no significant influence on serum cholesterol and triacylglycerides was found (Jahreis et al., 2002). In fecal samples, there was a statistically significant increase in the numbers of *Lb. paracasei* LTH 2579, but not in the feces of all volunteers. It is interesting to mention that the sausage matrix seems to protect the survival of probiotic lactobacilli through the gastrointestinal tract (Klingberg & Budde, 2006).

Another approach to produce healthier meat products is to make use of starter cultures that are able to produce micronutrients and nutraceuticals, e.g., vitamins and conjugated linoleic acid (CLA). A nutraceutical is defined as any substance that may be considered a food or part of a food that provides medical or health benefits, including the prevention and treatment of disease (Andlauer & Fürst, 2002). Although most LAB have limited biosynthetic capacities for vitamin production, careful selection could reveal strains that produce vitamins in considerable amounts (Lin & Young, 2000; Morishita, Tamura, Makino, & Kudo, 1999; Sybesma, Starrenburg, Tijsseling, Hoefnagel, & Hugenholtz, 2003). Moreover, through metabolic engineering it could be possible to develop starter cultures for the in situ production of vitamins, such as folic acid and riboflavin (Hugenholtz et al., 2002). Application of selected vitamin-producing LAB in meat has, however, to be investigated still. Concerning CLA, several health-promoting properties have been described, including antiatherogenic action, inhibition of carcinogenesis, antidiabetic effects, enhancement of immunological function, and reduction of body fat (Belury, 2002). Generally, CLA is found in meat from ruminants, mainly because of the bacterial hydrogenation of dietary linoleic acid in the rumen. It has been shown that lactobacilli, bifidobacteria, and propionibacteria can also produce CLA (Alonso, Cuesta, & Gilliland, 2003; Coakley et al., 2003; Jiang, Bjorck, & Fondén, 1998; Sieber, Collomb, Aeschlimann, Jelen, & Eyer, 2004), but further research is needed to investigate if this offers potential during meat fermentation.

For meat products to become healthier it is, obviously, important to also avoid the production of undesirable compounds such as toxins, biogenic amines, or D-lactic acid (Leroy et al., 2006). A general approach consists of using highly competitive starter cultures that overgrow producers of such undesirable compounds. For instance, the use of selected molds that are free of mycotoxin and antibiotic production as starter cultures could be useful in outcompeting mycotoxin- or antibiotic-producing strains from the house microbiota (Holzapfel, 2002; Laich, Fierro,

Cardoza, & Martin, 1999; López-Díaz et al., 2001; Sunesen & Stahnke, 2003). During ripening, bacteria that possess decarboxylases may produce biogenic amines such as tyramine, histamine, tryptamine, cadaverine, putrescine, and spermidine (Komprda et al., 2004). In general, starter bacteria have limited tyrosine-decarboxylating activity, but contaminant nonstarter LAB, in particular enterococci, are believed to be responsible for tyramine production (Ansorena et al., 2002). Highly competitive decarboxylase-negative starter cultures prevent the growth of biogenic amine producers and lead to end-products nearly free of biogenic amines (Bover-Cid, Hugas, Izquierdo-Pulido, & Vidal-Carou, 2000; Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2000; González-Fernández, Santos, Jaime, & Rovira, 2003; Suzzi & Gardini, 2003), as long as the raw material is of sufficient quality (Bover-Cid, Izquierdo-Pulido, & Vidal-Carou, 2001). Also, the introduction of starter strains that possess amine oxidase activity might be a way of further decreasing the amount of biogenic amines produced in situ (Fadda, Vignolo, & Oliver, 2001; Gardini, Matruscelli, Crudele, Paparella, & Suzzi, 2002; Leuschner & Hammes, 1998; Martuscelli, Crudele, Gardini, & Suzzi, 2000; Suzzi & Gardini, 2003). The production of amines by molds in fermented sausages does not appear significant but has not been fully studied yet (Bruna et al., 2003).

In view of a reduction of D-lactic acid by starter LAB, strains producing L-lactic acid should be selected (Holzapfel, 2002). The D-lactate isomer is not hydrolyzed by lactate dehydrogenase in the human body and is thus capable of causing acidosis, in particular in infants.

Chemical Acidulants

Role of Chemical Acidulants

As an alternative to adding a microbial starter culture and to accelerate the reduction in pH, chemical acidulants are sometimes used to manufacture salami-type sausages (Sebranek, 2004). Such acidulants decrease the pH without need for microbial lactic acid fermentation and contribute to the shortening of the ripening process. Because of their rapid acidification effect, they may reduce the risk of spoilage and bacterial contamination, lead to a prolonged shelf life, reduce the necessary amount of nitrite, improve color stability, and improve firmness and sliceability of the end-product. It has been shown that addition of GdL to meat decreases biogenic amine-producing enterococci and coliforms (Maijala, Eerola, Aho, & Hirn, 1993). In sausages, the most commonly applied acidifiers are GdL, citric acid, and lactic acid. The use of liquid lactic acid is not recommended because of its rapid protein denaturation effect (Barbut, 2006). GdL, in contrast to other chemical acidulants, leads to more progressive acidification and is therefore preferred by most manufacturers (see below). However, the acidulants can be encapsulated, so that the acid is released

during heating of the sausage and not during sausage manufacturing itself. The coating melts when heated to 57°C and slowly releases the acid into the meat mixture (Sebranek, 2004). Attention should be given to the type and quality of the encapsulating material (Barbut, 2006).

Glucono-delta-Lactone

For many manufacturers of undried or semidry sausages, GdL is the preferred acidulant. It is applied as a crystalline powder that is freely soluble in water, where it hydrolyzes to gluconic acid within a few hours and shows a progressive reduction of the pH (Barbut, 2006; Lücke, 1998). GdL is derived from gluconic acid by the removal of water. It is commercially produced by an aerobic fermentation process with *Aspergillus niger* (or its enzymes) to convert glucose into gluconic acid, after which a mixture of gluconic acid and GdL is separated by crystallization. In sausage, GdL is added at levels of up to 0.5% (Lücke, 1998). GdL is listed as a generally permitted food additive (E575) in the European Union (European Parliament and Council, 2006) and has received "generally recognized as safe" (GRAS) status from the U.S. Food and Drug Administration (Code of Federal Regulations [CFR], 2006). However, the U.S. Department of Agriculture only permits it in cured, comminuted meat products at a maximum level of 0.5%, or up to 1% in Genoa salami (United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 1995) (Chap. 1).

At room temperature, GdL displays a lactone structure without a free acid group. As a result, it can be safely added during the emulsifying stage of sausage making. Under the influence of heat in the smoking process, the ester hydrolyzes rapidly and is partly converted to gluconic acid. The rate of GdL hydrolysis is temperature-dependent, being lower at low temperature and increasing with higher temperature (Totosaus, Gault, & Guerrero, 2000). In this way, hydrolysis of GdL lowers the pH of the batter, enhancing the conversion of nitrite into nitric oxide by reducing compounds (e.g., sodium ascorbate). Nitric oxide, in turn, forms the desired red nitrosomyoglobin color. Because of its slow rate of acidification, compared to the instantaneous acidification which occurs with other acidulants, and because of its mild taste characteristics, GdL is more popular than other acidulants. However, if acidification is not done quickly enough, meat particles will crumble, making it difficult to stuff the batter (Barbut, 2006).

Other Chemical Acidulants

As an alternative to GdL, lactic acid can be used as an acidulant. In addition to their role as acidulants, lactic acid products are sometimes marketed as raw

sausage ingredients to fight *L. monocytogenes*. The application of liquid lactic acid in raw sausage causes an immediate pH drop, crumbling of meat particles, some moisture separation, and a microstructure with gaps among the meat particles after cooking (Barbut, 2006). Encapsulation of lactic acid does not produce such problems. Moreover, it results in lower cooking losses than when the sausage is produced with LAB that lead to slow acid release (Barbut, 2006). This procedure, however, is only valid in sausages that undergo a heating procedure, as is common in the United States, and that do not rely on dry-cured flavor development.

Drawbacks

The major drawback associated with the application of acidulants is that they generally lead to end-products that do not have a traditional dry-sausage flavor. Acidulants are thought to interfere with nitrite reduction and aroma formation by GCC in dry sausages (Campbell-Platt & Cook, 1995). GdL, for instance, is broken down into lactic acid and acetic acid by various LAB. These organic acids, and acetic acid in particular, are strongly inhibitory to GCC, which may lead to sausages that are prone to rancidity and color defects (Lücke, 1998). Moreover, sausages produced with GdL are less acceptable to some consumers due to an unpleasant sour flavor (Buncic et al., 1993). This is particularly undesirable in countries with a long tradition of dry-cured, fermented sausages.

Conclusions

Although excellent fermented sausages can be produced naturally, without starter culture, the addition of selected starter culture strains to the meat batter has several advantages, in particular with respect to the standardization and speed of the fermentation process. A still faster and, hence, more economical alternative is to use of chemical acidulants instead of a microbial fermentation, but this method interferes with flavor development and is only applicable in a limited amount of products that do not rely on the development of a typical dry sausage flavor. Further improvement is to be achieved by selecting strains that possess specific functional properties that may contribute to the quality, safety, and healthiness of the end-product. It seems particularly promising to investigate wild-type strains from traditional, artisan products and to evaluate how such strains can be applied in the industry.

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Chapter 12
Coating Ingredients

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Introduction

Batter- or breadcrumb-coated products are highly attractive foods. Coatings enhance the flavor, texture, and appearance, adding value to the substrate food by giving the fried product a pleasant golden color and a crispy exterior texture that is normally very appetizing.

Because "battering and frying" has been a traditional method for preparing foods, empiricism has dominated its application for decades. Although considerable geographical variations occasionally exist because of the raw materials available, versions of batter-coated or breaded foods are found in the traditional or regional cuisines of practically every part of the world.

Changes in lifestyle, particularly in the western world, have consolidated the availability and sales of convenience foods and frozen products, providing considerable support for the expansion and globalization of coated foods. Nowadays, the market for this type of product is growing steadily, and there are even major food service sector companies with a worldwide presence that only serve products of this kind.

Automation of manufacturing, innovations in the cooking methods, the demand for more sophisticated foods, diversification, and a concern to develop healthier products that contain less fat are the factors that dominate the latest research trends in this area. In addition, if the products are to go through the final cooking stage in the home there has to be a certain degree of versatility, as the consumer can vary the method or times and this should not lead to disappointment. The research fields have been increasing permanently in recent years. They cover developments in both coating formulations and coating application technology, which constitute the important challenges facing the technicians and researchers working in this area.

In the traditional process, the manufacturer batters the food pieces (chicken pieces, chicken nuggets, and strips, beef fritters and fingers, pork chunks, veal products, etc.), prefries them for a few seconds in order to give the batter a certain solidity of consistence, and then freezes them. The consumer buys them in this form and, in turn, fries them for a few minutes in order to cook them, normally until a golden external color has been reached. From the point of view of the industrial

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process, there is a choice between cooking fully before the application of the coating (95°C, 99% RH) or after it (180°C, 5% RH); in both cases the coating stage is followed by a prefrying step. The choice depends on the industrial machinery, the type of meat or other product to be coated, and its size, shape, etc., but it must also be remembered that yields can differ considerably, as can the final product quality.

While the batter is raw it must create a homogeneous layer that covers the food, which is normally also raw, and must adhere to it before and after coagulation – which takes place during the prefrying step – and during final frying; after the batter coagulates it must withstand freezing temperatures and normal handling (packaging and transportation) without cracking or breaking and without losing any portion of the external layer; during the final frying performed in the consumer's home it must create an outer crust with good acceptability in terms of texture (particularly crispness), flavor, and color. Coatings might also need to prevent oxidation, limit moisture and oil transfer, give freeze/thaw stability, and extend shelf life. Of course, they must also be cost-effective (Kuntz, 1997a). To achieve these objectives, research into the behavior of flours and starches has traveled a long way and a considerable array of ingredients with a broad range of functionalities has begun to be used. Methods for controlling the physical properties of both the raw and the fried products have also seen a tremendous boost in recent decades.

Although the fascinating world of battered or breaded foods involves many technological aspects, this chapter will pay particular attention to the use of ingredients and their properties in relation to good coated product performance and, more specifically, to the uses of polysaccharide hydrocolloids or gums, which have such a wide-ranging functionality that they feature in practically every approach to improving the quality of coated products. Most of the available information for studying the ingredients used in these types of coatings does not differ greatly between meat products and other types of foods although, in fact, most of the developments have been made for protein substrates. Nevertheless, developments involving specific aspects of meat products will be cited in detail in this chapter.

Types of Batter

Adhesion or Interface Batters

The adhesion coating or interface batter is used as an intermediate layer between the food to be coated and the exterior layer of bread crumbs or breading. It acts as the "glue" that holds the breading to the substrate. In this type of batter, stickiness is important and the viscosity must be strictly controlled, as the purpose of the batter is to hold a sufficiently thick layer of dry food coating made from flour, starch, seasonings, etc. uniformly in place. The ingredients, the batter characteristics, the uniformity

of the layer, its color, etc. do not need to be controlled so strictly, however, as all the final characteristics of the food depend on the outer layer of breading.

Tempura-Type or Leavened Batter

A tempura batter can be defined as a semiliquid dough, basically consisting of flour and water, into which a product is dipped before it is cooked, normally by frying. This batter is designed as a single outer covering for the substrate food. Unlike adhesion batters, this type of batter contains raising agents that produce carbon dioxide in the presence of water and heat, so it expands when fried, developing a number of gas cells and, consequently, a spongy structure.

The basic reaction that produces carbon dioxide comes from the interaction between sodium bicarbonate and acids or acid salts, which may be tartaric acid, tartrates, phosphates, glucono-delta-lactone, etc. The amount of acid used is controlled in order to regulate the rate of leavening and neutralization by sodium bicarbonate, generally so that this takes place after the mixing and beating of the ingredients (and continues during the battering of the substrate), but before the structure is set by frying. As bubbles expand, a surface-active material (emulsifier-like agent) must be present so that the bubble walls do not get too thin and the bubbles do not rupture or coalesce. During frying the batter foam becomes a completely set sponge structure.

Leavening reduces the density and increases the volume of the coatings, so they are lighter to eat (Brock, 2001). It must be borne in mind that the raising agent only expands pre-existing bubbles, so the beating stage is very important in this type of batter. The aeration caused by the raising agent contributes to crispness and facilitates steam release during the prefrying and final frying steps.

The structural characteristics of a tempura-like batter must be able, by themselves, to achieve a uniform external layer with good adhesion to the substrate. It must also be visually attractive, with good coverage, no defects, and an agreeable color. Tempura-type batters form a crisp, continuous, open, and uniform layer over the food substrate, constituting its final outer coating. They act as a barrier against loss of food substrate moisture as well by protecting the natural juices of foods from the effects of freeze/thaw cycles or reheating, thereby ensuring a final product that is tender and juicy on the inside while crisp on the outside.

Predusting

Predust is a dry ingredient or mixture of ingredients that is dusted onto the moist surface of the frozen, fresh, or steam- or heat-cooked food substrate before any other coating is applied. It improves batter adhesion because it absorbs part of the

water on the surface of the substrate, avoiding migration (Yang & Chen, 1979). If the batter is applied to a surface that is too moist, it can slip, leaving some areas uncovered or covered with a diluted material that will then form too thin a layer. The contrary occurs when a predust is applied on very dry product surfaces or deep frozen substrates, as the dust will not be absorbed by the product but will be dispersed in the liquid batter, increasing its viscosity, and resulting in the batter pick-up being too high.

In a pioneering work, Baker, Darfler, and Vadehra (1972) evaluated the predust material capacities of a series of ingredients from three large groups: starches, proteins, and gums. They concluded that the proteins produced crusts with better adhesion and, of these, dried egg albumin produced the best results in terms of yield and visual scores.

Predusting also reduces the voids that may be caused by entrapment of air pockets between the substrate and the batter during batter application, and also tends to increase batter pick-up. On occasion, consecutive layers of predust, batter, and breading are used to achieve the desired textural or functional results. The predust most commonly used is wheat flour. Native and modified starches, gums (hydroxypropyl methylcellulose or methylcellulose), and proteins (whey protein concentrate, egg albumin or gluten), alone or in combination, have also been used to absorb moisture and to help form a barrier against moisture and fat migration (Kuntz, 1997a; Usawakesmanee, Chinnan, Wuttijumnong, Jangchud, & Raksakulthai, 2004; Zhang, 2001). Not much research in this area has been published.

Batter Ingredients and Components

As a basis for suitable formulations of dry mixes, it is important to have a good understanding of the requirements that the raw batter and batter components must fulfill and of the characteristics they must develop in the product during processing, prefrying and freezing, and, finally, during final frying or cooking by the consumer.

In practice, however, the list of ingredients is far longer (starches, seasonings, gums, different proteins, such as egg or gluten, and many other items) and batters have therefore become highly complex systems in which the nature of the ingredients is very wide-ranging and their interactions, which determine the final performance of the product, are more difficult to study and characterize.

Wheat Flour

As previously mentioned, a batter is typically based on wheat flour, which determines its fundamental characteristics (degree of adhesion, viscosity, structure, starchy flavor note, color or absence of color, and fat absorption features). Flour

commonly makes up 80–90% (by weight) of dry batter mixes; and approximately 70–80% of breading formulas. The main contribution of wheat flour is body or structure (Van Beirendonck, 2001). Various characteristics of this flour base – moisture content, functionality of its proteins, and its amylose and amylopectin contents – have been correlated with the texture characteristics, oil absorption, appearance and overall acceptability of the external crust. In brief, higher water or amylopectin content and excess protein increase oil absorption and decrease crispness of the fried batter.

Flours from the various major wheat types have different functional properties that need to be taken into account when formulating a batter with particular properties. However, generally, no particular type of wheat is suggested in batter formulations, so multipurpose flour should be suitable.

Wheat flour proteins are highly efficient water binders. Owing to the functional properties that they develop when hydrated, they are able to form cohesive doughs or batters when subjected to kneading or beating forces. Because of its exceptional elastic properties, gluten can hold gas and expand during frying, producing a cellular structure and providing a desirable, spongy, porous, cooked coating in tempura-type batters, essential for a good, crispy texture. This type of structure also facilitates the passage of water and oil (Mukprasirt, Herald, Boyle, & Boyle, 2001). Hard wheat flours have a higher protein content than soft wheat flours and therefore need more water to produce a comparable viscosity when used to formulate a batter. Good viscosity will prevent the ingredients that are insoluble at ambient (or refrigerated) temperature from settling and causing undesired stratification. The batter will perform best when all the ingredients are in a balanced suspension.

Wheat starch is composed of two main fractions: amylose (a linear polymer) and amylopectin (a branched polymer). The functionality of the starch is determined to a great degree by the ratio of these two polymers. The linear structure of amylose has low water retention capacity and adds structure to the layers, while the branched amylopectin holds water (a possible cause of blow off) and disrupts layers, resulting in poor crisping action. In fact, commercially available high-amylose starches have various applications in batter coatings. Other factors must be borne in mind, such as the degree of starch damage. In the grains of cereal, starch granules are found in different sizes and embedded in a protein matrix; their degree of bonding and the milling process employed will determine the degree of starch damage and the particle size distribution. Damaged starch (as the granules that suffer mechanical changes during milling are called) is capable of absorbing higher quantities of water than intact granules (Olewnik & Kulp, 1993). Intact starch granules absorb around 30% of their weight in water and, because they have a natural tendency to settle out of solution, steps need to be taken to keep them uniformly distributed throughout the batter. Since damaged starch granules absorb greater quantities of water than intact ones, their use requires that the quantity of water in the formulation be increased.

When starch is heated in the presence of water, the gelatinization process begins. First, the granules swell as they absorb water into their structure. The crystalline zones of the starch that could not be reached by the water when cold now break and

all areas are exposed to the hydration process, resulting in swelling of the sample. At the same time, some of the material from the interior of the granule leaks out, increasing the viscosity of the system as a whole. Upon cooling, the gelatinized starch molecules rearrange themselves and form a gel that strengthens the firmness of the system as the temperature falls. The fraction responsible for this process is mainly amylose, which has a linear structure, rather than amylopectin, which has a branched structure. It is this heat-gelatinized starch that gives rise to the basic structure of the batter coating on the final product. Indeed, a formulation composed only of flour and water can work, even if it does not possess the optimum characteristics that are perfected by adding a whole series of other ingredients (Davis, 1983).

Mixing native wheat flour with steamed (heat treated) wheat flour (in which the gluten forming protein is partially or totally denatured), in a 1:1 ratio, has proved effective for controlling batter viscosity as well as textural and adhesion characteristics of the final product (Prakash & Rajalakshmi, 1999).

Nonwheat Flours and Starches

The effect of replacing wheat flour with flour made from other cereals and vegetable sources has been discussed in terms of changes in the composition and content of proteins and starch. The GM (genetically modified) issue, which principally affects corn, should also be considered. Flours from other sources include potato, rice, tapioca, pea, and barley, among others. The proteins of corn or rice flours are not capable of forming structures that retain gas or of contributing the viscosity that wheat proteins develop. Rice starch or corn starch, for example, are different from wheat starch in terms of the size and shape of their granules, so their gelatinization properties, water absorption rate, and swelling capacity are not the same. Consequently, replacing part of the wheat flour with rice flour changes the rheological properties of the batter, depending on the replacement ratio and the batter temperature (Mukprasirt, Herald, & Flores, 2000). Replacement of 50% of the wheat flour with rice flour, which is a poor thickening agent, leads to a batter that requires a greater proportion of solids or the addition of a thickener to achieve a suitable viscosity; this rice flour-based frying batter significantly reduces oil uptake during the frying process while retaining desirable organoleptic properties (Shih & Daigle, 1999). Batters composed of wheat–corn blends can be tailored by varying the ratio of these two ingredients; the diluting effect of corn on wheat gluten (which can cause leathery coatings) will increase crispness (Burge, 1990).

A recent study found that replacing 5% of the wheat flour with soy flour brought about an improvement in the quality of chicken nuggets in terms of crispness and color, while replacement with both soy flour (5%) and rice flour (5%) reduced oil absorption compared to a control that did not contain these additions; the study was conducted at different frying temperatures (Dogan, Sahin, & Sumnu, 2005). Biswas and Keshri (2003) used a batter prepared from mixtures of Bengal gram meal with other ingredients, including spices, carboxymethylcellulose, whole egg and skim

milk powder, for covering pork patties, and concluded that this mixture improved the quality of the final battered, fried patties. Wheat flour has been replaced with steam jet-cooked barley flour in batter formulations, producing batters with good rheological properties and coatings with low oil uptake and high moisture content, which could be related to the high water binding capacity of this ingredient (Lee & Inglett, 2006).

As ingredients, native starches present a wide range of functionalities that include improved adhesiveness, changes in tenderness and crunchiness, textural modifications, improvements in crispness, holding time under heat lamps, freeze/thaw stability, better moisture retention and decreased greasiness (Van Beirendonck, 2001).

Corn starch-based batters require continuous mixing during processing because the solids have a tendency to settle out easily, leading to changes in batter viscosity throughout the production period, and resulting in irregular batter pick-up (Suderman, 1993). The addition of a thickener to keep the solids in suspension helps to solve this problem.

High-amylose hybrid starches result in increased gelatinization temperatures; a film is formed in seconds, creating a uniform appearance and the desired final brittleness and crispiness of the coating (Bertram, 2001). These high-amylose starches, alone or in combination with other ingredients such as rice flour or flour from other cereals, dextrin, etc., have good film-forming properties, and help reduce oil absorption in fried, battered products (Higgins, Qian, & Williams, 1999; Van Beirendonck, 1998). In one study, native starches – high-amylose corn starch (HAC), normal corn starch (NCS), waxy corn starch (WCS), rice starch (RS), and waxy rice starch (WRS) – were used in wheat flour batter (20% starch). The crispness and hardness of the fried batter correlated positively with the amylose content of the starch (HAC > NCS > RS > WRS > WCS) and negatively with residual moisture content; however, WCS was the most effective in reducing oil uptake (Lee & Lim, 2004).

Modified starches find many applications in this area as a result of their wide-ranging functionality. Oxidized starches, for example, have functional carboxyl groups that bind with proteins in the substrate, and this bonding makes the batter stick (Shinsato, Hippleheuser, & Van Beirendonck, 1999). Lenchin and Bell (1985) used flour and corn starch with a high-amylose content to formulate a mix for coating prefried food products suitable for final cooking in a microwave oven which are characterized by improved crispness. Cold water-swelling, oil-resistant, rice-based starch products, such as pregelatinized rice flour, phosphorylated rice starch, and pregelatinized acetylated rice starch, are used in formulations to enhance viscosity and textural and sensory quality of the fried batter. Addition of pregelatinized rice flour gives a high crispness value but also increases oil absorption (Mohamed, Hamid, & Hamid, 1998). The effect of amylomaize, corn, waxy maize, and pregelatinized tapioca starches on the texture, moisture content, oil content, color, coating pick-up, cooking yield, volume, and porosity of deep-fat frying chicken nuggets was studied by Altunakar, Sahin, and Sumnu (2004). They concluded that starch addition to the formulation increased the crispness of the final product in the last

stages of frying, and the highest porosity and oil content were obtained when corn starch was used; pregelatinized tapioca starch resulted in the lowest oil content and the highest moisture content, coating pick-up and volume. Lee and Lim (2004) found that modified (oxidized, acid-treated, cross-linked, hydroxypropylated and acetylated) starches provided texture improvements (crispness and hardness) in fried products.

Gums and Hydrocolloids

One of the main challenges with the use of gums and hydrocolloids is that these perform functions that influence every stage of the manufacturing process: blending of the mix, coating, prefrying, freezing (if applicable), and final frying by the end consumer.

The use of gums has been one of the central issues in experimentation with batters to cover pieces of food. Their primary use is based on their ability to retain or immobilize water and their direct effect on the control of viscosity.

The addition of hydrocolloids is generally effective at levels as low as 1% or less (dry weight), of the formulation, so their addition does not "dilute" the functionality of proteins in the flour base. However, many gums are highly hydrophilic, so the formulation requires adjustment of the solids to water ratio, modifying the characteristics of the entire system. When hydrocolloids are used in a formulation, complete incorporation and hydration are essential if they are to be effective. Moreover, many authors recognize the need to study the numerous interactions that components of this kind develop in the presence of lipids, proteins, other hydrocolloids, or other batter ingredients. Since these components display the combined effect of surface tension, hydrophilic capability, ability to gel on heating, and ability to form films, it is necessary to take all these properties into account when predicting their performance in relation to the other ingredients of the batter (Annapure, Singhal, & Kulkarni, 1999).

The choice of the most appropriate gum for a particular function basically depends on its effectiveness, cost-effectiveness, ease of incorporation into the manufacturing process, and the sensory properties of the final product.

From the standpoint of their technological application also, there are several factors that are important to consider when choosing a gum for a specific effect within the batter. One of the most important of these is to achieve correct hydration. In this regard, when different alternatives are available, the preferred gum will be the one that can be incorporated into the batter by dry blending, as this requires no modification of the normal process.

The correct choice should also consider that other ingredients in the batter system can also affect gum performance, so the compatibility of the gum with those components must also be assessed. For example, the presence of soluble solids (i.e., sugar, salt, etc.) can reduce the solubility of hydrocolloids because of competition for available water (Meyers, 1990).

The preferred gum will also be cost-effective and that which gives the final batter-coated food the most suitable sensory characteristics.

Effects of Gums on Batter Viscosity

The best-known application of gums in batter-coated products, as a result of their water-immobilization properties, is related to their ability to control viscosity.

As previously mentioned the viscosity of the batter is critical to the quality of the coating and is recognized as one of the main factors that determine batter behavior during frying (Loewe, 1990, 1993). Higher viscosity increases the degree of coating and affects the quantity and quality of the batter that adheres to the substrate (Hsia, Smith, & Steffe, 1992). The degree of adhesion in turn determines the handling properties of the product during and after coating, as well as its final appearance and texture after frying.

The main reason why batter viscosity needs to be controlled is the inherent variability of the rheological properties of the main ingredients, principally flour (Mukprasirt, Herald, Boyle, & Rausch, 2000). The advantage of using gums rather than other hydrocolloids such as modified starches is their effectiveness at lower concentrations, thus reducing the dilution of the functional protein in the flour, which plays a critical role in the overall performance of the coating system.

At times, the need to increase batter viscosity is necessary due to the low inherent viscosity of the batter ingredients. For example, in rice flour-based batters, which are widely used in Asian countries and can be used as alternatives to traditional wheat flour-based batters, the absence of gluten limits their ability to provide viscosity. To obtain a batter with optimum properties, it is therefore necessary to add an agent that will give the formulation the required viscosity. Shih and Daygle (1999) reported that adding phosphorylated long grain rice starch esters to the batters was effective in enhancing their viscosity; these batters are an alternative for individuals with gluten allergies and, in addition, they absorbed substantially less oil than wheat flour-based batters. Another combination that has been used in this type of batter is oxidized starch, xanthan gum, and methylcellulose, which gave good results in a rice flour-based batter for chicken drumsticks as reported by Mukprasirt, Herald, Boyle, and Rausch (2000) and Mukprasirt et al. (2001).

The ability of gums to enhance viscosity also enables them to keep solids in suspension. This property has been related particularly to xanthan gum and tragacanth gum, which also provide yield value (i.e., the system begins to flow if the shear stress exceeds a certain threshold value) and are able to aid in the suspension of heavy particles under low shear conditions at low gum concentrations (0.10–0.25% w/w) (Hsia et al., 1992).

The greater viscosity conferred by gums also makes it possible to obtain batters with a greater proportion of water while maintaining appropriate viscosity. This property has been used to increase the quantity of water available for starch gelatinization (Davis, 1983).

Guar gum, xanthan gum, and the cellulose derivatives carboxymethylcellulose (CMC), methylcellulose (MC), and hydroxypropyl methylcellulose (HPMC) have been the most used. Evidently, the efficiency of viscosity enhancement varies according to the gum used and its concentration, but the interactions and resulting action of each product with other system components are also very important factors in the choice of gum. As an ionically charged gum, CMC can lose its effectiveness in the presence of salts (e.g., NaCl, leavening agents). In the case of MC and HPMC, temperature control is essential to assure the gum's functional properties. For these cellulose derivatives to be correctly hydrated, the temperature of the water used to reconstitute the dough must be between 10°C and 15°C, which ensures their gelling effectiveness during the subsequent step of coagulation in hot water. For this same reason, the dough must be kept at this temperature when it is used in the battering step.

The incorporation of hydrocolloids makes the flow behavior of batters more complex. However, only single shear rate rheological measurements are usually performed in industrial plants and they do not provide complete information. These batters generally present shear-thinning behavior, time dependency, and thixotropy, so a rheological characterization of their behavior over a range of shear stresses and times gives more complete information for optimizing the mixing, pumping, and coating processes, with a view to maintaining uniform batter properties, pick-up, and adhesion (Fiszman & Salvador, 2003). The sophisticated rheometers that are available nowadays make it possible to study the rheological behavior of batters in depth, although their use is generally confined to the field of research (Sanz, Fernández, Salvador, Muñoz, & Fiszman, 2004).

In a comparative study on the effects of guar gum, xanthan gum, and CMC on the rheological behavior of batters (Hsia et al., 1992), xanthan gum produced the batters with the greatest consistency, followed by guar gum and CMC. The concentration of the chosen hydrocolloid also affected its rheological behavior. In this case, for the concentration range studied (0.25–1%), increasing the concentration of xanthan gum and guar gum increased the consistency index of the batters as well as their pseudoplasticity, whereas the pseudoplastic behavior of CMC decreased at higher concentrations. A higher MC concentration in the batter, within the 1–2% range, increased the viscosity and shear thinning behavior of the batters, as reported by Sanz, Salvador, and Fiszman (2004a). The rheological behavior of these batters was particularly affected by temperature; a significant increase in pseudoplastic behavior was observed as the temperature was increased from 5°C to 25°C.

An important factor to consider when choosing the concentration of a hydrocolloid is its effect on the crispness of the final fried crust. An increased concentration of MC has been associated with greater moisture retention in the product during frying, causing a loss of crispness. The use of xanthan gum at levels higher than 0.2% imparts a chewy texture (Kuntz, 1995).

As mentioned below, in the particular case of MC and HPMC, their film-forming and thermal gelation properties also help maintain integrity and structure during frying, which is especially important for batters that use low quality flour or have low solids contents (Meyers, 1990).

The use of ingredients that do not develop much viscosity, such as rice flour, makes it necessary to incorporate a gum to ensure a quality similar to a classic formulation (Mukprasirt, Herald, & Flores, 2000).

Effects of Gums on Batter Pick-Up

Batter pick-up is a particularly important factor for batter-coated products. The appearance, thickness, and crispness of the fried external crust are critical to the sensory acceptance of fried batter-coated foods and they are all closely linked to the pick-up value. Pick-up is also important for industrial manufacturers of batter-coated products, as the weight of the coagulated batter in relation to the total weight of the batter-coated food determines the process yield, which in turn affects the final cost of the product.

Evidently, hydrocolloids influence the pick-up value of a batter-coated product because of their ability to confer viscosity. In principle, as the viscosity of the batter increases, so will the percentage of it that will adhere to the substrate. In batters formulated with xanthan gum, guar gum, and CMC at concentrations of 0.25%, 0.5% and 1.0%, a correlation between greater viscosity and greater adhesion of the batter to the food was observed (Hsia et al., 1992). However, this correlation is not always direct, as the increase in viscosity caused by a higher concentration of HPMC does not cause a proportional increase in the quantity of batter on the final product; the batter pick-up rate increased less than the batter viscosity with increasing levels of HPMC between 0.25% and 1.0% (Meyers & Conklin, 1990). Therefore, using the apparent viscosity values of the batter as a quality control check for batter pick-up may be misleading, so the actual pick-up should be monitored.

Apart from viscosity, some other properties of gums have been indirectly related to yield. The presence of HPMC in batter-coated and breaded chicken nuggets has been associated with increased product yields due to moisture loss reduction during frying (The Dow Chemical Co., 1991). Also, as remarked in a latter section, the body and integrity conferred by gums enhance batter performance before, during, and after the frying operation, leading to improved batter adhesion and, indirectly, to higher yields.

Effects of Gums on Oil Absorption During Frying

One of the main problems associated with consumption of batter-coated foods is the considerable amount of oil absorbed during the prefrying and frying steps. As is well known, a reduction in oil absorption leads to healthier products, because a high intake of fat is related to the risk of heart diseases (and, of course, taking into account that oils high in mono- or polyunsaturated fats are healthier options those high in saturated or trans-fats). In this regard, improved public and media awareness of the desirability of reducing fat in the average diet has prompted studies on

ways to lower the oil content of fried foods. Different ingredients have been proven to reduce oil absorption in fried foods. Among these, gums are of considerable interest, as they are the most effective. The gelling ability of hydrocolloids, together with their usually hydrophilic nature, makes them suitable for reducing oil uptake during frying of battered products (Annapure et al., 1999).

Use of MC and HPMC. Among the different gums employed as fat barriers, the cellulose ethers MC and HPMC, which possess the unique property of reversible thermal gelation, have been more widely investigated than other gums.

In contact with hot oil, the MC or HPMC in the batter gels to form a film. Together with their high water-retention capacity, this protects against moisture loss and the entry of oil during the frying process (Balasubramaniam, Chinnan, Mallikarjunan, & Phillips, 1997; Mallikarjunan, Chinnan, Balasubramaniam, & Phillips, 1997; Meyers, 1990).

The use of MC and HPMC in batters has been shown to be effective in a wide range of applications, such as chicken nuggets (Meyers & Conklin, 1990; The Dow Chemical Co., 1991), mushrooms, chicken breast strips and codfish fillets (Ang, 1993), chicken balls (Balasubramaniam et al., 1997), mashed potato balls (Mallikarjunan et al., 1997), and chicken strips (Holownia, Chinnan, Erickson, & Mallikarjunan, 2000).

Gelation of MC and HPMC is mainly caused by hydrophobic interaction between molecules containing methoxyl groups. However, the presence of a hydroxypropyl substitution significantly alters gelation properties. For the same degree of methoxyl substitution, an increase in hydroxypropyl substitution raises the gelation temperature and diminishes the strength of the resulting gel; therefore, gelation temperature is higher and gel strength is lower for HPMC than for MC. Regarding the influence of molecular weight, gel strength increases in line with molecular weight up to a molecular weight of approximately 140,000, at which point it stabilizes. Molecular weight does not affect gelation temperature (Sarkar, 1979).

Despite the differences in gelation properties between MC and HPMC, the studies currently available do not enable these to be linked to the barrier efficiency of the two hydrocolloids.

What has been linked to effectiveness as a barrier is the MC content of the batter. Raising the MC concentration from 1% to 2% has led to lower oil absorption and greater moisture reduction in the crust of batter-coated squid rings, both after the first 30 s of frying and after the final frying subsequent to freezing. At the three concentrations studied, the barrier effectiveness was more evident after 30 s of prefrying (Sanz et al., 2004a). Similar results regarding higher barrier effectiveness in the first 30 s of frying were found in batter-coated/breaded chicken nuggets containing HPMC levels from 0.25% to 1.0% w/w on a wet batter basis (The Dow Chemical Co., 1991).

When incorporating MC and HPMC into the batter, the two main ways of achieving correct hydrocolloid hydration provide two main alternatives. The simplest method is to disperse the hydrocolloid by dry blending with the other batter ingredients; it is then hydrated when they are mixed with water. When this method

is used, it must be remembered that for correct hydration, cold water must be used; the required temperature decreases as the number of methoxyl substitutions in the anhydroglucose ring rises. The other method is to hydrate the hydrocolloid before mixing it into the batter (The Dow Chemical Co., 1996).

These ways of blending MC or HPMC into the batter have been linked to their efficiency in reducing oil absorption. The barrier effect of HPMC in various batter-coated foods (pieces of chicken, fish, vegetables and cheese) has been found to be more efficient when using the prehydrated hydrocolloid method than when hydrated together with the other batter ingredients. The viscosity of the batters was also greater, which was linked to greater hydration efficiency and its effect on functional properties (Meyers & Conklin, 1990). Another factor that has been linked to barrier efficiency is the batter temperature at the moment of applying the coating. In batters with differing MC concentrations stored at 5°C, 15°C, and 25°C for an hour after preparation (dry-blending technique), a reduction in the barrier efficiency was observed as the temperature increased (Sanz et al., 2004a). Subsequent Cryo-SEM observation of the batters showed a greater density of eutectic artifacts at 5°C. This was associated with the development of greater viscosity and water-retention capacity by the batters which, in turn, was associated with the presence of a hydrocolloid that was more unfolded and, therefore, more efficient at performing its function (Llorca et al., 2005).

As an alternative to adding the hydrocolloids to the batter mix, another possibility that has been tested is to form an edible film around the pieces of food by dipping them in a solution of MC or HPMC. The influence of applying HPMC and MC as a film before breading or adding these substances to the breading formulation in order to reduce the amount of oil absorbed by the crust has been evaluated in marinated chicken strips (Holownia et al., 2000). The most efficient method was to add the dry hydrocolloid to the breading formulation. In this work, application as a film before breading did not reduce the amount of oil absorbed by the crust but did reduce its moisture; this was associated with an inhibition of moisture migration from the substrate into the crust. Applying the film after breading was rejected owing to adhesion problems.

Use of Other Cellulose Derivatives. Although less efficient than MC and HPMC, another cellulose-derived hydrocolloid that has also been used to reduce oil absorption is sodium carboxymethylcellulose (CMC). Adding CMC to the formulation of "boondis," a deep-fried batter-based legume snack food popular in India reduced the amount of oil in the final product (Priya, Singhal, & Kulkami, 1996). This study analyzed different concentrations of CMC (0.5–3%), adjusting the proportion of water to obtain adequate viscosity. The greatest barrier efficiency was obtained at a concentration of 2%; higher concentrations (3%) were not effective.

Microcrystalline cellulose codried with whey (to allow it to be used in dry form) increased moisture retention and reduced the oil absorbed by the bread coating of fried batter-coated and breaded fish (Anonymous, 1980).

Cellulose derivatives are not always the most effective barriers to oil absorption. A study that evaluated the oil uptake barrier efficiency of various gums at concentrations between 0.25% and 2% in model systems based on chickpea flour (gram

flour) classified HPMC as the most effective at 0.25%, although its efficiency fell as concentration increased. For a 2% admixture, gum arabic proved the most effective, followed by carrageenate and karaya gum. Similarly to HPMC, CMC showed decreased effectiveness with increasing gum concentration. In both cases, the effect was thought to be the consequence of the formation of a thick coating at higher concentrations resulting in rupture of the film from excessive pressure built up during frying. Other gums studied, such as xanthan gum, ghatti gum, tragacanth gum, and locust bean gum, were not effective. In this study all the gums were incorporated by dry-mixing with chickpea flour and the water level was adjusted to obtain a soft dough (Annapure et al., 1999).

Noncellulose Hydrocolloid Barriers. In addition to MC and HPMC, other gel forming hydrocolloids which have been employed as barriers against fat absorption are gellan gum and pectin.

Gellan gum, the anionic linear heteropolysaccharide produced by *Pseudomonas elodea*, can be applied as a hot solution. The food is dipped into the solution and the film forms as it cools. Another possibility is to dip the food into a cold gellan solution; gelation takes place after ions such as Na^{+}, Ca^{++}, Mg^{++} or K^{+} have been added (Duxbury, 1993).

Gellan gum has also been added with calcium chloride to the dry ingredient mixture of batters for chicken, fish, cheese, and vegetables, resulting in low oil absorption and the development of appropriate crispness, and gellan solutions have been used to coat the crumbs used for breading. The use of these rather than conventional breading crumbs resulted in a final product with excellent crispness and lower oil absorption (Chalupa & Sanderson, 1994).

Pectin, a substance that gels in contact with calcium, forms a film that has proven effective for reducing oil absorption in a batter-coated and breaded product. Ca^{++} is added to the breading (by dry blending, spray drying, agglomerating, baking the calcium source into the bread crumb or any combination of these methods) and the batter-coated and breaded food is treated with a solution of calcium-reactive pectin. A hydrophilic film that reduces oil absorption forms during the reaction between Ca^{++} and the pectin. The level of Ca^{++} added to the breading is critical, as sufficient calcium must be available for efficient reaction with the pectin. The preferred pectin types are characterized by very strong calcium reactivity, selected from the group of conventional low methoxy pectins and amidated low methoxy pectins (Gerrish, Higgins, & Kresl, 1997).

Methylcellulose Application in a Method Without Prefrying

The most recent application of gums to batter-coated products involves the thermogelling property of MC to create a new industrial manufacturing process for frozen batter-coated foods that eliminates the prefrying stage and thus provides a healthier and more environmentally friendly alternative (Fiszman et al., 2003; Sanz, Salvador, & Fiszman, 2004b).

The purpose of the prefrying stage of industrial manufacture is to coagulate the coating around the food. It is the most problem-ridden stage of the process and one in which the batter-coated food absorbs a considerable amount of oil.

The patented process adds MC to the batter and basically consists of the same stages as the traditional process except that the prefrying process is replaced by immersing the batter-coated food in hot (70–80°C) water for 30 s and then quickly heating it in a microwave, conventional, or infrared oven. The aim of the hot water is to prompt the gelation of the MC so that the batter develops the appropriate consistency. A further objective of the heating step is to "fix" the resulting structure to preclude thermo-reversibility of the gelation process and make the batter-coated food stable at any temperature.

The new product, batter-coated by the new process, offers the additional advantage of absorbing significantly less oil during the final frying by the consumer while having a moisture content similar to that of traditional batter-coated products, thus giving it adequate crispness.

Similarly to commercial wheat flour-based formulations already available, the batter mix employed allows great flexibility and, in addition to wheat flour and MC, it may include other ingredients, allowing the final batter-coated products to be given different sensory properties and, consequently, to cover a wider range of consumer requirements.

The basic ingredients of the batter should preferably be chosen for their low ability to confer viscosity, in order to make it possible to obtain an appropriate batter viscosity despite the MC concentrations used.

An essential prerequisite for correct coagulation of the batter in the water bath is that the MC be well hydrated. Dispersion of the MC by dry blending with the rest of the ingredients and hydration with cold (10–14°C) water has given good results. A reduction in the gelling efficiency of the reconstituted batter has been observed as the temperature rises, so the batter must be kept refrigerated during use to ensure that the process proceeds correctly.

Unlike the use of cellulose ethers as oil barriers, this application is limited exclusively to MC, since the lower strength of the gels that develop when using HPMC is insufficient for correct coagulation.

The choice of MC concentration is influenced by the balance between the viscosity-conferring effect and the subsequent gelling action. A gum concentration that confers to the batter appropriate viscosity and correct coagulation when placed in the hot water bath must be used. The addition of 1.5–2% (dry ingredients) of MC for a 1.2:1 (w/w) ratio of water to dry ingredients has given satisfactory results. Lower levels of MC were insufficient to obtain an adequate consistency after freezing. Although higher concentrations provided excellent coagulation, the viscosity of the batters after mixing was excessive, preventing an adequate coating from being applied.

The thermal process undergone by batters in this innovative process has been studied by SAOS (small amplitude oscillatory shear). The evolution of their viscoelastic behavior with temperature showed that the main feature associated with MC batters was a decrease in the temperature at which an increase in consistency

appears (Sanz, Fernández, et al., 2004). The influence of a number of ingredients (wheat starch, corn starch, dextrin, gluten, and dried egg) on the viscous behavior of these MC batters was also studied by dynamic rheological techniques (Sanz, Salvador, Vélez, Muñoz, & Fiszman, 2005).

Effects of Gums on Adhesion Properties

In a batter system, adhesion can be defined as the chemical and physical bonding of a food coating with the food substrate (Suderman & Cunningham, 1983). The batter must cover the substrate evenly and remain adhered to it throughout all the stages of the manufacturing process. It must then withstand the frozen storage and transport conditions without becoming detached from the substrate. Finally, during final cooking by the consumer, the batter must continue to remain attached to the substrate and must not have any holes or cracks in its surface. Factors affecting batter adhesion to food products include the properties of the food used (Suderman & Cunningham, 1980), cooking methods (Baker et al., 1972; Hale & Goodwin, 1968), and batter ingredients (Baker et al., 1972; Hale & Goodwin, 1968; Hanson & Fletcher, 1963).

Adhesion is another critical characteristic of batter-coated foods which has been associated with gums. Similarly to their use as barriers against fat absorption, the effectiveness of gums in improving adhesion has been linked mainly to their gel-forming properties. The gels or films they form provide strength and integrity, which have a positive effect on adhesion, preventing “blow-off” and “pillowing.”. In the particular case of MC and HPMC, cohesion is conferred to the raw dough in a primary state; structural integrity is then conferred by the activation of thermal gelation during frying. Gelling of gellan gum, pectin, carrageenan, and alginates in the presence of ionic salts has also been associated with improved adhesion (Kuntz, 1997b). The method of adding gellan and pectin to a batter-coated food is the same as described above for their use as oil absorption reduction agents. For alginates, a possible method of application is that described by Earle and McKee (1985); the food substrate is first coated with an algin solution and is then dusted with a dry mixture which contains Ca^{++}. Subsequently, once the algin gel has formed, the food is dipped into the tempura batter and prefried.

Although it does not possess gel forming properties, CMC has also been significantly correlated to improvements in the adhesion of a commercial breading mix to poultry skin, as measured by a mechanical method. Other nongelling gums tested in the study (guar, tragacanth and xanthan) did not significantly affect adhesion. In no case were significant differences in adhesion found when the concentration of the gums was increased (Suderman, Wiker, & Cunningham, 1981). In another study that evaluated the relationship between the rheological properties of batters and the breading adhesion properties of different nongelling hydrocolloids (xanthan, guar and CMC), a high correlation between apparent viscosity and breading adhesion (measured as overall yield and cooking yield) was found. Xanthan gum was the only one that significantly improved adhesion.

In this case adhesion was found to increase with hydrocolloid concentration (Hsia et al., 1992).

The use of gums to improve adhesion, however, is not without controversy. For example, the addition of MC to a rice flour-based batter provided good adhesion immediately after frying but was not suitable for frozen storage (Mukprasirt et al., 2001). Langan (1988) found that in the presence of adhesion starches (e.g., hypochlorite-treated dent corn starch; cross-linked and pregelatinized waxy corn starch), some gums may actually inhibit adhesive strength. Similarly, the addition of MC alone at 0.3% has been found to not form a film that adhered to chicken skin, as was the case for xanthan gum at 0.2%. In contrast, the combined effect of oxidized cornstarch, xanthan gum, and MC significantly improved the binding force between the rice flour-based batter and the chicken drumsticks. In this study, adhesion was evaluated by texture analysis and laser scanning confocal microscopy (Mukprasirt, Herald, Boyle, & Rausch, 2000). The negative effect on adhesion was explained by excessive moisture binding by the batters, which may cause excessive steam pressure to build up between the batter and the substrate during frying.

Effects of Gums on Freezing and Handling Stability

Freezing stability is another property that has been generally associated with the use of gums in batter-coated products. This property has been mainly attributed to their ability (associated with their hydrophilic nature) to bind the free water generated during freeze–thaw cycles, thereby preventing ice crystal formation and growth, and water migration from the substrate to the coating, which are the main causes of quality loss during frozen storage or freeze/thaw cycles.

There is currently a need for more scientific research in this area, as there continues to be disagreement regarding the use of gums for the purpose of adhesion. For example, lack of adhesion in rice flour-based batters during frozen storage has been explained as being due to their sensitivity to freezing damage; structural analysis has shown that number and size of voids increased within the fried batter due to water retention, which upon slow freezing would lead to ice crystal growth, however the MC-added batter exhibited more damage compared to other rice-flour based formulations (Mukprasirt, Herald, & Flores, 2000).

Effects of Gums on Microwaveability

The microwave oven is a well-established method for cooking or heating food today. However, a problem that remains unsolved is that microwave cooking or heating of traditional batter-coated foods results in undesirably soft and soggy textures, due to the conduction of water from the inside to the outside of the product (Fiszman & Salvador, 2003). One way to solve this problem would be to prevent moisture from migrating from the food substrate to the batter (Meyers, 1990). The use of gums batter-coated foods has been shown to assist in this regard. Pickford

(1993) presented a method of producing a coated fried foodstuff which may be heated by microwave radiation prior to consumption. Pickford's method is based on adding a high-amylose starch and a cellulose gum able to gel upon heating (MC or HPMC) to the predust and the dry batter mix. The exact composition will be formulated taking into account the amount of moisture vapor which will be released into the batter during frying and subsequent microwave reheating, thus ensuring that the batter does not become soggy. In general, as the cellulose gum content of the predust increases, so does the allowable moisture loss through the gel during heating. A further advantage of the formulation is an improvement of batter adhesion and of product stability during freezing and thawing cycles.

Some gums can be gelled by various mechanisms (presence of specific ions, etc.) and the structure that they form can make the coating more resistant and durable, allowing it to withstand handling (Loewe, 1993) and the occurrence of blow-outs (or air pockets), which cause the batter to fall off the food due to the pressure built up between the batter and the product during frying (Ang, Miller, & Blais, 1991).

Effects of Gums on Frying Oil Life

The frying process exposes the oil to conditions that cause its degradation if good hygienic practices are not followed, and can give rise to undesirable constituents that can not only jeopardize the quality and nutritional value of the food, but also pose a potential occupational hazard. The chemical reactions that contribute to these unwanted by-products include triacylglycerol hydrolysis, oxidation, and polymerization. Heating the oil to high temperatures in the presence of air and the degree of unsaturation of the oil are among the major factors that contribute to the progress of these breakdown reactions. The food being fried may also accelerate these reactions, when moisture and other constituents migrate from the food into the oil. A basic tool to assure food and oil quality is oil filtration. Filter materials help maintain oil quality by preventing solid particles from accumulating in the bottom of the fryer. By the other hand use, but not abused, frying oils may be topped up or diluted with fresh oil.

The film and gel forming ability of certain gums has also found an application in extending the useful life of frying oils. By conferring batter integrity barrier properties, they are able to prevent the migration of moisture and other components from the food into the frying medium.

Holownia et al. (2000) and Holownia, Erickson, Chinnan, and Eitenmiller (2001) evaluated the effectiveness of using HPMC as a barrier in marinated chicken strips to extend the useful life of frying oil. It was found that the application of HPMC, either as a film or as a breading mix ingredient, created an effective barrier to the migration of water and acetic acid from the food into the frying oil. Through the resulting reduction in the pro-oxidant action of the acetic acid, the useful life of the frying oil was extended.

The application of a pectin solution around a breaded food and of calcium to the breading has been found to reduce batter/breading detachment and fall-off into the

oil, as well as to maintain batter and breading integrity and adhesion during the prefrying and final frying stages (Gerrish et al., 1997).

The hydrocolloids most commonly used as barriers are MC and HPMC (Ang, 1989; Lee & Han, 1988; Stypula & Buckholz, 1989). Meyers and Conklin (1990) proposed the use of a prehydrated solution of HPMC to ensure its effectiveness. Clearly, one must not lose sight of the fact that oil absorption also depends on factors such as the shape, porosity, composition (especially initial water content), weight to surface ratio, and surface roughness of the product, as well as oil composition, frying time, and oil and food temperature (Pinthus, Weinberg, & Saguy, 1993).

Proteins

The use of proteins in batter formulations also has a long history. Egg derivatives are among the most utilized proteins in these applications. The use of egg white in batters to coat broiler drumsticks was reported to improve coating pick-up, final yield values, and sensory scores, compared to formulations without this ingredient (Baker & Scott-Kline, 1988). Mohamed et al. (1998) observed that adding ovalbumin to a batter increased its crispness and improved its color, and attributed this to the presence of protein amino groups that take part in the Maillard reaction. On the subject of using egg in coating batters, Loewe (1993) considers that albumin helps bond the coating to the substrate while the lecithin in the yolk could act as an emulsifier, improving the stability of the system. Soy concentrate has produced good adhesion, although not as high as albumen or gluten (Baker et al., 1972). The rheology of batters containing dried whole egg has been studied, with the observation that the proteins contributed a more pronounced shear-thinning behavior during flow and a higher elastic component value in oscillatory dynamic measurements of the systems studied (Baixauli, Sanz, Salvador, & Fiszman, 2003).

According to Baker and Scott-Kline (1988), batters with high protein contents produce coatings that may be perceived as more nutritious (some nutrition-conscious consumers feel that high carbohydrate coatings contribute to obesity). Egg albumen batters have a lower caloric content (due to lower solids contents than control samples) than batters based purely on flour. However, a high proportion of egg albumen in batter formulations for coating chicken nuggets can cause gummy texture or color problems, so in order to formulate acceptable coating batters it has proven necessary to make certain process modifications (e.g., to the cooking system). Coating pick-up values, final fried product yield, and sensory scores were found to be slightly better in formulations containing egg white than in those that did not. Mohamed et al. (1998) found that egg yolk increased the hardness of the coating as well as oil absorption, probably because the proteins largely take the form of lipoproteins and phosphoproteins, which can reduce the surface tension between water and oil. Other proteins, such as powdered milk or whey solids, also provide structure and contribute lactose, a reducing sugar that takes part in nonenzymatic browning reactions. Bhardwaj (1990) proposed a batter with a high concentration of a pulse (Moong Dal, a

leguminose seed) of a particular particle size in order to obtain fried battered products of high quality with a crispy, chewy crust.

Soluble proteins extracted from animal or fish muscle by a concentration and ultrafiltering process were used to obtain products with high moisture and protein contents that blocked the absorption of fats; these liquid proteins were added to the batter or applied as a coating by waterfall or spraying over the substrate prior to frying, or by dipping the product after it had been breaded, forming a barrier that avoided water evaporation during final frying (Pszczola, 2005). Fully cooked, breaded chicken patties treated with NutraPure® (proteins extracted from pollock fish) applied either as a batter additive or as a coating have been observed to contain 23– 48% less fat than a similar commercial product; their flavor was rated clean by a sensory panel, the substrate food was found to be moist, the final product underwent little shrinkage and held up well under lamps, and the crumb had increased crunch and bronze color (Kelleher, 2005).

Other developments have used soy protein isolate, egg white or whey protein isolate applied as a postbreading dip. In formed chicken patties that were battered/breaded and then dipped in these protein solutions (10%), the best results were obtained with egg white proteins, which reduced oil pick-up by 34% and caused an 11% increase in moisture retention (Brannan & Teyke, 2006).

Dextrins

Dextrins are usually obtained by hydrolysis of starch with enzymes, followed by anhydrous heating, which results in repolymerization. They may also be obtained from starch by controlled hydrolysis with acids (Chap. 2). Compared with the original starch, dextrins produce less viscous solutions and are water-soluble.

The use of dextrins in batter formulations is associated with crispness and snap retention and improvement in the fried product (Shinsato et al., 1999), with tenderness and browning, and with providing cohesion to the batter system. The dextrins used generally have medium to high viscosity and aid in the formation of a continuous, uniform batter. In addition to increasing the crispness of the fried product, they help maintain crispness under infrared heating lamps. Battered squid rings prepared with a dextrin-containing batter remained crisper for a longer period of time after frying (Baixauli et al., 2003).

Fiber and Fiber Sources

Powdered cellulose with specific fiber lengths (>100 mm) has also been described as a batter ingredient that reduces oil absorption and increases moisture retention in the final product after frying (Ang et al., 1991). As the result of a strong interaction due to hydrogen bonding between water molecules and the cellulose fibers in the

batter, the displacement of water by oil during drying is restricted. The nonenzymatic browning properties of powdered cellulose can also be advantageous for controlling the development of color (Ang, 1993). Other dietary fibers, such as oat, soy, pea, or sugar beet fibers, can be used for the same purpose. These substances develop greater mechanical resistance than conventional batters, making it easier for the coagulated product to stay intact during handling, and they improve product appearance by giving the coating an even, golden color after frying (Ang, 1991).

Microcrystalline cellulose codried with whey demonstrated a similar functionality in tempura-battered food items (Anonymous, 1980). Fibers have been used to promote adhesion of batter to the substrate. Polydextrose, considered a soluble fiber, has also been shown, alone or in combination with other fibers, soy protein, or a cellulose derivative, to reduce absorption of oil in a batter formulation (Kilibwa, 1999).

Other Ingredients

A number of other minor ingredients can be added to batter mixes.

Seasonings

The current market for coated products is being driven by convenience, and diversity of seasoning adds value and influences the growth of this product sector. Herbs and spices are widely used in predusting systems, as are oleoresins and extracts. The predusting stage is a good point at which to add flavorings, as they remain reasonably protected from the cooking process and do not penetrate into the substrate, as in a marinade. Adding flavorings to the batter is another possibility, but it must be remembered that insoluble ingredients can give the batter a gritty texture and can cause blockages in pump tubes, volatile oils can easily escape during the frying step, and although they add sweetness, sugars can also dramatically affect the external color during frying. If the product is to be breaded, the addition of delicate and volatile components should be avoided and it should be remembered that the presence of sugars can cause spotting upon frying. Breadings frequently contain white, red, and black peppers. Cayenne pepper is popular in many Cajun dishes. A good option is to use vegetable powders like onion or garlic, due to their low volatility and neutral color. The color that can be imparted by some oleoresins should also be taken into account, if they give the batter a green color, for instance, the product will not be attractive. On the other hand, flavor is developed during frying even without flavor addition, particularly via Maillard and browning reactions. Flavors transfer from the product into the cooking oil and then back to the product again. Because of this, and also because frying is a great contributor to flavor loss, strict oil management procedures must be used if on e is to maintain a certain load of flavor profiles (Calver, 2001).

In such a wide realm as seasonings and flavor systems, it will be deduced that while some general remarks can be made, each case must be studied individually. The current trend is diversity, adding visual appeal and unique flavors (cereal crumbs, tortilla crumbs, potato shreds, bean thread noodles, etc.). Golden brown has always has been the benchmark color for fried foods; but now consumers are demanding more variety, so designers can also add caramel colors to enhance brown notes, paprika to enhance redness, or annatto to contribute yellow or orange notes (Gerdes, 2001).

Antioxidants are another category of ingredients that could have interesting technical applications. Biswas, Keshri, and Bisht (2003) used a 50:50 mixture of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in a batter mix for coating pork patties and evaluated product quality after long refrigerated and frozen storage times. Instrumental quality attributes and sensory scores of the final battered, fried products were higher for the treated simples than for the controls throughout storage.

Raw Batter Quality Factors

Control of Raw Batter Viscosity

The consistency of a batter is a key quality attribute of coatings, in that it is a critical determinant of their performance during frying (Shih & Daigle, 1999). Viscosity affects the thickness and quality of the adhering batter layer, the handling properties of the battered product, and its appearance and final texture. For decades, the apparent viscosity of reconstituted doughs has been measured at a single shear rate (Cunningham & Tiede, 1981; Mohamed et al., 1998; Olewnik & Kulp, 1993; Prakash & Rajalakshmi, 1999). However, these doughs generally present thixotropy, shear-thinning behavior, and time-dependency, so a rheological characterization of their flow behavior over a wide range of shear stresses, temperatures, and time gives more complete information. This makes it possible to predict their behavior during the processes of mixing, pumping, and coating, with a view to keeping batter properties, pick-up, process tolerance and adhesion uniform, as well as to discover the possible variations that any change in composition or process might introduce (Balasubramaniam et al., 1997; Hsia et al., 1992; Mukprasirt, Herald, & Flores, 2000). For example, the incorporation of proteins or gums makes flow characterization more complex. The technique of dynamic rheology at very low strains, introduced into this field of food technology research a relatively short time ago, is a very useful tool because it provides valuable information that may be related to the structure that these batter systems develop. By applying low degrees of strain, indicating the degree of solidness and structuring that have developed, these techniques are able to show the relationship between the viscous and elastic components, and how they change with composition. Structural changes in batters

at increasing temperatures, corresponding to the transition from a paste to a gelled state, and the effects of adding proteins or dextrins, can be clearly followed by studying their mechanical spectra at different temperatures (65°C and 75°C) (Baixauli et al., 2003). The influence of several ingredients (wheat starch, corn starch, dextrin, gluten or dried egg) on the thermo-rheological behavior of batters containing methylcellulose has been studied by dynamic methods (Sanz et al., 2005). However, these methods for monitoring batter quality are not yet in general use, although their application is becoming more widespread.

The most important factors that affect the rheological properties of batters are the properties and levels of the ingredients, the solids to water ratio, and temperature. Also, as in any fluid system, rheological properties depend on factors such as shear rate, duration of shearing, and previous thermal and shear histories (Steffe, 1996). A widely used model to characterize raw batter flow is that of Ostwald–deWaele:

$$\eta_{ap} = k\gamma^{n-1}$$

where η_{ap} = apparent viscosity
k = consistency index
γ = shear rate
n = flow index, which varies between 0 and 1 and indicates when the system deviates from Newtonian behavior (n = 1 corresponds to a Newtonian liquid, i.e., one where apparent viscosity does not vary with shear rate)

In general, batters exhibit shear-thinning behavior, i.e., an increase in shear rate lowers the viscosity. Baixauli et al. (2003) and Salvador, Sanz, and Fiszman (2003) found that an increase in temperature resulted in lower consistency index values in several tempura batter formulations containing different ingredients. The effect of soy flour (5%) and rice flour (5%) on the rheological behavior of chicken nugget batter formulations was studied by Dogan et al. (2005). Soy flour provided the highest apparent viscosity, measured in a parallel plate rotational viscometer and modeled as a power-law fluid. These investigators also evaluated the time dependency of the batters by determining the change in apparent viscosity under constant shear rate over a fixed time. In order to achieve optimum consistency and water distribution, another key factor that should be controlled is the solids to water ratio. In deep-frying batters, the volume fraction of water is very critical in terms of oil absorption, since oil uptake increases linearly with percentage moisture removed (Shukla, 1993).

Degree of Raw Batter Adhesion

The importance of viscosity in raw batter coatings has already been mentioned. In principle, the better the raw batter adheres to the substrate food, the lower the losses from breakage or partial detachment of the batter during the subsequent steps

(freezing, packing, transport, etc.). From this point of view, it is important for substrate proteins to be available for setting up protein/starch interactions. The availability of substrate proteins can be increased by extraction through mechanical treatments such as mincing, tumbling, etc.

On the other hand, it is important that pieces of food be prevented from sticking together and forming what are known as "marriages," "twins," "doubles," or "clusters"; for this, as well as for avoiding excessive stickiness, it is very important to use mechanical handling methods that do not allow the batter-coated pieces to touch or overlap prior to freezing or final cooking. The thickness of the adhered layer is also important financially, as it is directly related to yield and to sensory acceptance by consumers.

Various indices are commonly used to express the degree of batter adhesion. Pick-up is defined as the percentage of raw batter that adheres to an uncoated piece of food:

$$\% \text{ Coating pick-up} = (C–I) \times 100/I$$

where C = mass of coated food item after dipping in raw batter

I = initial mass of raw uncoated food item

This pick-up rate expresses the quantity of raw batter that adheres to a given weight of an uncoated food.

Pick-up can also be expressed as the quantity of raw batter that adheres to a given weight of an already coated food:

$$\% \text{ Coating pick-up} = (C–I) \times 100/C+I$$

Moreover, partial batter losses during manufacture, frozen storage, or handling by the consumer cause financial losses and create a poor general impression of the product. A layer that is too thick can lead to an incompletely cooked final product, lack of crispness, and a generally hard, lumpy appearance.

The surface and structural characteristics, moisture content, and shape and size of the substrate food are a further set of factors that are not without importance. For instance, a layer of ice on the food to be coated reduces the degree of adhesion (Kuntz, 1997b). Rounded substrate shapes favor uniform coating. Other factors that also have an effect are the sequence of steps, degree and type of mechanization used in the coating procedures, thermal treatment of the food pieces and their external temperature at the moment of coating them, temperature of the batter, and type of predusting, if any. In general these aspects have not received much attention as subjects of study nor have they been described in the literature. Meiron, Marmur, and Saguy (2004) developed an experimental system that allows the quantification of the effect of surface roughness on mechanical adhesion. They used a model system simulating rough hydrophobic food surfaces and coatings to develop a theoretical basis for studying related phenomena such as breading and battering.

During frying, a batter can suffer blow-outs as a result of the pressure generated between the batter and the substrate food, causing the batter to fall off. In this case,

increasing the mechanical resistance of the exterior layer by adding fiber, for example, would improve the appearance of the final fried product (Ang, 1991). Appearance can also be detrimentally affected by the product's surface looking either too smooth (in which case some way of enhancing the effect of the leavening should be considered) or too rough. Sometimes too many bubbles can appear on the surface, giving the product a blistered or flaky appearance. All these appearance properties can be controlled by adjusting the ingredients and water content of the batter, as well as the temperature of the frying oil.

Control of Final Texture of Battered, Fried Products

The most highly appreciated texture of a fried product is crispness. Chewy toughness and mushy softness are equally undesirable sensations caused by lack of crispness. Ideally, the coating should exhibit a structure that offers some resistance to the initial bite but then quickly melts away in the mouth (Loewe, 1993). The crisp final texture of the fried product can be evaluated by instrumental or sensory techniques. Parameters such as crispness or crunchiness, fragility, tenderness, etc., however, are hard to quantify using empirical mechanical methods because what is perceived in the mouth is a complex combination of sensations.

Measurement of puncture by a plunger is the technique most often used (Fan, Singh, & Pinthus, 1997; Mohamed et al., 1998). Baixauli et al. (2003) performed penetration measurements of the final crust removed from the food, and recorded the maximum force on penetration by a 4 mm diameter cylindrical probe. The test was performed on recently fried foods 15 and 30 min after frying to ascertain the retention of crispness over time, a factor of some interest when foods are kept warm by infrared lamps. In this study, a crisp texture was identified as a penetration curve with a multipeak profile. Lee et al. (2004) measured crispness as the number of peaks in a texturometer curve obtained for a deep-fried batter.

The Kramer shear press has been used to measure the instrumental texture of the fried crust of battered onion rings up to breaking point. Specific shear force (maximum force/mass) and toughness [work (area under the loading portion of the curve)/mass] were calculated (Ling, Gennadios, Hanna, & Cuppet, 1998).

It is hard to imagine that a single instrumental parameter could be capable of discriminating between differences which are sometimes very subtle. Consequently, Salvador, Sanz, and Fiszman (2002) proposed evaluating the complete penetration curve of a fried coating in order to appreciate various aspects of texture. To compare different methods of evaluating fried crusts, Lima and Singh (2001) found that the results obtained with a puncture test (1.07 mm diameter rigid flat probe) and a three-point bending cell in a restructured potato model system were sensitive to both frying time and frying temperature. It should be pointed out that in this case different frying times (5, 10 and 15 min) and temperatures (170°C, 180°C and 190°CC) were used, so the differences between the products would be considerable.

Other approaches to measuring the crispness of foods include ultrasound or sound measurements, alone or in combination with mechanical determinations. However, studies related to crispness in high-moisture breaded or battered foods have been very limited. Antonova, Mallikarjunan, and Duncan (2003) investigated the relationship between ultrasonic and mechanical parameters for chicken nuggets and sensory crispness results obtained with a trained panel. They found significant differences in ultrasonic velocity, transmission loss, peak force, and total energy in the breaded chicken nuggets cooked by different methods, and concluded that sensory crispness could be reasonably well predicted by ultrasonic velocity. An attempt to correlate mechanical and acoustic measurements with the moisture content of the different layers of breaded fried shrimps has been described by Tahnpoonsuk and Hung (1998). These investigators used a modified Warner-Bratzler blade to cut through breaded fried shrimps (after holding under heat lamps for different lengths of time) while simultaneously recording the sound with a microphone. The sound was then analyzed by Fast Fourier Transformation; however, no clear results were obtained since the authors found that the crispness loss in this multilayer food did not correlate with the changes in mechanical properties. This was mainly due to the occurrence of a middle layer between the outer and inner crust layers, which developed after 30 min of holding and with an intermediate moisture level.

Since there is a causal connection between structure and functionality, knowledge of a food's microstructure can be very useful for understanding, and hence controlling, its physical properties. From a structural viewpoint, battered foods are very complex systems, as they tend to contain a wide range of different kinds of components, with both the coating and the food substrate undergoing substantial changes as a result of frying. Very few studies have analyzed the microstructure of these food types. Llorca, Hernando, Pérez-Munuera, Fiszman, and Lluch (2001) evaluated the effects of frying on the microstructure of battered squid rings and observed that in the prefried products the batter and the squid muscle were interconnected, whereas in the final fried product the two layers were separated. The same team also used electron microscope techniques to investigate how formulation ingredients (corn flour, salt, leavening, etc.) affected fat absorption (Llorca et al., 2003). Results showed that a leavening agent significantly increased fat content (due to the generation of gas cells that lodge oil), salt also increased the fat content, but replacement of wheat flour by corn flour caused fat content to decrease. The batter–substrate interaction can also be investigated by electron microscopy, which has shown the adherent structures that form between the two, depending on the ingredients used (Mukprasirt, Herald, Boyle, & Rausch, 2000). In a study of breaded, fried chicken breasts, scanning electronic microscopy (SEM) demonstrated greater merging between the breadcrumbs and the batter with decreasing breadcrumb particle size (Maskat & Kerr, 2004a). The field of microscopic observation is a point that requires greater attention from researchers of batter or bread-coated products. Factors such as degree of adhesion and types of batter/substrate bond could be analyzed in greater depth.

Sensory methods are very valuable for assessing various attributes of batter or bread-coated products. Attributes such as greasiness, juiciness, oiliness, and

mealiness of coatings have been assessed with trained panelists (Prakash & Rajalakshimi, 1999). A specially trained sensory panel to evaluate sensory crispness was able to determine significant differences in crispness intensity among breaded fried chicken samples cooked by different methods and stored under different conditions (Antonova, Mallikarjunan, & Duncan, 2004). In some products attention should be given to moistness, since the product progressively becomes drier over time, resulting in poor acceptability. In one study, different moisture-releasing ingredients (milk and vegetable ingredient combinations, several moisture levels) in mackerel mince-based nuggets were evaluated by a sensory panel for firmness, moistness, and overall desirability (Lee, Joaquin, & Lee, 2006). A consumer study evaluated the acceptability of fried batter-coated squid rings prepared by an innovative method that reduces their fat content, and examined how the provision of favorable nutritional information (nil industrial fat content and lower absorption of frying oil) or a familiarization period (home consumption) influences the sensory acceptability of fried batter-coated squid rings (Salvador, Hough, & Fiszman, 2005). Specifically, the exterior batter layer attributes evaluated were appearance, crispness, and coating thickness. Another study compared the characteristics of nuggets made from emulsion or restructured buffalo meat. Results showed that despite higher overall acceptance for freshly prepared emulsion nuggets, panelists rated them considerably lower compared to restructured nuggets after subsequent storage (Thomas, Anjaneyulu, & Kondaiah, 2006).

Control of Coating Adhesion in Final Product

As previously discussed, coating adhesion is one of the main quality factors for coated products. The batter or coating pick-up of the final product is defined as (Baixauli et al., 2003):

$$\% \text{ Batter pick-up} = (B/B + S) \times 100$$

where B = mass of batter coating of food item after final frying
S = mass of food item excluding batter ("peeled") after final frying

The final product batter or coating pick-up index expresses the proportion of the final weight of the fried product comprised by the coating. It should be remembered that these weights obviously include the weight of oil absorbed and that both the food and the external crust have lost moisture during the frying process. The same index can be calculated after prefrying, where it provides a more faithful picture of the yield of the coating process as it indicates how much of the weight of the product, batter-coated and ready for final frying (as it reaches the end user), is made up of the external crust. This index is of great practical value to manufacturers.

In breaded products there is a natural tendency for the bread crumbs or particles to fall off the coated product. The % cooked yield and % overall yield (Hsia et al., 1992) have been defined as

$$\% \text{ Cooked yield} = CM \times 100/S$$
$$\% \text{ Cooked yield} = S \times 100/I$$

where CM = mass of cooked breaded food item
S = mass of cooked breaded item after shaking (normalized sieve and process)
I = initial mass of raw unbreaded food item

In this case, the % overall yield takes into account possible losses in the adhered breading layer due to postcook handling. Many studies on coated products have calculated how the addition of certain products affects some of these indices. Higher fat content has been suggested as a possible cause of higher coating loss during shaking in fried, battered, and breaded chicken breasts prepared with different levels of surfactant (Tween 80) and batter mix to solvent ratios (Maskat & Kerr, 2004b).

In tempura-type batters, a thin, not very viscous batter produces a weak, porous coating that is capable of absorbing large quantities of oil. It is a well-studied fact that there is a strong relationship between oil uptake and water removal (Gamble, Rice, & Selman, 1987), so a layer that is too thin is difficult to handle and has a poor barrier effect, which means that it is not very good at retaining food juices during frying.

Control of Color: Raw and Final Product

Depending on the market, coated products are sold with the final appearance of a "raw" food or with golden tones that simulate the color of fully fried foods. The former are usually sold with instructions for a final frying step, during which they acquire their final golden color (although their color will already range from yellowish to orangey), whereas the latter may allow the final stage to take place in a conventional or microwave oven as well as by the traditional final frying. Prefrying is the stage of the manufacturing process that gives the food its initial slightly golden color; for this, the coating must possess certain characteristics. Products covered with tempura-type batters acquire a yellowish color when yellow corn flours or starches are used (Burge, 1990) and a golden-brown note when potato starch is used. Color may also be controlled by adjusting the balance between the types of flour, starches, and other ingredients that tend to take part in browning reactions (reducing sugars in milk solids, for instance) (Suderman, 1983). In these products, it is normal to use colorings to obtain yellow or orange tones, which are more attractive than the pale white of a raw batter. Baixauli, Salvador, Fiszman, and Calvo (2002) reported that the final color of battered squid rings depended significantly on frying times and temperatures. These investigators also observed that, although the color of the frying oil darkened to a certain extent as the number of fryings increased, this factor did not affect the color of the final fried product, contrary to the belief that dark oil is detrimental. Most of the color present in an oil comes from the food and not from oil breakdown products; therefore, the poor

cooking performance of an older oil is not directly related to the fact that it is dark (Friedman, 1991). Color evenness is also an important factor; therefore, it is important that spices or small lumps not be allowed to darken during frying and impart a "spotted" final color; this could be prevented by adjusting frying temperature.

Breadings

Breading, as currently understood, can be defined as a covering made of bread crumbs that are applied in dry form to a previously moistened or batter-coated food. While batters may be similar to glue, breadings are compared to the icing on the cake (Gerdes, 2001). This covering uses particles of different sizes to form crusts with particular textures; normally, the larger the particle size the crunchier the bite and the higher the pick-up rate. The product's surface appearance depends on breading granulation or the size of the crumb pieces; greater than 4.0 mm in diameter for coarse breadings, between 1.4 and 4.0 mm for medium ones and below 1.4 mm for fine breadings. The bread crumb shapes can create a coating that is both attractive and economical. The current industry trend is towards more textural variety.

The raw material breadings is generally regular bread, both fresh and that which is returned by shops after the sell-by date. Another option is to bake breads with certain properties that the manufacturers require (e.g., spices, higher shortening content, cheese, flavor enhancers, etc.). The loaves go through a tempering stage that makes them firm and hard so that they can handle the machines without sticking. This is done by leaving them unwrapped for a couple of days at ambient refrigeration temperatures, or by blowing dehumidified air over the loaves as they circulate on conveyor belts. The following step is to cut up the loaves, normally into cubes, and dry them in an oven (conventional or otherwise) to a moisture content of 3–6% (Jackel, 1993).

Particle size is important for the preparation process because fine breading has a high area to volume ratio and absorbs moisture very fast, so it becomes embedded more easily in the matrix, which then needs to be dried before further handling. At the other extreme, very coarse particles provide weight and are visually very attractive but have a small area to volume ratio, so their moisture absorption rate is low and they also require longer drying times. Porosity is another factor that needs to be taken into account. A breading with a more open and porous structure absorbs moisture more rapidly and exchanges it more rapidly for oil during the frying process; however, excess frying oil also drains out faster during the postfrying draining stage. Bouchon and Pyle (2005) established that the largest proportion of oil which ends up in a deep-fried food is sucked into the porous crust region after the product is removed from the oil bath, highlighting the relevance of both the characteristics of the crust and the time interval. Evidently, a balance between all these stages needs to be struck by adapting the ingredients to the type of end product required.

As regards the shape of the substrate (where this can be modified), a high area to volume ratio is preferable to achieve good coverage without negative effects on

appearance. Breaded products are highly dependent on the size and shape of the particles and on their tolerance to frying; a mixture of particles of different sizes is often the best choice.

Many particle sizes and shapes have been tested in recent years and different breading styles are beginning to appear, such as "home-made," "country," etc., with large particle sizes. Characteristics of coatings formed from breadings of three particle sizes were investigated in breaded, fried chicken breast by Maskat and Kerr (2002). They found that smaller breading produced smoother and more uniform coatings, but acoustic measurements taken during compression of coatings did not show any significant differences due to different particle sizes. In another study (Maskat & Kerr, 2004a), the same researchers found that coating adhesion was highest in a coating formed from smaller particles that also had a higher moisture content in the surface of the meat as well as in the coatings; no significant differences were found in coating pick-up, cooking loss or yield between samples with different breadcrumb particle sizes.

A recent development is the use of fresh Japanese-style bread crumbs or "nama panko," which give a special texture and appearance. The bread is made by passing an electric current through the dough, so it does not develop a crust. The breadcrumb product has much higher spike levels (sliver-like texture) than those of a standard dry crumb.

Some alternative breading materials to bread or other wheat-flour products have been described. For example, precooked rice dried to moisture content of 11–12% before milling (1 mm particle size) was used on chicken breast. The resulting product had better sensory acceptability and lower fat absorption than that made with a commercial breader, in addition to other good performance features (Gastélum-Benavides, Félix-Gocobachi, Fimbres-López, Vargas-Robles, & Cinco-Moroyoqui, 2004).

The use of certain particulates adds interest to coated foods, enhancing some finished product attributes. They act synergistically with ethnic flavors, as in the case of sesame seeds in oriental foods, contribute to texture (e.g., shredded coconut), or add color (e.g., visible green parsley particles).

Alternative Cooking and Modified Frying Methods

Microwave

In food consumption, the increasing trend towards preparation in less time has given rise to a great demand for frozen products that are ready to heat and eat. The microwave oven is now a well-established method for cooking or heating food, primarily because of its quickness. However, prefried products cooked in microwave ovens tend to be undesirably soft and soggy, because in this type of heating water is conducted from the inside to the outside. There is a need for more research

focused specifically on developing batter mix formulations that develop crispness during microwave cooking or heating. Several patents have made advances in this direction. Lenchin and Bell (1985) developed a dry mix containing 60–70% high-amylose corn flour, which provides generally acceptable prefried foodstuffs that have crispy coatings after microwave cooking. Pickford (1993) presented a method of producing a coated fried foodstuff which may be heated by microwave radiation prior to consumption; in addition to a high-amylose starch, the predust and the batter dry mix contain a cellulose derivative that gels when heated; the product was prepared following a standard manufacturing process (prefrying in oil at 180–200°C for approx. 30 s and subsequent freezing, or direct frying and freezing).

Cooking Method Modifications

New possibilities and improvements in conventional cooking methods have been researched recently. One is to use nitrogen instead of the vapor generated by the product to create the necessary pressure in the fryer; this avoids the limitation of working with a large food load (necessary to create the proper pressure). The increase in pressure applied during frying in a restaurant-type fryer resulted in more tender and juicier fried product, due to a reduction in both moisture loss and oil uptake by the product, and in an extension of oil (Innawong and Mallikarjunan, 2002). Alexander and Alexander (2000) presented a method for preparing a frozen, battered food product which, in order to shorten the final cooking process, involves steam and heat-cooking and chilling the substrate prior to the battering, prefrying, and freezing steps. The effects of changes in formulation or in ingredient proportions when utilizing these innovative cooking methods need to be studied.

Future Research Needs

Research on batters is tending towards the creation of highly complex systems that incorporate combinations of increasingly sophisticated ingredients, developed for their multifunctionality. The incorporation of dietary fibers that absorb less fat, starches that develop crisp textures and are less susceptible to human digestion, celluloses that eliminate the prefrying step and also absorb less fat during final frying, edible films and coatings that preserve frying oil quality by creating a barrier against migration of food marinades or juices, or minor ingredients with antioxidant properties that extend the shelf life of the final product are already a reality.

The dramatic increase in the development of new ingredients brings with it a need for research to clarify their mechanisms of action and the interactions between their functionalities. Improved methods for the control of raw batters need to be applied, such as the current dynamic rheology techniques used to characterize complex rheological behavior, associated in many cases with the development of

three-dimensional structures that condition the entire performance of the coating. In addition, quality control of final products involves the development of trained sensory panels to determine their most important attributes and how they are impacted by changes in ingredients and cooking methods, as well as their shelf life. Nowadays, the use of a combination of several microscopic techniques and the application of modern digital image acquisition and analysis techniques are essential tools for studying the structures that are developed.

On the other hand, no satisfactory answer has yet been found to such a simple question as "what is the best wheat flour for a wheat flour-based batter?" This is an indication of the need for research in an area that has been based on empiricism for many decades.

More research needs to focus on frozen, coagulated fat-free or low fat products, microwaveable products or products that can be cooked in conventional ovens, ways of improving crispness and juiciness and of maintaining these characteristics over long storage times, and batter adhesion and performance during frozen storage including the effect of gum use.

With regard to the new trends, consumer studies based on well-planned surveys assist in centering the market preferences. Nowadays, the concern about health embraces a number of driving issues, needs, and opportunities which may be approached by designing specific products based in different food raw materials. These tailor-made products provide physiological benefits that could be targeted at particular consumer groups.

The ultimate goal that should inspire research in the field of coated products today is a greater choice of meat products that add value and are safe for consumers.

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Chapter 13
Antioxidants

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Introduction

Spoilage prevention and shelf-life extension of products will always remain an important goal to the meat industry. While the greatest emphasis is placed on the prevention of microbial spoilage, chemical deterioration, specifically oxidative spoilage, is an important consideration for fresh meats and manufactured meat products as well; hence the inclusion of a chapter on antioxidants in this book.

Antioxidants are among the food ingredients most easily recognized by the public as ingredients with a specific function in foods, largely due to the considerable news coverage of the purported health benefits of antioxidants, rather than to their functionality in foods. However, while magazines are filled with articles about natural antioxidants found in fruits and vegetables, considerably less information is available on antioxidants that are either naturally present in (endogenous) or added to meats and meat products.

Many meat preservation methods, including the addition of antioxidants, have long-standing historical roots. For example, while smoking is first and foremost considered a preservation method against microbial spoilage, smoke also contains antioxidants in the form of phenolics (Kjallstrand & Petersson, 2001), although their importance in regard to delaying oxidation in smoked meats has not yet been fully investigated. The reader is referred to Chap. 10 for a detailed discussion of smoke as a functional meat ingredient.

Oxidative deterioration can take place before and after cooking. Lipid oxidation in meats prior to cooking is well understood and essentially follows the established lipid autoxidation scheme, which affects the flavor and color of meat products (McMillin, 1996). Lipid oxidation in meats after cooking and during subsequent refrigerated storage, which is commonly termed "warmed-over flavor" (WOF), has been thoroughly investigated and is quite well understood (Vercellotti, 1988). Because small but significant increases in markers of oxidative deterioration have been observed in uncooked meats, which is indicative of flavor deterioration prior to cooking, the concept of meat flavor deterioration is sometimes being used as a more inclusive term than WOF because it considers the increase in nondesirable oxidative compounds in meats before and after cooking (Spanier, Edwards, &

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Dupuy, 1988). In practice, it is difficult to distinguish between pre- and postcooking oxidation because the oxidation products are virtually identical and the various approaches to dealing with oxidation prior to cooking are often effective against WOF development or meat flavor deterioration as well.

When discussing antioxidants it is important to distinguish between extrinsic or exogenous antioxidants and intrinsic or endogenous antioxidants, as well as antioxidants that are formed during cooking. While numerous effective antioxidant systems are already known, the increased demand for convenience foods and the evolving markets for precooked meats call for more options to prevent lipid oxidation in meat and meat products before and after cooking.

Endogenous Antioxidants in Meats and Meat Products

Because this book is focused on the use of functional non-meat ingredients, this section is kept intentionally short and readers are referred to the excellent review by Decker and Mei (1996) for additional details on antioxidant systems endogenous to meats. Well-known endogenous systems in fresh meats include tocopherols, carnosine, lipoic acid, and various enzymatic systems (Decker & Mei, 1996). In addition, numerous Maillard products formed during cooking have also been shown to have antioxidant activities (Bailey, 1988). However, none of these systems, individually or combined, have been shown to sufficiently delay oxidation in meat products under most commonly used processing conditions (Decker & Mei, 1996). Therefore, strategies to reduce oxidation almost always include addition of exogenous antioxidants.

Exogenous Antioxidants Added to Meats and Meat Products

When discussing exogenous or extrinsic (i.e. added) antioxidants, it is possible to categorize such antioxidants in several different ways. The most obvious way of distinguishing these antioxidants is based on their source, i.e. whether they are synthetic or derived from a natural source. However, another approach of categorizing the antioxidants is by their legal status, i.e. whether they have received U.S. Department of Agriculture (USDA) or Food and Drug Administration (FDA) approval for use in meats and meat products. It is important to note, however, that unless FDA's legal notification specifically includes meat and meat products, the "substances listed approved in FDA regulations for use in food generally (21 CFR, Parts 172–180) or listed as Generally Recognized As Safe (GRAS) for use in food (21 CFR, Parts 182 and 184) are not automatically acceptable as safe and suitable for use in meat and poultry products" (Derfler, 2002). However, a letter by the FDA to USDA Food Safety and Inspection Service (FSIS) stating that meat and meat products were considered

during the safety assessment could suffice to receive permission of using such additives in meats and meat products, unless FSIS considers that specific rulemaking is required (Derfler, 2002). An example of such a situation might be the GRAS assessment of grape seed extract (Tarantino, 2003).

One very common meat ingredient, nitrite, will not be discussed in this chapter. While it is well known that nitrites in cured meats are highly effective antioxidants (Younathan, 1985), a separate chapter (Chap. 1) discusses its use in meat products. In addition, because curing imparts flavor characteristics that are not desirable for all meat products, other antioxidants are commonly used in meats.

Synthetic Antioxidants

Although powerful synthetic antioxidants such as BHA (butylated hydroxyl anisole) and BHT (butylated hydroxyl toluene) have been permitted for use in selected meat products for a fairly long time (Code of Federal Regulations [CFR], 2007), consumer concern has become a driving force for considering the use of natural antioxidants. Besides BHA and BHT, other synthetic antioxidants permitted for use in certain meat products include TBHQ (tertiary butylhydroquinone) and tocopherols (vitamin E). Vitamin E is unique because it is often treated as a natural additive, even though the tocopherol used in most studies is not derived from a natural source.

According to USDA's *Food Standards and Labeling Policy Book* (USDA-FSIS, 2005), antioxidants such as BHA and BHT can be used in cooked fresh sausages, also called "Brown N Serve" sausages. More specifically, BHA and BHT can be used at up to 0.02% of essential oil content in spice mixtures, as well as in bacon (0.01% of fat content if used individually or 0.02% if used in combination), while TBHQ can be used only in bacon in combination with either BHA or BHT. Because their antioxidant activity in meat products is well established, the synthetic antioxidants are also commonly used as comparators or controls in studies investigating the antioxidant activity of spices, herbs, and plant extracts. The efficacy of other food additives that are considered to have antioxidant activity, such as tripolyphosphate, is not as clear-cut. Phosphates were one of the first food ingredients investigated for their potential antioxidant activities in meat products (Tims & Watts 1958). While in one comparative study that included BHA/BHT, tripolyphosphate, rosemary oleoresin, and sodium citrate, tripolyphosphate was found to be the most effective antioxidant during refrigerated storage of precooked roast beef slices (Murphy, Kerry, Buckley, & Gray, 1998), other studies drawing similar comparisons found tripolyphosphate to be the least effective antioxidant (Ahn, Grün, & Fernando, 2002). Considering that concentration levels in both studies were similar, the most likely explanation for the opposing results is the way the antioxidants were incorporated. In the first study, because intact muscle slices were used, brining was the mode of antioxidant delivery, while in the second study the antioxidants were mixed into ground beef.

Natural Antioxidants

The use of natural antioxidant systems to accomplish a reduction in lipid oxidation in meats is not a new concept either. Research published up to the mid-1980s was reviewed by Rhee (1987), who discussed the potential use of vegetable extracts, citrus juice concentrates, and oilseed products for delaying or reducing lipid oxidation in meat products.

The most logical differentiation between studies in this area is the timing of antioxidant addition. Antioxidants may be added to the feed, not for the purpose of preventing autoxidation in the feed, but for the discreet purpose of decreasing autoxidation in the meat of the animal, or during the manufacturing process of the meat product. Adding antioxidants to the feed has the distinct advantage that they can be more evenly distributed in the meat, whereas addition after harvest requires the disruption of the meat matrix, i.e. it is only possible to add them to a manufactured product. As previously mentioned, because vitamin E is a well-studied antioxidant, it is frequently used either as a positive control or in combination with other natural antioxidant systems in both types of studies, whereas the BHA/BHT system is used as a positive control only in studies where antioxidants are added during meat product manufacture.

Feeding trials have revealed that the best time for adding antioxidants, specifically vitamin E, is not after slaughter (postmortem) but during the finishing period of the animals, such as cattle or pigs (Buckley, Morrissey, & Gray, 1995; Faustman et al., 1989; Phillips et al., 2001). For poultry, this concept of dietary antioxidant supplementation has been known for many years (Marusich et al., 1975). While the efficacy of vitamin E as an antioxidant when provided as a feed supplement has been extensively investigated (Liu, Lanari, & Schaefer, 1995), the investigation of the antioxidative effect of spices and spice extracts as feed ingredients has begun only recently (Botsoglou, Govaris, Giannenas, Botsoglou, & Papageorgiou, 2007; Botsoglou, Grigoropoulou, Botsoglou, Govaris, & Papageorgiou, 2003; Florou-Paneri et al., 2005; Govaris, Botsoglou, Papageorgiou, Botsoglou, & Ambrosiadis, 2004). Studying lipid oxidation in turkey breast and thigh muscles using TBARS after feed supplementation with oregano oil, α-tocopherol, and a combination thereof, Botsoglou et al. (2003) found that the antioxidant activities of oregano oil and α-tocopherol are comparable when fed at the same level (200 ppm), but that the combination of the two antioxidant treatments exhibits synergistic effects. A comparison of the effects of dietary versus postmortem tocopherol and oregano oil addition on the oxidative status of cooked turkey breast and thigh patties showed that all antioxidant treatments reduced TBARS values over time compared to the control, but dietary supplementation was more effective than postmortem addition (Govaris et al., 2004). No differences were found between vitamin E and oregano oil supplementation via either diet or postmortem, but thigh patties showed significantly greater oxidation than breast patties, which was explained by thigh meat's higher heme iron and polyunsaturated fatty acid content. An important finding in this study was that dietary oregano oil showed an in vivo tocopherol-sparing effect,

as indicated by higher levels of tocopherol in turkey meat from oregano-fed turkeys, which could not be accounted for by the natural presence of vitamin E in the oregano oil. However, a similar vitamin E sparing effect was observed in meat when the antioxidants, specifically BHA/BHT and rosemary, were added to the minced beef harvested from cattle that were fed vitamin E supplements prior to slaughter (Formanek et al., 2001).

The most widely investigated natural antioxidant systems used during the manufacture of meat products are probably rosemary and various rosemary extracts (Barbut, Josephson, & Maurer, 1995) and it is starting to become a positive control, similar to vitamin E, in many studies. However, many other herbs, spices, and plant extracts have been investigated as well. A review of the general use of spices as antioxidants was published by Madsen and Bertelsen (1995). Specific investigations of spices, herbs, and plant extracts for meat applications include aloe vera, fenugreek, ginseng, mustard, sage (McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001), horseradish (Delaquis, Ward, Holley, Cliff, & Mazza, 1999), oregano (Govaris et al., 2004), potato peel (Mansour & Khalil, 2000), hyssop (Fernández-López et al., 2003), marjoram, basil, thyme, ginger, caraway, clove, peppermint, nutmeg, curry, cinnamon (Abd El-Alim, Lugasi, Hovari, & Dworschak, 1999), honey (Johnston, Sepe, Miano, Brannan, & Alderton, 2005), tea catechins, vitamin C, borage (Mitsumoto, O'Grady, Kerry, & Buckley, 2005; Sánchez-Escalante, Djeanane, Torrescano, Beltrán, & Roncalés, 2003), orange, lemon (Fernández-López, Zhi, Aleson-Carbonell, Pérez-Alvarez, & Kuri, 2005), black pepper (Tipsrisukond, Fernando, & Clarke, 1998), green tea, coffee, grape skin (Nissen, Byrne, Bertelsen, & Skibsted, 2004), grapeseed, and pinebark extracts (Ahn et al., 2002).

The search for excellent antioxidants is not limited to plant extracts, however, but includes animal sources as well. Chitosan has been recognized as an effective antioxidant in meat products (Darmadji & Izumimoto, 1994; Georgantelis, Blekas, Katikou, Ambrosiadis, & Fletouris, 2007). In a recent study by Georgantelis et al. (2007), chitosan by itself at 10 ppm or in combination with rosemary or α-tocopherol was found to be a better antioxidant for delaying lipid oxidation in ground beef burgers than rosemary at 200 ppm or α-tocopherol at 60 ppm. In fact, there was no difference in malonaldehyde content of the burgers between the chitosan and the chitosan with either rosemary or vitamin E treatments, indicating that addition of rosemary or vitamin E did not contribute much to chitosan's antioxidant activity. However, it would be premature to conclude that no synergistic effects exist among the various antioxidant systems. It was already mentioned above that oregano oil and α-tocopherol show synergistic effects, while other studies have shown similar synergism between rosemary extract or tocopherols and ascorbic acid, while ascorbic acid alone often shows a pro-oxidant effect (Mitsumoto et al., 1991; Sánchez-Escalante et al. 2001, 2003). Such synergistic effects can be attributed to the regeneration of one antioxidant system by the other, which is a well-known biochemical effect in the physiology of animals and has been studied in many model systems. It is apparent that the same synergistic effects can be observed in meat applications.

The applicability of herbs and spices in meat products depends, of course, on their sensory compatibilities with meats, as was already noted 20 years ago (Rhee, 1987), and has resulted in research into using deodorized oleoresin extracts of spices and herbs. A distinct advantage of using spice and herb extracts is that they do not fall under the USDA's antioxidant regulation (Hazen, 2005). However, many other natural extracts are affirmed as GRAS by the FDA and are also permitted for use in meat and meat products, such as grape seed extract (Tarantino, 2003).

Numerous studies have been published on the use of natural antioxidant systems in meat systems. This review will highlight selected studies that will illustrate the complexity of the subject.

As mentioned above, rosemary and rosemary extracts have been carefully researched and are the most widely used natural antioxidants in the meat industry. While rosemary has a distinct flavor profile, several deodorized and standardized rosemary extracts are available, which allow the manufacture of a consistent product. For example, in a comparison of a patented natural rosemary extract (Fortium®, Kemin Industries, Des Moines, IA) to a BHA/BHT (100 ppm or 0.01% each) antioxidant system in fresh, frozen, and precooked pork sausages over a 16-week period, the rosemary extract performed better than the BHA/BHT treatment (Sebranek, Sewalt, Robbins, & Houser, 2005). Oxidative changes were evaluated by colorimetry, a sensory panel, and the thiobarbituric acid reactive substances (TBARS) method. The raw-frozen pork sausages containing rosemary extract showed a strong treatment × time interaction. After 28 and 42 days, respectively, the sausages with 2,500 and 1,500 ppm of rosemary extract had significantly lower TBARS values than the BHA/BHT-treated sausages. However, not all the experiments were as clear-cut in their study. In an experiment with fresh-refrigerated sausages containing rosemary extract concentrations at up to 3,000 ppm, all three treatments showed significant delay of oxidation compared to the control. Sausages with rosemary extracts showed only a slight, nonsignificant advantage over the BHA/BHT treatment, and no treatment × time interaction was observed. In studies that use standardized and deodorized rosemary extracts, flavor is usually not negatively affected. In fact, panelists rated the raw-frozen sausage with 2,500 ppm rosemary extract as superior in pork flavor after cooking. In this study, warmed-over flavors in treated precooked-frozen sausages were generally low, but they were significantly lower in the sausages treated with BHA/BHT and 2,500 ppm rosemary extract in comparison to the control. Similarly, a study with precooked beef patties showed that addition of rosemary extract did not affect color, odor, appearance, or texture, but increased liking scores (Thongtan, Toma, Reiboldt, & Daoud, 2005).

A very extensive study on the effect of numerous plant extracts on the oxidative stability of pork patties in comparison to BHA/BHT and postmortem as well as dietary vitamin E supplementation was conducted by McCarthy et al. (2001). They compared the antioxidant activity of aloe vera, fenugreek, ginseng, mustard, rosemary, sage, soy protein, tea catechins, whey proteins, vitamin E, and BHA/BHT in raw and cooked pork patties. Besides the fact that rosemary, tea catechins, vitamin E, and BHA/BHT always ranked among the five most effective antioxidants, two other

important observations were noted: (1) whey proteins exhibited strong antioxidant activity but only in the cooked pork patties and (2) the efficacy of the antioxidant systems varied over time, e.g., for cooked pork patties ginseng ranked higher than aloe vera on day 3, but after 9 days aloe vera showed more efficacy than ginseng. These results indicate that in addition to processing effects, duration of storage affects the effectiveness of antioxidant systems.

In addition to the various food plant extracts, research on using nonfood based antioxidant systems has commenced as well. For example, nonfood products that are being marketed as dietary supplements, such as grape seed extracts, have been investigated for use in meat products (Ahn et al., 2002; Lau & King, 2003). Ahn et al. (2002) studied two popular dietary supplements, the grapeseed extract ActiVin® (Dry Creek Nutrition, Inc., Modesto, CA) and the pine bark extract Pycnogenol® (Natural Health Science, Hillside, NJ) and compared them to BHA/BHT, vitamin E, a rosemary oleoresin (Herbalox® Kalsec Inc., Kalamazoo, Mich.), and sodium tripolyphosphate (FMC Corporation, Philadelphia, PA). In a study with ground beef, the antioxidants were initially compared to BHA/BHT at the allowable level of 200 ppm, using TBARS and hexanal analysis, as well as WOF scores, which showed excellent correlation as oxidation indicators. This first part of the study showed significant antioxidant effects for all the antioxidant systems, with the exception of sodium tripolyphosphate. However, the BHA/BHT combination surpassed any of the natural antioxidant systems. In the second part of the study, levels of the natural antioxidant systems were increased to 500 and 1,000 ppm. ActiVin® and Pycnogenol® at both levels outperformed not only the vitamin E system but also the rosemary extract, with neither one being significantly different from the BHA/BHT combination. A study conducted at about the same time by Lau and King (2003), using a different grape seed extract, showed similar results in turkey patties. Interest in investigating similar non-food-based antioxidant systems in meats is spreading. For example, Han and Rhee (2005) investigated the antioxidant potential of various nonculinary herbs, such as peony roots, in ground beef. Using such dietary supplements as antioxidant systems in meat may enable meat products to become vehicles of delivering antioxidants for human consumption. For example, for the above study (Ahn et al. 2002), it was calculated that one meat patty would supply the approximate recommended amounts of grape seed extract and pine bark extract, as indicated on the dietary supplement packages (Gruen & Ahn, 2005). While the research area of using meat products as functional foods to deliver health benefits beyond the nutritional value of the meat product itself is still in its infancy in the USA, in other countries, such as Germany, it has become an important area of research (K. Troeger, personal communication, 2007) (Chap. 4).

A fair number of the bioactive constituents of the spices, herbs, and plant extracts that exhibit antioxidant activity are known, such as rosmanol and rosmarinic acid in rosemary, and a still useful review of these chemicals is that by Shahidi and Wanasundara (1992). These chemicals for the most part belong to the large group of polyphenolic compounds. However, this information is of little value to the industry because only the spices, herbs, and plant extracts them-

selves are exempt from the strict food additive laws, and any intended use of pure chemicals, such as rosmarinic acid, would trigger regulations requiring considerable research into the safety of such chemical. There are many strategies and ingredients to reduce lipid oxidation in meat products. The decision on which strategy to use depends on numerous factors, including animal species, muscle type, product type, processing method, type and duration of storage, and intended use.

Among these factors, the type of product may be one of the most important considerations, because recent research (Estévez & Cava, 2006) showed that the antioxidant effect of natural systems can turn into a pro-oxidant effect in specific products, an effect that is fairly well known for vitamins E and C. In addition to reduced lipid oxidation, most antioxidant systems also improve flavor and color stability, unless the extract itself imparts a flavor or color to the meat product, which then may limit the use of these natural antioxidant systems.

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Chapter 14
Antimicrobial Ingredients

Catherine A. Simpson and John N. Sofos

Microorganisms Associated with Meat and Poultry Products

There are five classes of foodborne biological hazards: (1) bacteria, (2) parasites, (3) fungi, (4) viruses, and (5) prions (the inclusion of which is debatable). Depending on type, in addition to adverse human health issues, bacteria present in meat or poultry products or other foods may lead to fermentation or spoilage. This chapter focuses on controlling spoilage and pathogenic bacterial and fungal agents, as other biological hazards lack the ability to multiply in foods at the postharvest stage. Specific pathogens and spoilage organisms present and of concern may vary between fresh and ready-to-eat (RTE) meat and poultry products. Pathogenic *Escherichia coli*, *Salmonella*, and *Campylobacter* are those of greatest current concern in fresh products, while *Listeria monocytogenes* contamination is of primary concern in processed and RTE meat products (Bacon & Sofos, 2003; Sofos, 2002, 2004).

The most common sources of foodborne bacteria, molds, and yeasts include gastrointestinal tracts, animal hides, water, soil, feces, vegetation, air, dust, food contact surfaces, and food handlers (Sofos, 1994, 2002, 2004). The primary factors affecting survival and growth of microorganisms are pH, a_w, temperature, antimicrobials, and atmospheric conditions. Unfavorable adjustments to one or more of these factors may inhibit growth or lead to destruction of microbial contamination (Sofos, 2004). In general, optimal microbial growth occurs at pH values between 6.5 and 7.0 (pH of fresh meat is 5.0–6.5, with an average of 5.5), although most organisms continue to grow within the pH range of 4.0 and 9.5. Many bacterial species can survive (some yeasts and molds may actively replicate) below pH 4.0 (International Commission on Microbiological Specifications for Foods [ICMSF], 1980, 1996). When the a_w exceeds 0.90, bacteria tend to outcompete yeasts and molds, which are more commonly associated with lower a_w food products in which growth of bacteria is limited and fungi are able to establish themselves (Leistner & Gould, 2002). A deficit of available water inhibits proper metabolic function and cellular division of pathogenic, spoilage, and fermentative microorganisms (Leistner, Rödel, & Krispien, 1981). As a_w decreases, microorganisms may exhibit an extended lag phase, and grow more slowly, if at all (Jay, Loessner, & Golden, 2005). Aerobes require oxygen for respiration while anaerobes survive and grow in the absence of oxygen. Under optimal conditions, facultative anaerobes use oxygen, but can also replace oxygen with nitrite as the final electron receptor of the electron transport chain (denitrification), and

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therefore, survive in its absence (ICMSF, 1996; Singleton, 2004). Mesophiles replicate at temperatures between 20°C and 45°C with optimal growth occurring at 30°C–40°C. Psychrotrophs are quite dynamic in their ability to survive and slowly replicate under refrigeration (≤7°C), although optimal growth occurs between 20°C and 30°C. Thermophiles are found at ≥45°C and grow optimally at 55°C–65°C (ICMSF, 1980, 1996; Jay et al., 2005).

The inactivation or inhibition of one species of microorganisms does not necessarily indicate reductions in other microbial populations. Many antimicrobials act differently on gram-negative vs. gram-positive bacteria, or fungi due to differences in cell membranes and/or metabolic requirements (Nychas, Marshall, & Sofos, 2007; Sofos, 1984); gram-negative bacteria possess an outer cell membrane and gram-positive bacteria do not. Organisms within each classification may also react differently in the presence of an antimicrobial agent, and treatment effects should be investigated for specific microbial types (Sofos, Simpson, Belk, Scanga, & Smith, 2006). Characteristics and growth/survival parameters of common meat-associated pathogens and spoilage organisms can be found in Tables 14.1, 14.2, respectively.

Antimicrobials in Meat and Poultry Products

Need for Antimicrobial Agents

Fresh Meat

For ease of interpretation, "meat" refers to all cattle, swine, sheep, goat, and equine carcasses or derived products, as defined by the Food Safety and Inspection Service (FSIS). In 1994, the FSIS, a branch of the United States Department of Agriculture (USDA) took, what seemed at the time to be, appropriate recourse to the health concerns, extensive media coverage, and consumer unease which stemmed from a 1992–1993 outbreak of *E. coli* O157:H7 infections associated with consumption of undercooked ground beef patties (Bell et al., 1994). As part of such actions, *E. coli* O157:H7 was classified as an "adulterant" in nonintact fresh beef cuts including ground beef, and a "zero tolerance" policy was enacted to address visible contamination (soil, feces, milk, or ingesta) on beef carcasses during slaughter (USDA/FSIS, 1996). One of the most influential aspects of such efforts has been the implementation of hazard analysis critical control point (HACCP) programs, which manage processes or interventions applied to control foodborne hazards (Sofos, 2002, 2004; Sofos & Smith, 1998). These developments led to exploration of various technologies, including chemical antimicrobial solutions, for application in commercial settings in order to control contamination and allow compliance with regulatory requirements as well as commercial specifications (Koutsoumanis & Sofos, 2004; Sofos & Smith, 1998; Stopforth & Sofos, 2006).

Table 14.1 Characteristics and survival/growth parameters of pathogenic microorganisms commonly associated with fresh and processed meat products[1]

Organism	Growth temperatures; optimal (°C)	pH range	Minimum a_w	Maximum salt (%)	Oxygen requirements	Associated meat products
Gram positive						
Bacillus cereus	4–49; 30	4.3–9.3	0.91	10	Aerobic to facultative	Meats, soups, sauces
Clostridium botulinum					Anaerobic	Meat, poultry and seafood, low acid moist foods
Group I	3–48; 30	4.6–9.0	0.94	5		
Group II	10–48; 37	5.0–9.0	0.97	10		
Clostridium perfringens	12–50; 43–45	5.0–9.0	0.95	6	Anaerobic to aerotolerant	Raw and undercooked meat and poultry, gravies and sauces
Listeria monocytogenes	0–45; 30	4.4–9.0	0.92	10	Facultative	Delicatessen meats, frankfurters, seafood
Staphylococcus aureus	7–48; 37	4.0–10.0	0.86	10–18	Facultative	Delicatessen meats, meat salads
Gram negative						
Aeromonas	0–45; 28	4.5 (min)		6	Facultative	Meat, poultry and seafood, and water contaminated food
Campylobacter	32–45; 42–45	4.9–9.0	0.98	3.5	Microaerophilic	Fresh meat
Enterohemorrhagic *Escherichia coli*	7–45; 35–40	4.4–10	0.95	6–8	Facultative	Fresh ground beef
Salmonella	5–47; 37	4.0–9.0	0.93–0.95	4–8	Facultative	Fresh meat
Vibrio	5–43; 30–37	4.5–11.0	0.94–0.96	0.5–8.7	Facultative	Raw and undercooked seafood, water-contaminated foods
Yersinia	0–44; 32–34	4.6–9.0	0.95	5–8	Facultative	Raw pork, beef, chitterlings

As derived from Knøchel (1990); ICMSF (1980, 1996); Kirov, Anderson, and McMeekin (1990).

[1]Each factor is considered individually, assuming all other factors are within ideal parameters.

Table 14.2 Characteristics and survival/growth parameters of spoilage microorganisms commonly associated with fresh and processed-meat products[1]

Organism	Temperature requirement	pH range	Minimum a_w	Maximum salt (%)	Oxygen requirements	Signs of spoilage	Product associations[2]
Gram–positive bacteria							
Cornyforms	Mesophilic Psychrotrophic	5.0–9.0	0.94	8.0	Facultative	Slime, souring	B, E
Lactobacillus	Psychrotrophic	2.8–6.8	0.90	6.5	Microaerophilic to facultative	Slime/ropey slime, souring, greening, off-odor	C, E, F
Enterococcus *Pediococcus* *Carnobacterium*	Mesophilic Psychrotrophic	4.3–9.0 (6.8–9.0)	0.90	10.0 (26.4)	Facultative	Souring, greening, yellowing, bone taints (bone sours), slime	B, G, F
Leuconostoc *Weisella*	Psychrotrophic	5.5 (min)	0.90	14.0	Facultative	Slime, ropey slime, gas production, sour-off odor, greening	B, G, F
Staphylococcus *Micrococcus*	Mesophilic	4.0–10.0	0.86	10.0–18.0	Facultative	Souring	B, C, F
Gram–negative bacteria							
Aeromonas	Mesophilic Psychrotrophic	4.5 (min)		6	Faculative	Gas production	I
Acientobacter *Moraxella* *Psychrobacter*	Mesophilic Psychrotrophic Psychrophilic	3.3 (min)	0.96	10.0–25.0	Aerobic to facultative	Off-odor	A
Enterobacteriaceae	Mesophilic	4.4–9.0	0.94	4.0–8.0	Facultative	Gas production, off-odor, taint	A
Pseudomonas *Shewanella*	Psychrotrophic	5.0–8.0	0.95	4.8	Obligate anaerobes	Off-odor, slime, greening	A, D

Yeasts							
Candida *Saccharomyces* *Torulopsis* *Zygosacchromyces*	Mesophilic Psychrotrophic	1.6–4.5	0.88 (0.62)	12.0	Facultative	Off-odor, slime	H
Molds							
Aspergillis *Penicllium* *Rhizopus*	Psychrotrophic	2.0–8.5	0.61–0.80	–	Strict aerobes	Black, blue, green, or white spots	H

As derived from Baylis (2006); Betts (2006); Gould and Russell (2003); Hammes and Vogel (1995); Hocking (2006); Holley, Guan, Peirson, and Yost (2002); Jay et al. (2005); Kirov et al. (1990); Knøchel (1990); Kurtzman and James (2006); Liao (2006); Moss (2006); Mountney and Gould (1988); Samelis (2006); Schillinger and Holzapfel (2006).

[1]Each factor is considered individually, assuming all other factors are within ideal parameters.

[2]A: Raw protein-rich foods; B: cured meats, sausages; C: fermented meats, sausages; D: under refrigeration; E: under vacuum, low O_2 conditions; F: high salt concentrations; G: low salt concentrations; H: low pH, low a_w; I: fish, seafood.

The majority of antimicrobials used to decontaminate fresh meat or poultry products are not considered "ingredients," and are typically referred to as antimicrobial interventions. Interventions used to decontaminate carcasses are approved by the FSIS if they (1) are generally recognized as safe (GRAS), (2) do not lead to adulteration, (3) do not create labeling issues (i.e. added ingredients), (4) are scientifically proven to be efficacious, and (5) do not pose human health issues to workers or consumers (USDA/FSIS, 2003a, 2008). Moreover, ideal raw meat decontamination treatments should be environmentally friendly, should not alter the organoleptic properties of products, leave residues, or be of concern to consumers, public health officials, regulators, or legislators. Such treatments should also be effective against a range of pathogens, economical, and simple to apply (Koutsoumanis, Geornaras, & Sofos, 2006; Koutsoumanis & Sofos, 2004; Sofos, 2005a; Sofos & Smith, 1998; Stopforth & Sofos, 2006). Common carcass decontaminants include hot water and steam treatments, organic acids, and other chemical solutions. Care should be taken to ensure that only the outer surfaces of food and animal carcasses are treated during the application of harvest-floor chemical intervention solutions. Rips or cuts in the subcutaneous fat layer created during processing, specifically hide removal, may become reservoirs for collection of decontamination fluids and/or microorganisms. Simpson et al. (2006) found that 5.0% of pockets formed by such surface cuts contained decontamination fluids positive for *E. coli* O157:H7 when tested after carcass chilling and before fabrication. In addition to efforts to avoid the creation of such reservoirs, plant personnel may consider covering subcutaneous fat surface cuts with plastic film to avoid collection of harvest floor fluids and/or bacteria. The most common meat and poultry decontaminants are discussed in this chapter.

Processed Meats

The characteristics of fresh meat and poultry products (i.e., nutrient-rich, pH above 5.0, high a_w) encourage growth of microorganisms involved in rapid spoilage and potentially, if not properly refrigerated or inadequately cooked, in foodborne illness. For this reason, preservation methods may be utilized to modify physical, chemical, and/or biological properties in order to extend shelf life and improve safety. Physical preservation methods include heat, refrigeration, drying, smoking, packaging, and irradiation processes. This chapter deals with chemical and biological antimicrobial agents or preservatives which may be used as ingredients. The U.S. Food and Drug Administration (FDA) defines chemical preservatives as "any chemical that, when added to food, tends to prevent or retard deterioration"; sodium chloride, sugars, vinegar, spices and oil extracts of spices, naturally occurring components of wood smoke, and chemicals originally applied for insecticidal/herbicidal purposes, may have antimicrobial properties but are not considered chemical preservatives (Code of Federal Regulations [CFR], 2007a). Most ingredients incorporated into processed meat and poultry product formulations exhibit some degree of antimicrobial activity.

As part of the interim rule issued in response to multistate outbreaks of listeriosis associated with consumption of RTE meat and poultry products (Centers for Disease Control and Prevention, 1998, 2000, 2002), manufacturers of products, which are capable of supporting growth of *L. monocytogenes* and may be exposed to postlethality treatment contamination, must address control of the pathogen in their HACCP plans or prerequisite programs (USDA/FSIS, 2003b). The use of antimicrobial agents and other postlethality treatments, comprehensive sanitation programs, and intensive microbiological testing are encouraged. In addition, the antilisterial properties of food ingredients with GRAS status may be investigated for use as antimicrobial agents in such products when appropriate.

Characteristics and Applications of Antimicrobial Agents

Organic Acids and Salts

Characteristics and Properties

Short-chain organic acid antimicrobial agents are naturally present in foods or chemically synthesized, and some are also formed during microbial fermentation of carbohydrates present in foods (Beuchat, 1998; Stratford & Eklund, 2003). Commonly used organic acids in food applications include acetic, citric, lactic, benzoic, sorbic, fumaric, malic, and propionic acid. Salts of organic acids may also be used as antimicrobials as their anions are released when dissolved in water solutions or in foods (Kuntz, 1999). As weak acids, organic acids are less likely than stronger acids to dissociate and donate a proton to neighboring water molecules. In the undissociated state, weak acids enter into cells where they dissociate, forcing the organism to expend energy in efforts to remove excess protons and maintain homeostasis (Doores, 2005). The cellular machinery responsible for the uptake of extracellular nutrients may also be shut down as cellular proteins, nucleic acids, and membrane components are affected by exposure to low intracellular pH (Beuchat, 1998; Langworthy, 1978). In addition to the above, microbial contaminants may be indirectly inhibited as meat proteins are denatured under low pH conditions leading to reduced water-holding capacity, and thus, a lowering of the a_w of a product (Kuntz, 1999).

The antimicrobial activity of organic acids is pH-dependent, and also influenced by degree of water and lipid solubility (Davidson, 2001; Doores, 2005; Freese, Sheu, & Galliers, 1973; Leo, Hansch, & Elkins, 1971). The dissociation constant (pK_a) of an acid (Table 14.3) is the pH at which equal proportions of dissociated and undissociated molecules are present in a solution, and the antimicrobial activity of organic acids increases as the environmental pH approaches the pK_a (Stratford & Eklund, 2003). The partition coefficient ($\log P_{oct}$) of a compound is a measurement of its solubility within a biphasic system and indicates the preference of a com-

Table 14.3 Molecular weight (MW) and dissociation constant (pK_a) of organic acids commonly used in meat processing

Organic acid	MW	pK_a[a]
Acetic	60.50	4.75
Propionic	74.08	4.88
Lactic	90.08	3.08
Phosphoric	98.00	2.21
Sorbic	112.13	4.80
Fumaric	116.70	3.03
Benzoic	122.12	4.19
Malic	134.09	3.40
Caprylic (octanoic)	144.21	4.89
Citric	192.12	3.14

As derived from Smith (2003); Sofos et al. (1998).
[a]pK_a: Dissociation constant (negative logarithm of the acid dissociation constant [K_a]).

pound toward the octanol vs. water phase fractions of a solution; positive log P_{oct} values are indicative of the lipophilicity of a compound (Leo et al., 1971). Organic acids with lipid-soluble properties (e.g., sorbic, propionic acid) are superior antimicrobial agents as these acids are able to penetrate the outer lipid membrane of microbial cells (Davidson, 2001; Freese et al., 1973); in high-fat products, however, their lipid solubility may reduce amounts present in the aquatic phase and, thus, limit antimicrobial activity. Sodium chloride may reduce the log P_{oct} of organic acids (Stratford & Eklund, 2003).

Sublethal acid concentrations may lead to selection of acid-tolerant pathogens, the evolution of acid-resistance in previously susceptible microorganisms, or to development of organisms which are more tolerant to ensuing treatments such as cooking (Davidson & Harrison, 2002; Duffy, Grau, & Vanderlinde, 2000; Samelis & Sofos, 2003; Sofos & Smith, 1998). Additional concerns associated with use of organic acids as antimicrobial agents include undesirable effects on meat quality and accelerated corrosion of food processing equipment (Samelis & Sofos, 2003; Sofos & Smith, 1998; Sofos et al., 2006). Thus, selection and application of organic acids in single or multiple hurdle preservation strategies should be optimized to avoid creation of potential risks.

Acids are generally more effective against a broader range of microorganisms when used in combination with additional antimicrobial agents, including other organic acids (Lueck, 1980; Malicki, Jarmoluk, & Bruzewicz, 2004). Adams and Hall (1988) demonstrated the synergistic effects of organic acid combinations, while greater activity was also observed when organic acids were applied as warm solutions (Hardin, Acuff, Lucia, Oman, & Savell, 1995; Venkitanarayanan, Ezeike, Zhoa, & Doyle, 1999) or when applied in the presence of other antimicrobial compounds, and especially those which interfere with cell membrane function and/or increase membrane permeability (Kadner, 1996). Other chapters and reviews dedicated to the characteristics and uses of organic acids, esters, and salts are available,

and include those by Bogaert and Naidu (2000), Chipley (2005), Doores (2005), Drosinos, Mataragas, Kampani, Kritikos, and Metaxopoulos (2000), Glass, Smith, Granberg, and Johnson (1999), Marshall, Cotton, and Bal'a (2000), Sharma (2000), Sofos (2000), Stratford and Eklund (2003), Wicklund, Stetzer, Tucker, Nicolalde, and Brewer (2005), and Zhu, Du, Cordray, and Ahn (2005).

Acetic Acid. Acetic or ethanoic acid (CH_3-COOH; log P_{oct} – 0.319) is a monocarboxylic acid which occurs naturally in plant and animal tissues and is also a by-product of ethanol oxidation by *Acetobacter*, *Gluconobacter* and other heterofermentative strains of lactic acid bacteria (LAB) (Doores, 2003; Leo et al., 1971). Homofermentative LAB, gram-negative and gram-positive bacteria, yeasts, and some mold species exhibit various degrees of sensitivity to acetic acid (Table 14.4) (Doores, 2005). Vinegar, a 5% acetic acid solution, is known for its strong sensory characteristics, and thus acetic acid is self-limiting in food applications. As an antimicrobial agent, acetic acid (2–5% solutions) is used to decontaminate food animal carcasses and potassium and sodium acetate/diacetate may also be added to processed meat and poultry products to control *L. monocytogenes* in combination with sodium/potassium lactate (Tables 14.5 and 14.6). Other organisms inhibited by sodium diacetate include pathogenic *E. coli*, *Pseudomonas fluorescens*, *Salmonella*, *Shewanella putrefaciens*, *Bacillus cereus*, and some aerobic microorganisms involved in spoilage (Ajjarapu & Shelef, 1999; Shelef & Addala, 1994). Acetic and lactic acid as well as lactate and diacetate blends may demonstrate synergistic

Table 14.4 Antimicrobial spectrum of common antimicrobial agents

	Bacteria			
Antimicrobial agent	Gram-positive	Gram-negative	Yeast	Mold
Acetic acid/acetates	●	●	●	◑
Benzoic acid/benzoates	◑	◑	●	●
Citric acid/citrates	●	●	○	◑
Lactic acid/lactates	●	●	◑	◑
Sorbic acid/sorbates	●	●	●	●
Chlorine compounds	●	●	◑	●
Cetylpyridinium chloride	●	●	●	●
Ethanol	●	●	●	●
Lactoferrin	●	●	●	●
Lysozyme	●	○	○	○
Ozone	●	●	○	●
Hydrogen peroxide	●	●	●	●
Pediocin	●	○	○	○
Polyphosphates	●	●	●	●
Parabens	●	○	●	●
Peroxyacids	●	●	●	●
Natamycin	○	○	●	●
Nisin	●	○	○	○
Nitrite	●	●	○	○
Medium chain fatty acids/esters	●	◑	●	●

Symbols: (●) effective to highly effective; (◑) slightly to moderately effective; (○) ineffective.

Table 14.5 Fresh meat and poultry decontamination treatments approved for use in the United States

Antimicrobial agent	Maximum level	Approval	Uses
Organic acids (acetic, citric, lactic)	2.0–5.0% aqueous acetic acid, ambient or 55°C	21 CFR 184.1005, 1033, 1061	Acidifier, flavoring agent
Chlorine compounds including chlorine dioxide	3–50 ppm free available chlorine	21 CFR 173.300	Antimicrobial, bleaching agent, oxidizing agent
Acidified sodium chlorite	50–1,200 ppm, pH 2.3–3.2, 45–55°C	21 CR 173.325	Antimicrobial agent
Cetylpyridinium chloride (CPC)	0.3 g CPC/lb raw poultry carcass; shall contain propylene glycol at concentration 1.5 times that of CPC	21 CFR 173.375	Antimicrobial agent
Lactoferrin	Up to 2% lactoferrin bound to GRAS glucosamine	FSIS Directive 7120.1	Antifungal, antiviral, antibacterial, and anti-inflammatory agent
Octanoic acid (caprylic acid)	As described in 21 CFR 170.329	21 CFR 184.1025	Adjuvant, antimicrobial, defoamer, flavoring agent
Peroxyacid (peroxyacetic acid (PA), acetic acid, octanoic acid, hydrogen peroxide (HP), and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP)	Meat (220 ppm PA, 75 ppm HP); poultry (220 ppm PA, 110 HP, 13 ppm HEDP)	21 CFR 173.370	Antimicrobial agent
Ozone	Antimicrobial application described in 21 CFR 170.3(o)(2)	21 CFR 184.1563	Antimicrobial, oxidizer, water disinfection and purification
Trisodium phosphate	8–12% TSP in aqueous solution containing 20 ppm chlorine, ≤15 s	21 CFR 182.1778	Binder, emulsifier, color stabilizer, antimicrobial agent
Acidic calcium sulfate	As described in FSFS Directive 7120.1 Amendment 6	21 CFR 184.1230	Dough conditioner, nutrient, pH control agent, stabilizer, thickener

As derived from CFR (2001, 2002); IOM (2003); USDA/FSIS (2007).

properties when used in combination (Adams & Hall, 1988; Barmpalia et al., 2005; Blom et al., 1997). Daily intake of acetic acid and calcium, potassium or sodium acetate is not limited for humans of any age and acetic acid may be used in infant formulas (Food and Agriculture Organization/World Health Organization [FAO/WHO], 1966, 1974). The acceptable daily intake of sodium diacetate for humans is 15 mg/kg of body weight (FAO/WHO, 1974).

Table 14.6 Processed meat antimicrobials approved for use in the United States

Antimicrobial agent	Maximum level	Approval	Uses
Acidified calcium sulfate	<0.07% of final product	21 CFR 184.1230	Nonacidified form: tofu coagulant, nutrient, dough conditioner, firming agent, sequestrant, pH control agent
Buffered sodium citrate	pH 5.6; ≤1.3% of final product formulation	21 CFR 184.1751	Flavoring agent, pH control agent, preservative, antioxidant
Citric acid	≤10% solution applied before slicing (bologna)	FSIS Directive 7120.1 Amendment 9	Acidifier, flavoring agent, preservative
Eugenol	As described in 21 CFR 170(o)(12), 184.1(b)(1)	21 CFR 184.1257	Flavoring agent
Hops β-acids	Casings: 2.5 mg/lb of finished product; 2.0 mg/lb within product formulation	FSIS Directive 7120.1 Amendment 2	Flavoring agent
Laurimide arginine ethyl ester (LAE)	≤200 ppm as determined by final product weight	FSIS Directive 7120.1 Amendment 5	Preservative, antimicrobial agent, surfactant
Lysozyme	2.0–2.5 mg/lb of finished product	FSIS Directive 7120.1	Antimicrobial agent
Nisin	As described in 21 CFR 133.180, FSIS Directive 7120.1 Amendment 2	21 CFR 184.1563	Antimicrobial agent
Octanoic acid solution	≤400 ppm as determined by final product weight	21 CFR 172.860	Adjuvant, antimicrobial, defoamer, flavoring agent
Potassium benzoates Sodium benzoate	Apply according to current good manufacturing practices	21 CFR 184.1733	Preservative, antimicrobial agent
Potassium lactate Sodium lactate	4.8% (wt) of total formulation; singly or in combination and in accordance with 21 CFR 184.1	9 CFR 424.21	Emulsifier, flavor enhancer, acidifier, antimicrobial agent
Potassium nitrite Sodium nitrite	<200 ppm alone or with sodium nitrate	21 CFR181.34	Curing agent, preservative, antimicrobial agent
Potassium sorbate	58.2 g/100 ml water	21 CFR 182.3225	Preservative, antimicrobial agent
Sodium diacetate	0.25% (wt) of total formulation	21 CFR 184.1754	Emulsifier, acidifier, antimicrobial agent
Sodium tripolyphosphate	Apply according to current good manufacturing practices	21 CFR 182.1810	Moisture binder, emulsifier, preservative, antioxidant

As derived from CFR (2001, 2002); IOM (2003); USDA/FSIS (2007).

Citric Acid. Citric acid ($C_6H_3O_7$; log P_{oct} – 0.172) can be isolated from citrus fruits and is produced during normal metabolic processes of most living organisms (Doores, 2003; Leo et al., 1971). It is a powerful chelator which sequesters metal ions (Ca^{2+}, Mg^{2+}, Fe^{3+}) involved in oxidative rancidity and also required for microbial homeostasis (Stratford & Eklund, 2003). Citric acid is used throughout the food industry as a sequesterant, dispersing agent, acidifier, flavoring agent, preservative, or as a dietary supplement (manganese citrate) and may also be used in infant formulas (Doores, 2003; Institute of Medicine [IOM], 2003). Aqueous solutions (2–5%) may be used to decontaminate animal carcasses (Table 14.5) and multiple citric acid derivatives (isopropyl and monostyryl citrate) are used to preserve the color of cured meats and/or promote antioxidant efficacy (Doores, 2003). As applied in commercial decontamination treatments, citric acid may provide less antimicrobial activity because (1) it is nonlipophilic (Leo et al., 1971), (2) its pK_a is quite low (3.14), (3) it is produced during normal metabolic processes and may be easily metabolized by numerous microorganisms (Foegeding & Busta, 1991), and (4) it has a higher molecular weight than other organic acids (Table 14.3). Therefore, it may be less likely to be taken up into cells or be applied at lower concentrations than other acids when using percentage-based working solutions. When compared at equal molar concentrations, citric acid was more effective as an antimicrobial than lactic or acetic acid (Durán & Sofos, 2006; Miller, Call, & Whiting, 1993). Buchanan and Golden (1994) found that the antilisterial activity of citric acid solutions was reduced at pH values >5, especially when the concentration of citric acid was low (≤0.5%). The daily intake of citric acid/derivatives is not limited (FAO/WHO, 1966).

Lactic Acid. Lactic acid (CH_3-CH(OH)-COOH; log P_{oct} – 0.620) is produced during fermentation by LAB, and is available as a hygroscopic, syrupy liquid, with a moderately strong acidic taste (Doores, 2005; IOM, 2003; Leo et al., 1971). In addition to the decontamination of beef, pork, and poultry carcass, lactic acid is a multifunctional ingredient and approved for use as an acidulant, flavoring agent, emulsifier, and antimicrobial agent in various meat and poultry product formulations (Tables 14.5 and 14.6) (Shelef, 1994; Sofos, Belk, & Smith, 1999; Sofos, Beuchat, Davidson, & Johnson, 1998; Sofos, Kochevar, Bellinger, et al., 1999; Sofos, Kochevar, Reagan, & Smith, 1999; Sofos et al., 2006; Wederquist, Sofos, & Schmidt, 1994). Organisms inhibited by lactic acid include gram-negative and -positive bacteria and fungi (Table 14.4) (Sofos, Maga, & Boyle, 1988). Two isomers of lactate (D- and L-lactate) exist, either of which may be produced during the normal metabolic processes of some microorganisms (FAO/WHO, 1966), and commercial solutions containing the individual isomers, or equal concentrations of D- and L-lactate, are available. In one study, the antimicrobial effect of D-lactate (≤150 mM) against *E. coli* O157 and non-O157 strains was inferior to equal concentrations of L-lactate; however, both isoforms were equally lethal at concentrations ≥200 mM (McWilliam Leitch & Stewart, 2002). This phenomenon is attributed, in part, to the generation of D-lactate by *E. coli* during fermentation, requiring these organisms to possess cellular mechanisms to regulate its presence inside the cell (Bunch, Mat-Jan, Lee, & Clark, 1997; McWilliam Leitch & Stewart, 2002).

In general, the efficacy of lactic acid is enhanced in the presence of other bacteriostatic factors produced by LAB (e.g., bacteriocins), acetates and diacetates, potassium sorbate, nisin, nitrites, glucono-delta-lactone, ethanol, and under modified atmosphere conditions (Doores, 2005; Jordan et al., 1999; Malicki et al., 2004; Scannell, Hill, Buckley, & Arendt, 1997). The acceptable daily intake of lactic acid is not limited, although its calcium, potassium, and sodium salts are limited to 100 mg/kg/day of DL-lactate (FAO/WHO, 1966). L- and DL-Lactic acid/lactates should not be used in infant formulas (Doores, 2003).

Benzoic Acid. Sodium benzoate, a derivative of benzoic acid (C_6H_5-COOH), is a chemical preservative frequently added to food, pharmaceutical and cosmetic product formulations, although the use of sodium benzoate in food products has been scrutinized as some individuals have experienced skin rashes or asthma attacks after consuming associated food products (Sax & Lewis, 1989). Fungi are most susceptible to benzoic acid and benzoates, although some bacteria, including pathogens and spore-formers, are also susceptible (Table 14.4) (Lueck, 1980; Sofos, 1994). Some spoilage bacteria are impervious to benzoate compounds (Smith, 2003). Synergistic antimicrobial activity between benzoates and other preservatives/ingredients is also pH-dependent, and has been observed in the presence boric acid, carbon dioxide, chitosan, formic acid, parabens, sulfur dioxide, sorbic acid/sorbates, sodium chloride, and sucrose. Benzoates are also increasingly effective in thermally processed or refrigerated food products (Lueck, 1980; Sagoo, Board, & Roller, 2002; Smith, 2003). Lipids, ferric salts, and exposure to anionic surfactants tend to antagonize antimicrobial activity (Smith, 2003). Topical exposures to benzoic acid may result in eye and/or dermal irritation (Sax & Lewis, 1989). Benzoates may possess mutagenic and teratogenic properties, and when introduced orally, may induce allergic reactions in humans (Schaubschlager, Becker, Schade, Zabel, & Schlaak, 1991). While benzoic acid does not accumulate in the body, the acceptable daily intake of benzoic acid and its salts is limited to 0–5 mg/kg of body weight (FAO/WHO, 1966).

Sorbic Acid. The compound (C_5H_7-COOH) was originally isolated from the oil of unripened rowanberries (Sofos, 1989) but now it is manufactured synthetically. Sorbates, including the free acid and salt forms, are commonly used to preserve cosmetics, pharmaceuticals, and food products (Stopforth, Sofos, & Busta, 2005) and are effective against many gram-positive and -negative bacteria and fungi (Table 14.4) (Sofos, 2000). Resistance to sorbate inhibition has been observed in catalase-negative LAB, yeasts, and molds including *Candida*, *Saccharomyces*, *Torulopsis*, *Zygosaccharomyces bailii*, *Aspergillus*, and also in some species of *Pseudomonas* (Smith, 2003; Sofos & Busta, 1993; Warth, 1988). In one study, the addition of sorbate to a broth system did not affect growth, but did interfere with the ability of *L. monocytogenes* to secrete the toxin listeriolysin O (McKellar, 1993). Of the sorbate salts, potassium sorbate is considerably more popular than calcium or sodium counterparts as it is significantly more water-soluble. Potassium sorbate is nearly 400 times more water-soluble than sorbic acid, although sorbic acid exhibits better solubility in other solvents (Sofos, 1989). While optimal activity is observed at a pH of 4.8 or less, sorbates show measurable

activity at higher (up to neutral) pH values (Smith, 2003). Antimicrobial activity is also enhanced in the presence of sulfur dioxide, carbon dioxide, sodium chloride, sucrose, propionic acid, nisin plus polyphosphates, benzoates, and in low a_w foods; activity may be impaired following exposure to nonionic surfactants (Smith, 2003). Regarding human health, sorbates are among the safest compounds used to preserve food products (Stopforth, Sofos et al., 2005). Sorbates do not appear to be mutagenic, are nontoxic at concentrations found in food products, and nonirritating for most consumers at levels used to preserve cosmetics and pharmaceuticals (Lück, 1976; Stopforth, Sofos et al., 2005). Sorbate-induced skin irritation has been observed in sensitive persons, with concentrations of 1% or less being sufficient to induce adverse, although minor reactions (Lück, 1976). The acceptable daily intake of sorbic acid and its salts is limited to 0–25 mg/kg of body weight (FAO/WHO, 1974).

Other Organic Acids. Fumaric acid (HOOC-$[CH]_2$-COOH) is a short-chain fatty (carboxylic) acid, which may be used to stabilize cured meat color, is effective against bacteria and molds, and has been more effective as a preservative when used in combination with sorbates (Beuchat, 1998; Doores, 2003; Podolak, Zayas, Kastner, & Fung, 1996). Acceptable daily intake of fumaric acid is limited to 0–6 mg/kg body weight (FAO/WHO, 1974). Malic acid (HO-CH-COOH-CH_2-COOH), the primary acid recovered from apples, cherries, and plums, is effective against bacteria and fungi and is most commonly used as a flavoring or pH control agent or to enhance the ability of antioxidants to stabilize animal fats (Doores, 2003, 2005; Stratford & Eklund, 2003). Propionic acid (CH_3-CH_2-COOH) is effective against certain bacteria and fungi, and may be used as an antimycotic agent in food products, including processed meats (Doores, 2003, 2005). The daily intake of malic and propionic acid is not limited (FAO/WHO, 1966). All previously mentioned compounds have received GRAS approval.

Applications

Fresh Meat Decontamination. Organic acid solutions are used to reduce microbial contamination on meat and poultry carcasses (Table 14.5), and the application of such treatments is widely practiced in the United States (Cutter & Rivera-Betancourt, 2000; Sofos, 2005a; Sofos, Belk et al., 1999; Sofos, Kochevar, Bellinger et al., 1999; Sofos, Kochevar, Reagan et al., 1999; Sofos et al., 2006). Solutions (2–5%) of organic acids (acetic, citric and lactic acid) may be applied at temperatures of up to 55°C as water-based sprays/rinses or dips (Table 14.5). Lactic and acetic acid are the most commonly used organic acids in the decontamination of animal carcasses and their efficacy (2–5%), alone and in combination with other interventions, has been scientifically validated (Dickson & Siragusa, 1994; Hardin et al., 1995; Prasai et al., 1991, 1997; Sofos, 2005a; Stopforth & Sofos, 2006). For instance, Cutter and Rivera-Betancourt (2000) estimated that lactic or acetic acid (2%) reduced levels of *E. coli* O157:H7 and *Salmonella* Typhimurium DT104 populations by ≥2 log CFU/cm^2.

The antimicrobial activity of organic acid decontamination treatments depends on temperature, pressure, and duration of application, acid concentration, type of tissue being treated, and product storage conditions prior to and following treatment (Cords, Burnett, Hilgren, Finley, & Magnuson, 2005; Durán & Sofos, 2006; Hardin et al., 1995; Sofos et al., 2006). In general, greater microbial reductions are observed when organic acids are applied at elevated temperatures (55°C) (Hardin et al., 1995). In one study, postevisceration lactic acid spraying treatments (1.0%, 55°C) were more effective than pre-evisceration treatments in reducing microbial contamination on inoculated beef tissue (Prasai et al., 1991). Correspondingly, lactic acid treatments (1.5%, spray) were more effective in reducing *Salmonella*, *Listeria*, and total aerobic counts on beef strip loins when applied after storage, compared to prestorage treatments (Prasai et al., 1997). Deumier (2006) investigated the effect of commercial-scale vacuum-tumbling (1 min) deboned chicken legs in a 1% lactic acid solution on post-tumbling prevalence of *Salmonella*. Prevalence of *Salmonella*-positive batches prior to implementation (January–August) was 13.15% and decreased to 4.83% (August–February) following implementation (Deumeir, 2006). To minimize water evaporation and accelerate surface temperature decline during chilling, beef carcasses may receive short systematic showers of water during the first 10–12 h in the cooler (Allen, Hunt, Luchiari Filho, Danler, & Goll, 1987; Doyle & Schoeni, 1984; Jones & Robertson, 1988). In one study, greater reductions in acid-adapted and non-acid-adapted *E. coli* O157:H7 populations were observed during chilling when lactic acid (2%) was added to the water spray applied to beef carcass tissue (vs. water-only spray), and after 48 h, initial populations (4.8 log CFU/cm^2) were reduced by 2.3 and 4 log cycles, respectively (Stopforth et al., 2004). Counts on samples receiving a water-only spray were reduced by about 1 log after 48 h (Stopforth et al., 2004).

In one study, 2% acetic acid was shown to be capable of sublethally injuring approximately 65% of various *S.* Typhimurium inoculum levels, followed by a residual 1 log unit reduction 4 h later (Dickson, 1992). A combination of acetic (1.5%) and propionic acid (1.5%) significantly extended shelf life, and was more effective than lactic acid (2%) against total viable counts, *Staphylococcus aureus*, *L. monocytogenes*, *E. coli*, and *S.* Typhimurium on sheep and goat meat (Dubal et al., 2004). Fumaric acid (1.0–1.5%) was also more effective against *L. monocytogenes* and *E. coli* O157:H7 on lean beef tissue than lactic or acetic acid (Podolak et al., 1996).

Citric acid and citric acid blends containing hydrochloric acid, and/or phosphoric acid, are also approved for use as poultry decontamination solutions, and may also be used to lower the pH of meat and poultry products (Tables 14.5 and 14.6), although conflicting reports have been published relative to the antimicrobial effects of citric acid (Doores, 1993). As indicated, citric acid has a much higher molecular weight than acetic or lactic acid (Table 14.3) and variations in efficacy may be related to the method used to dilute concentrated citric acid into working solutions. Growth of *E. coli* O157:H7 in fresh ground beef was inhibited following addition of sodium diacetate (0.1% or 0.2%) or sodium lactate (0.9% or 1.8%), but did not respond to buffered sodium citrate (BSC, 1% or 2%) treatments (Ajjarapu & Shelef, 1999). Ceylan,

Hajmeer, and Marsden (2002) found that 1% BSC alone did not inhibit growth of aerobic microorganisms in fresh ground beef, although 1% BSC plus 0.1% sodium diacetate maintained subspoilage levels of aerobic populations throughout storage (10 days, 4°C); the use of sodium diacetate alone was not included as a control.

Processed Meats. Sodium and calcium acetates have GRAS status and are approved for miscellaneous and general use, and also for use (≤0.25%) as antimicrobial agents in meat and poultry products (Table 14.6). Sodium and potassium lactate, individually or in combination with diacetate, may be used as antimicrobial agents in fully cooked meat, meat products, and poultry and poultry products, except those included in infant foods/formulas (Table 14.6). Citric acid may be applied to bologna in edible and inedible casings and to other meat and poultry products in fibrous casings, just before the product is sliced (Table 14.6).

A mounting body of evidence indicates that approved levels of lactate and sodium diacetate are both necessary and sufficient to control *L. monocytogenes* in RTE meat products (Blom et al., 1997; Glass et al., 1999; Schlyter, Glass, Loeffelholz, Degnan, & Luchansky, 1993; Seman, Borger, Meyer, Hall, & Milkowski, 2002), and that *L. monocytogenes* is most effectively controlled by combinations of lactate and sodium diacetate (Barmpalia et al., 2005; Blom et al., 1997; Seman et al., 2002). Geornaras et al. (2006) found that potassium lactate (1.5%) plus sodium diacetate (0.05%) extended the lag phase (10 days) of *L. monocytogenes* (3.2 log CFU/cm^2) on frankfurters compared to controls (0 days) and inhibited growth throughout storage (10°C, 48 days). The effect of potassium lactate plus sodium diacetate on commercial ham inoculated with *L. monocytogenes* following two contamination scenarios (manufacturing- vs. retail-level), and during three storage scenarios (processing, retail, home) involving various temperature (refrigerated storage, 4°C or 7°C; intermittent temperature abuse, 25°C, 90 min) and atmospheric conditions (vacuum- and aerobic packaging) was also examined (Lianou et al., 2007). In general, pathogen counts were lower on ham formulated with antimicrobials compared to controls, level of inhibition depended on contamination scenario and packaging method, and counts associated with manufacturing-level contamination and aerobic packaging were higher than others (Lianou et al., 2007). Combinations of lactate and sodium diacetate may be even more effective against *L. monocytogenes* when used in conjunction with other antimicrobial agents (Scannell et al., 1997). For example, postprocessing dipping treatments (0.5% nisaplin, the commercial form of nisin followed by 2.5% lactic acid, 2.5% acetic acid, or 5.0% potassium benzoate, or lactic or acetic acid alone) were inhibitory on frankfurters without antimicrobials and antilisterial on frankfurters formulated with potassium lactate and sodium diacetate (Geornaras et al., 2006). The appearance, color, odor, flavor, texture, and overall acceptability of treated frankfurters were not significantly different from controls (Geornaras et al., 2006). Others have also reported the synergistic nature of lactate and nisin combinations against *S. aureus* and *Salmonella* Kentucky in fresh pork sausage (Scannell et al., 1997).

Juneja and Thippareddi (2004) found that a commercially available form of BSC (Ional^{tm}, 1.3%) inhibited the growth of *C. perfringens* in cooked model roast beef samples during chilling. When used in combination with *Bifidobacterium breve*,

sodium acetate and potassium sorbate inhibited growth of spoilage organisms associated with fresh camel meat (Al-Sheddy, Al-Dagal, & Bazaraa, 1999). Malic acid (2.6%), incorporated into soy protein film, reduced initial *L. monocytogenes*, *Salmonella* Gaminara, and *E. coli* O157:H7 populations (approximately 8–9 log CFU/ml) by 5.5, 3.0, and 6.8 log CFU/ml, respectively (Eswaranandam, Hettiarachacy, & Johnson, 2004), although soy protein films containing citric, lactic, or tartaric acid, or acids plus nisin were ineffective.

Parabens

Characteristics and Properties

The alkyl esters (methyl, ethyl, propyl, butyl, heptyl) of *para*-hydroxybenzoic acid (parabens) are commonly used to preserve cosmetics and other toiletries and drugs, and, in some instances, food products (Davidson, 2005; Padilla-Zakour, 1998). With the exception of methyl esters, parabens are odorless, and all impart a distinct flavor even when used at low concentrations (Davidson, 2005; Lueck, 1980). As antimicrobials, parabens disorder key membrane lipids and cease membrane functions including the uptake of metabolically required nutrients and/or efflux of metabolic by-products (Bredin, Davin-Regli, & Pages, 2005; Tatsaguchi, Kuwamoto, Ogomori, Ide, & Watanabe, 1991). Fungi are more susceptible to parabens than bacteria, and gram-positive bacteria are more susceptible than gram-negative types (Table 14.4) (Stratford & Eklund, 2003). Alterations in cell membrane composition and subsequent resistance may be consequences of applying parabens at sublethal levels (Juneja & Davidson, 1993; Russell & Chopra, 1996). Parabens with longer alkyl chains are increasingly superior as antimicrobials. Thus, the order of effectiveness is heptyl > butyl > propyl > ethyl > methyl (Aalto, Firman, & Rigler, 1953; Stratford & Eklund, 2003). Potassium salts of parabens are highly soluble, and therefore, more effective as antimicrobials than other less soluble paraben salts (Mizuba & Sheikh, 1987). Activity depends on pH, temperature, time, and substrate (Aalto et al., 1953; Chichester & Tanner, 1972; Davidson, 2005). Parabens are heat tolerant and increasingly soluble at elevated temperatures, yet remain active at low temperatures (Gould, 2000; Padilla-Zakour, 1998; Smith, 2003). While parabens are effective over a wide pH range (3.0–8.0), antimicrobial activity is generally optimized at a neutral to slightly alkaline pH; parabens also tend to inhibit a larger spectrum of microorganisms when used in combination (e.g., 2:1 methyl and propyl paraben) (Gould, 2000; Padilla-Zakour, 1998; Smith, 2003). In contrast to other parabens which are most active at a neutral pH, potassium butyl paraben exhibits optimal activity at pH values between 4 and 6 (Mizuba & Sheikh, 1987). Moir and Eyles (1992) also reported that methyl paraben was more inhibitory against *L. monocytogenes* at pH 5 than at pH 6. The consideration for the use of parabens to preserve food products has raised health concerns, and although their involvement in cases of chronic dermatitis, gastrointestinal irritation, and other

forms of allergic response have been questioned, limited data are available which link these compounds to such afflictions (Soni, Burdock, Taylor, & Greenberg, 2001; Soni, Taylor, Greenberg, & Burdock, 2002).

Applications

The inhibitory effects of ethyl, propyl, and butyl parabens (0.1%) against *C. botulinum* toxin production in canned pork slurry were evaluated and while propyl and butyl paraben were ineffective, ethyl paraben inhibited toxin production for up to 4 weeks at 27°C (Draughon, Sung, Mount, & Davidson, 1982). Parabens may be more effective against low levels of microbial contamination and become increasingly less effective in the presence of higher contamination levels (Padilla-Zakour, 1998). While the use of parabens in meat products is not approved in the United States, such use is practiced in other regions of the world.

Medium- and Long-Chain Fatty Acids and Esters

Characteristics and Properties

Medium and long-chain-length fatty acids have exhibited the ability to inhibit/inactivate foodborne pathogens, but are more commonly used as adjuvants and wetting agents or as components of hygiene and sanitation programs (Burnett et al., 2007; IOM, 2003; Kabara & Marshall, 2005; Mbandi, Brywig, & Shelef, 2004). Chemically, medium-chain fatty acids are lipids with carbon chains of eight to fourteen carbons in length (Sofos et al., 1998) and include caprylic or octanoic (8:0), capric (10:0), and lauric (12:0) acid; long-chain fatty acids (≥16 carbon chain length) include palmitic (16:0), steric (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acid. Both, the degree of saturation and carbon chain length influence antimicrobial activity and spectrum of microorganisms affected by these acids (Kabara, 1993; Nieman, 1954; Sofos et al., 1998). In general, those with chains of 14 carbons or less, branched-chain fatty acids, those which contain hydroxyl groups, and saturated fatty acids are less effective as antimicrobials (Conley & Kabara, 1973; Nieman, 1954).

Fatty acids are esterified by replacing the hydrogen atom of the acid group with an alcohol (e.g., ethanol) or a sugar group (e.g., glycerol). Conley and Kabara (1973) found that antimicrobial activity is lost when fatty acids are esterified with hydrophobic compounds. In contrast, the addition of hydrophilic compounds compliments the hydrophobic nature of fatty acids, and subsequent antimicrobial activity may be intensified. The same is also true for short-chain fatty acids (Davidson, 2001). In general, esterified fatty acids convey more intense flavors/aromas and provide more potent antimicrobial activity against a broader spectrum of microorganisms than their unesterified counterparts (Sofos et al., 1998).

Collectively, medium- and long-chain fatty acids do not respond uniformly to environmental conditions or food properties, nor do they act on similar types

of microorganisms. However, antimicrobial activity is generally optimized at pH values of 4.6 or less and involves cell membrane interactions which result in impaired function and leakage or the intracellular dissociation of fatty acid molecules (Burnett et al., 2007; Monk, Beuchat, & Hathcox, 1996; Oh & Marshall, 1992). Gram-positive bacteria, yeasts, and molds are generally fatty acid-sensitive (Table 14.4) (Nieman, 1954). Gram-negative bacteria are not usually inhibited by fatty acids of more than eight carbons in length (Conley & Kabara, 1973), although previous exposure to other types of stress may increase the likelihood of inhibition by medium- and long-chain fatty acids (Sofos et al., 1998). The antimicrobial activity of fatty acids may be reduced in the presence of serum albumin and other protein compounds, starches, cholesterol, lecithin, and calcium or magnesium ions (Sofos et al., 1998). Fatty acids are also less likely to be homogenously distributed within products which contain lipids (Sofos et al., 1998). Wang and Johnson (1997) described the limitations of coconut-derived monoglycerides in regard to lack of distribution in lipid phases of food products, and found that ethanol, sodium citrate (0.1%), propylene glycol, (0.1%) and glycine (0.1–0.2%) could also be used as carriers of fatty acids, without antagonizing antimicrobial activity.

Applications

Octanoic or caprylic acid (CH_3-$[CH_2]_6$-COOH; log P_{oct}, 2.845) has GRAS approval and may be used to decontaminate the surfaces of beef carcasses and as an antimicrobial on RTE meat and poultry products (Tables 14.5 and 14.6) (Leo et al., 1971). The compound is characterized by a moderately sweet odor/taste, reminiscent of butter or cheese and, at neutral pH values, is more effective as an antimicrobial than other acids (Doores, 2003). Lauramide arginine ethyl ester (LAE) dissolved in propylene glycol may be applied to the surfaces (200 ppm) of fresh meat and poultry cuts, and to nonstandardized RTE meat and poultry products (Table 14.6). When the antilisterial activities of several medium- and long-chain fatty acids (50–700 μg/ml of conjugated lauric acid, monolaurin, capric, lauric, myristic, palmitic, stearic, oleic, linoleic, or linolenic acid) were compared, lauric acid > monolaurin > capric acid most effectively inhibited the growth of *L. monocytogenes* in a broth system (Mbandi et al., 2004). The antilisterial activity of lauric acid, monolaurin, and capric acid was greater when used in combination compared to individual applications, although no combination (≤500 μg/ml) adequately inhibited growth of *L. monocytogenes* in emulsified beef tissue or in frankfurters during storage (5°C, 21 days) (Mbandi et al., 2004). Surface treatments of lauric arginate (4 or 8 ml of 5.0% or 2 ml of 10.0% solution placed into shrink wrap bags just before vacuum-packaging) substantially reduced *L. monocytogenes* counts on "table brown" hams after 24 h (4°C), and inhibited growth during extended storage (60 days) (Luchansky et al., 2005). Others have also reported the success of monolaurin treatments in the inhibition of *L. monocytogenes* associated with processed meat products (Doyle, 1999). The antimicrobial activity of monolaurin is temperature- and pH-dependent,

and increases as both parameters are lowered (Doyle, 1999; Monk et al., 1996; Oh & Marshall, 1992). When placed in pouches containing whole muscle oven-roasted turkey breast, oil-browned turkey breast, cured ham, roast beef, and comminuted roast beef just before vacuum-packaging or shrink-wrapping (93.3°C, 2 s), octanoic acid (1%; adjusted to pH 2 or 4 with phosphoric or citric acid, respectively) reduced *L. monocytogenes* counts (5 log CFU/sample reduced by 0.85–2.89 log CFU/sample) after 24 h at 5°C (Burnett et al., 2007). Octanoic acid was more effective at pH values of 4 compared to 2 and greater microbial reductions were observed after longer heat-shrink exposures (Burnett et al., 2007).

Quaternary Ammonium Compounds

Characteristics and Properties

Quaternary ammonium compounds (QAC) are surface active agents (surfactants) commonly added to personal hygiene, pharmaceutical, food industry, and environmental sanitation products to act as antiseptics, disinfectants, conditioners, and emulsifiers (International Programme on Chemical Safety [IPCS], n.d.). QAC contain ampiphilic regions that interact with and disrupt/damage cell membranes, ultimately resulting in the loss of action potential and death (Oyarzabal, 2005). Viruses, fungi, and bacteria are susceptible to QAC (Table 14.4), although gram-negative bacteria are less susceptible than gram-positive types especially at sublethal concentrations (Maxcy, Tiwari, & Soprey, 1971). Maxcy et al. (1971) examined acquired QAC-tolerance in *E. coli* and found that tolerant cultures grew more slowly in growth medium and were more sensitive than parental cultures and unlikely to persist in the environment. The development of QAC-tolerance was dependent upon the structure and degree of affinity of each compound for microbial cells, as determined by adsorption, filtration, and elution of remaining QAC after treatment (Maxcy et al., 1971).

Like many sanitizing solutions, the efficacy of QAC increases at higher temperatures, and the negative impact of organic matter on antimicrobial activity is enhanced at low temperatures (Table 14.7). Activity is reduced in the presence of metallic ions (hard water source) and optimal pH during application is food product- and microorganism-specific, although the highest level of activity is usually observed at acidic pH values (Cords et al., 2005). QAC are not compatible with anionic surfactants/detergents or soaps and form a film with residual antimicrobial activity (Chmielewski & Frank, 2003); thus, fermentative lactic acid cultures may be inhibited in meat products previously treated with QAC compounds. Cetylpyridinium chloride (CPC) is a surface-active QAC commonly added to toothpaste, mouthwash, and cough drop formulations due to its ability to impede attachment of plaque-forming bacteria to tooth enamel (McDonnell & Russell, 1999; Oyarzabal, 2005).

Table 14.7 Extraneous factors known to directly optimize (+) or antagonize (–) the antimicrobial activity of solutions used to decontaminate whole animal carcasses and/or parts

Classification	Antimicrobial agent	Temperature		pH			Organic soil	Hard water
		Low	High	<7	7	>7		
Organic acids	Acetic acid		+	+	–	–		
	Lactic acid		+	+	–	–		
	Citric acid		+	+	–	–		–
Chlorine compounds	Chlorine gas	–				–	–	–
	Hypochlorite	–	+			–	–	
	Chlorine dioxide					–	–	
	Acidified sodium chlorite			+	–	–	–	
	Monochloramine					–		
Oxidizing agents	Ozone	+	–	+		–	–	
	Hydrogen peroxide	–	+	+			–	–
	Peroxyacid		+	+		–	–	
	Electrolyzed oxidizing water			+		–	–	
Other	Cetylpyridinium chloride	–	+			+	–	–
	Trisodium phosphate							–
	Lactoferrin		+	+				

As derived from Axtell et al. (2006); Ayebah et al. (2005); Chmielewski and Frank (2003); El-Kest and Marth (1988); Hardin et al. (1995); Kaczur and Caulfield (1994); Leistner and Gouldm (2002); Moore, Griffiths, and Peters (2000); Naidu (2002); Prakash (2000); Restaino, Frampton, Hemphill, and Palnikar (1995); Russell and Axtell (2005); Schumb et al. (1955); Sofos Busta (1991); Stratford and Eklund (2003); Taylor, Rogers, and Holah (1999); White (1992); WHO (2004); Yang and Chen (1979).
Absence of symbol indicates negligible effect of a factor on the activity of the corresponding antimicrobial agent.

Applications

The efficacy of CPC as a decontamination fluid has been scientifically validated, and available data indicate that approved levels of CPC (Table 14.5) are capable of significantly reducing levels of pathogens encountered at multiple stages of meat and poultry processing (Belk, 2001; Huffman, 2002; Sofos & Smith, 1998; Sofos, Kochevar, Bellinger et al., 1999). Cecure^{tm} is a patented CPC solution (pH 7.1) developed by Safe Foods Corporation (North Little Rock, AR), and approved for use on fresh, processed, and RTE meat products (Safe Foods Corporation, 2008) (Table 14.5).

Prior to its approval as an antimicrobial, Ransom et al. (2003) compared the ability of CPC (0.5%) and other approved intervention technologies to inactivate *E. coli* O157:H7 populations on inoculated beef carcass tissue samples. CPC induced greater reductions (4.8 log CFU/cm^2) than 2.0% lactic acid, 0.02% acidified sodium chlorite, 2.0% acetic acid, 0.02% peroxyacetic acid, 1.0% lactoferricin B, or 0.001%

acidified chlorine (reductions of 3.3, 1.9, 1.6, 1.4, 0.7, or 0.4 log CFU/cm^2, respectively); reductions in pathogen counts on CPC-treated lean beef tissue were also greater than other treatments, although differences were less extreme (Ransom et al., 2003). Although applications of a 0.1% CPC solution were ineffective against *C. jejuni* attached to poultry skin, higher concentrations of CPC (0.5%) reduced pathogen counts more effectively than 10% trisodium phosphate (TSP) or 1.0% acidified sodium chlorite solutions (Arrit, Eifert, Pierson, Sumner, 2002). Following longer exposures (30 s vs. 3–10 min), CPC was also more effective than TSP or acidified sodium chlorite against *C. jejuni* on poultry skin (Arrit et al., 2002). In another study, 0.1% CPC preinoculation treatments (ambient temperature, 10 min) were sufficient to inhibit the attachment of *S.* Typhimurium to poultry skin (Breen et al., 1995), indicating that very low concentrations of CPC may mitigate future contamination events.

During simulated chilling (4°C) of beef carcass tissue, CPC (0.1% or 0.5%) added to the water spray (1 min, every 30 min for 10 h) reduced initial non acid-adapted *E. coli* O157:H7 counts (5.1 log CFU/cm^2) to below the level of detection after 10 h and acid-adapted cells were no longer detected after 24 h (Stopforth et al., 2004). Water-only spray treatments reduced populations by about 1 log CFU/cm^2 (Stopforth et al., 2004), while activity of CPC and other antimicrobial solutions (e.g., lactic acid) decreased against acid-adapted inocula of the pathogen. When applied to RTE polish sausages inoculated with *L. monocytogenes* (3–7 log CFU/g), CPC treatments (1%, 25°C, 30 s, 20 psi spray) immediately reduced pathogen counts (by 1–3 log CFU/g) and inhibited survivors for 42 days (0°C and 4°C) (Singh et al., 2005); no negative impacts on product color or texture were observed.

Chlorine Compounds

Characteristics and Properties

Chlorine gas (Cl_2) is formed during the electrolysis of hydrochloric acid or sodium chloride, and has a distinct odor which is discernible even at minute concentrations (0.001 mg/l) (Lueck, 1980). In general, chlorine compounds are effective against gram-positive, gram-negative, and acid-fast bacteria, fungi, and viruses (Table 14.4) (McDonnell & Russell, 1999). In contrast to many other decontamination solutions, gram-positive bacteria may be less susceptible to chlorine treatments than gram-negative types; bacteria associated with biofilms are also less sensitive to chlorine compounds (Chmielewski & Frank, 2003). Chlorine compounds are highly sensitive to alkaline pH and excess organic matter and slightly sensitive to water hardness (Table 14.7). Antimicrobial activity is also reduced at lower temperatures especially when applied at low concentrations (Khanna & Naidu, 2000). Thus, chlorine compounds are effective for relatively short periods of time and must be continually replaced with fresh solution (Lillard, 1980).

Multiple chlorine compounds, at various levels of free available chlorine (Cl_2, HOCl, OCl^-), are approved for use as food animal carcass decontaminants (Table 14.5). Hypochlorite (OCl^-), or bleach, is formed when a single chlorine binds a single oxygen; sodium hypochlorite (NaOCl) is the most popular chlorine derivative for commercial use (Khanna & Naidu, 2000). Sodium chlorite dissociates when combined with a GRAS weak acid to produce chlorous acid (HO_2Cl) and chlorine dioxide (ClO_2) (Warf & Kemp, 2001). The chlorous acid derivative, acidified sodium chlorite (ASC), is also approved for use as a carcass decontamination solution (Table 14.5). Chlorine dioxide is water-soluble, has 2.6 times the oxidizing capacity (1.57 eV) of chlorine, and exhibits a bactericidal action similar to that of ASC (Berg, Roberts, & Martin, 1986; Science Applications International Corporation, 2002). Chlorine dioxide is also more stable over a wider pH spectrum and in the presence of excess organic material, and is less corrosive than chlorine (Kaczur & Caulfield, 1994). Unlike chlorine, chlorine dioxide does not produce carcinogenic trihalomethane or haloacetic acid by-products (Khanna & Naidu, 2000). Monochloramine (NH_2Cl) is a tasteless, odorless mixture of water, chlorine, and ammonia which also generates fewer trihalomethanes than traditional chlorine and has been found to prevent formation and facilitate the breakdown of pre-existing biofilms (Axtell, Russell, & Berman, 2006; Moore et al., 2006; Russell & Axtell, 2005; White, 1992). Although a surface contact time of at least 1 h is required for monochloramine to equal chlorine in bactericidal activity, monochloramine is less reactive than other chlorine compounds, and, therefore, highly resistant to elevated concentrations of organic material (Russell & Axtell, 2005; White, 1992).

Applications

Chlorine compounds are highly effective oxidizing agents to which many microorganisms are susceptible (Cords et al., 2005; Doyle, 2005; McDonnell & Russell, 1999). For this reason, the use of chlorinated solutions for carcass decontamination purposes has been investigated with varying degrees of success (Axtell et al., 2006; Belk, 2001; Lim & Mustapha, 2004; Pohlman, Stivarius, McElyea, Johnson, & Johnson, 2002; Yang & Johnson, 2001). Calcium hypochlorite, chlorine gas, and electrically stimulated hypochlorous acid are approved for use in the United States for decontamination of: (1) whole red meat carcasses, quarters, and primals; (2) whole and eviscerated poultry carcasses as well as salvage parts; and (3) the water used in meat and poultry processing (Table 14.5). Chlorine dioxide is also approved for use in water used to process poultry carcasses (Table 14.5), and packaging materials, impregnated with chlorine dioxide, are available for commercial use (Appendini & Hotchkiss, 2002). ASC may also be applied to beef carcasses, parts and organs, poultry carcasses, parts and organs, and poultry carcass prechiller and chiller water systems (Table 14.5).

In one study, ASC controlled *Campylobacter* on poultry skin more effectively when applied after chilling, compared to prechilling applications, although the efficacy of prechilling applications may have been less apparent due to the presence

of *Campylobacter* in chiller tank water (Kemp, Aldrich, & Waldroup, 2000; Kemp & Schneider, 2002; Mead, Hudson, & Hinton, 1995). When added to the water source applied to beef carcasses during chilling (1 min, every 30 min for 10 h), ASC (0.12%) reduced *E. coli* O157:H7 counts (5.0 reduced to 1.6 log CFU/cm^2) on inoculated beef brisket adipose tissue during simulated spray-chilling (48 h) better than water alone (1 log reduction) (Stopforth et al., 2004). The application (1 oz/lb or 62.5 g/kg) of 300 ppm ASC to inoculated boneless 50/50 lean beef trimmings reduced *Enterobacteriaceae* counts by 1–3 log CFU/g and suppressed growth of total aerobic bacteria in ground beef derived from treated trimmings (90/10 and 73/27 lean) during storage (20 days) as chubs or under modified atmospheres (Bosilevac, Shackelford, Fahle, Biela, & Koohmaraie, 2004). Although higher concentrations of ASC have been associated with unappealing color and organoleptic properties in treated products, 300 ppm did not negatively affect the color, taste, or odor of ground beef made from treated trimmings (Bosilevac et al., 2004). At 1,200 ppm, ASC treatments resulted in the loss of redness and light surface color of fresh beef, which were increasingly evident as the pH of treatments was lowered (Lim & Mustapha, 2004).

Russell and Axtell (2005) estimated that the chlorine demand of typical poultry chiller water may be as high as 1,000–2,000 ppm. Such high concentrations of organic carbon found in recirculated chiller water deplete chlorine almost immediately, and approved levels of chlorine for use in chiller water (≤50 ppm) are quickly inactivated, providing little, if any effect against microorganisms (Lillard, 1980). In one study, fresh chlorinated (10 ppm) chiller water was significantly more effective against *C. jejuni* than recirculated (8 h) chlorinated (10 ppm) chiller water (Yang & Johnson, 2001). This attribute of chlorine compounds and other oxidizers deserves serious consideration, as the application of sublethal levels of antimicrobial compounds allows pathogens to survive and may potentiate subsequent resistance to higher concentrations (Yang & Johnson, 2001).

The chemical instability of chlorine derivatives and health concerns regarding potential carcinogenic by-products created during their degradation have led to increased use of nontraditional chlorine compounds (Moore et al., 2006). Nearly one quarter of U.S. municipal water treatment facilities have replaced traditional chlorine with monochloramine (NH_2Cl) in water disinfection for two reasons: (1) monochloramine remains reactive much longer and (2) monochloramine produces fewer trihalomethanes than chlorine (Moore et al., 2006); as an aside to potential health benefits, monochloramine-treated water is not accompanied by the taste of chlorine. Thus, the ability of sodium hypochlorite (50 ppm) vs. monochloramine (50 ppm) solutions to inactivate *E. coli* and *Salmonella* in commercial poultry carcass chiller water was investigated (Russell & Axtell, 2005). Although samples were collected from different lots of carcasses, pathogen counts associated with monochloramine treated water were lower than those recovered from sodium hypochlorite-treated chiller water (2.5– 8.6 vs. 6.7–25.7 CFU/ml, respectively) (Russell & Axtell, 2005). Monochloramine is recommended for use in high organic load, high oxygen demand situations which allow longer contact times (>1 h), like those encountered in poultry scalders, pickers, immersion chillers, and during the recycling of process

water, while the use of monochloramine in processes with short contact times (<1 h) is not recommended (Axtell et al., 2006).

Ozone

Characteristics and Properties

Ozone (O_3) is a naturally occurring, highly unstable colorless gas with an odor that can be detected at the end of a rainstorm. Chemical synthesis is possible via in situ introduction of ionizing radiation or intense electrical discharges through air (reacts with O_2), though ozone rapidly reverts back to O_2 plus O^- at ambient temperature (IOM, 2003). Ozone was originally used to decontaminate municipal water supplies in nineteenth-century Europe (Sopher, Graham, Rice, & Strasser, 2002). Ozone has an oxidation capacity of 2.07 eV, and is active against gram-negative and -positive bacteria, fungi, viruses, and spores (Table 14.4) (Muthukumarappan, Halaweish, & Naidu, 2000; Sopher et al., 2002). Yang and Chen (1979) found that temperature, pH, and organic load affect overall activity (Table 14.7), and efficacy increases during longer contact times. In general, the solubility of ozone decreases at higher temperatures, with the highest level of solubility occurring at 2°C (Yang & Chen, 1979). The antimicrobial activity of ozone is depleted in the presence of ≥2.5% sodium chloride (Yang & Chen, 1979). As a surface decontamination solution, ozone leaves very few harmful residues, although harmful bromide by-products may be produced during its decomposition (Sopher et al., 2002), and ozone is not compatible with rubber products (Smith & Hong-Shum, 2003). Ozone is a strong oxidizer which may irritate eyes and skin and other mucous membranes or impair respiratory tract or cardiovascular function (Smith & Hong-Shum, 2003). Very low levels (as low as 0.1 ppm) are sufficient to induce uncomfortable side effects in humans; at 1 ppm concentrations, humans begin to encounter temporary and reversible headaches and eye irritation, while exposure to ≥50 ppm may result in death (Masaki, 1986; Muthukumarappan et al., 2000; Smith & Hong-Shum, 2003).

Applications

Aqueous ozone may be applied as a wash or rinse to decontaminate meat and poultry products (Table 14.5). Like many oxidizers, ozone is less effective in the presence of proteins or other organic materials (Table 14.7) and approved levels of ozone have not been successful as antimicrobial interventions for meat products (Castillo, McKenzie, Lucia, & Acuff, 2003; Smith et al., 2001). When applied to the surfaces of inoculated (10^6–10^7 CFU) beef carcass tissue, an aqueous ozone (95 mg/l, 80 lb/in.^{2}, 28°C) solution followed by a water wash (28°C) was equally effective against *E. coli* O157:H7 and *S.* Typhimurium as water washing alone (Castillo et al., 2003). Smith et al. (2001) found that activated ozone, as generated by the Deligen II system (Bioxide Corporation), was not effective in inactivating *L.*

monocytogenes or *Salmonella* on beef hides, carcasses, or in ground beef during blending. However, when used in combination with 0.05% CPC or 5.0% acetic acid, ozone (1%, 15 min, 7.2°C) reduced total aerobic populations, coliforms, *E. coli*, and *S.* Typhimurium in inoculated ground beef, and did not adversely affect beef-odor or generate off-odors in treated samples (Pohlman, Stivarius, McElyea, Johnson et al., 2002). Gorman et al. (1995) found that aqueous ozone treatments, which followed water-spray treatments (16°C or 35°C), were effective against *E. coli* on beef adipose tissue; however, ozone was not effective when applied before water-spray treatments. Reagan et al. (1996) reported similar findings, and in both studies, hot water treatments (≥74°C) were more effective than combinations of hot water and ozone, or ozone-only treatments (Gorman et al., 1995; Reagan et al., 1996). When applied to carcasses during aging, ozone treatments (0.03 ppm) led to increased carcass shrinkage and discoloration, and while bacterial growth on treated carcass sides was inhibited, bacterial growth at the retail level and subsequent retail case life was not delayed in ozone-treated products (Greer & Jones, 1989).

The efficacy of ozone treatments also differs across species, strains, and planktonic vs. sessile cells. In one study, ozone was less effective against different strains of *L. monocytogenes*, and significantly higher concentrations were required to inactivate cells incorporated into biofilms (as compared to planktonic cells) (Robbins, Fisher, Moltz, & Martin, 2005). Although ozone treatments do not leave a residue or appear to alter sensory attributes of treated food products, the effect of ozone treatments on vitamin, mineral, and other nutritive factors is not well understood and requires further investigation. As an oxidizing agent it may affect these as well as well as other quality characteristics of foods, especially those containing lipids.

Peroxides and Peroxyacids

Characteristics and Properties

The most common peroxide compounds that may be used to decontaminate meat and poultry products are hydrogen peroxide and peroxyacid. Hydrogen peroxide (H_2O_2), also referred to as oxygenated or oxidizing water, is naturally found inside mucous membranes, neutrophils, or as a component of milk and honey (Cords et al., 2005). Hydrogen peroxide is also generated in food products during irradiation processes or during fermentation by LAB (Cords et al., 2005; Kuby, 1997; Lewis, Velásquez, Cuppett, & McKee, 2002). The decomposition of hydrogen peroxide generates hydroxyl radicals which interfere with or puncture cell membranes and/or damage DNA; such damage may directly result in death or initiate cellular suicide mechanisms (Cords & Dychdala, 1993). Susceptible microbes include viruses, fungi, and bacterial cells and spores (Table 14.4), although gram-negative bacteria are more susceptible than gram-positive types (Cords & Dychdala, 1993). Antimicrobial activity is optimized at high temperatures, an

acidic pH, and in the absence of organic contaminants (Table 14.7). Hydrogen peroxide in solutions also generates greater quantities of hydroxyl radicals when used in combination with salts and/or metallic ions (Schumb, Satterfield, & Wentworth, 1955). Cold temperatures, alkaline pH, fats, and proteins may also impede the activity of hydrogen peroxide (Table 14.7). Hydrogen peroxide does not generate carcinogenic or mutagenic compounds (Gleason, Gosselin, Hodge, & Smith, 1969).

Peroxyacetic acid (PAA) is a powerful oxidizer with a mechanism of killing and antimicrobial spectrum similar to that of hydrogen peroxide (Cords & Dychdala, 1993; Gagnaire, Marignac, Hecht, & Héry, 2002; WHO, 2004). Optimal applications of PAA occur under acidic pH and in the absence of organic material (Table 14.7). Cold to ambient temperature applications are effective, although greater efficacy is observed at higher temperatures; the presence of metallic ions (hard water) does not reduce activity (Table 14.7) (Cords et al., 2005; Gutzmann et al., 2000).

Applications

Very low concentrations of hydrogen peroxide may result in the bloating and/or bleaching of lean tissue and the oxidation of fats and muscle pigments (Hilgren & Gutzmann, 2002). This type of damage is observed less frequently when carcasses are treated with PAA instead of hydrogen peroxide (Hilgren & Gutzmann, 2002). Corrosion of food processing equipment is also less problematic when using PAA compared to other chemical solutions (Bricher, 2005). While PAA has been proven as a successful antimicrobial intervention for the decontamination of fruits and vegetables (Parish et al., 2003; Park & Beuchat, 1999; WHO, 2004), the majority of published data indicate that PAA is less effective in removing/inactivating microbial contamination associated with meat products (WHO, 2004). PAA treatments were less effective than 2% lactic acid in reducing *E. coli* O157:H7 on beef cuts and trimmings (Ransom et al., 2003). Similar observations were reported by Ellebracht et al. (2005). As part of a larger investigation (Stopforth et al., 2004), PAA was added to the water spray applied to beef carcass tissue (1 min, every 30 s for 10 h) during simulated chilling (–1 ± 2°C, 48 h). While it is unclear if freshly mixed PAA was applied throughout chilling, *E. coli* O157:H7 counts on PAA-treated tissue were generally similar to nontreated and water-treated controls (Stopforth et al., 2004). Castillo et al. (2005) investigated the use of PAA as a postchilling antimicrobial intervention for beef carcasses and trimmings. While PAA (1,000 ppm, 43–55°C, spray) reduced *E. coli* O157:H7 and *S.* Typhimurium by 1.7 and 1.3 log CFU/cm^2, respectively, lactic acid treatments (4%, 55°C, spray) resulted in significantly higher reductions (Castillo et al., 2005). The FDA has approved Inspexx^{tm}, a 2% peroxyacetic acid solution mixture manufactured by Ecolab Inc. (Saint Paul, MN) (Ecolab, 2008). The manufactures of Inspexx™ recommend use of PAA-specific dispensing equipment in order to optimize results.

Electrolyzed Oxidizing Water

Characteristics and Properties

Electrodes placed in aqueous solutions of sodium chloride (approximately 0.1%) generate acidic (pH 2.6) and alkaline (pH 11.6) electrolyzed oxidizing water (EO) (Kim, Hung, & Russell, 2005). An anode electrode generates acidic EO which is essentially diluted hypochlorous acid (10–100 ppm free available chlorine) with an oxidation reduction potential of 1,100 mV (Kim et al., 2005). Higher initial concentrations of sodium chloride (>0.1%) generate acidic EO with a lower pH and more free available chlorine (Ezeike & Hung, 2004; Kim, Hung, & Brackett, 2000). In general, the antimicrobial activity of acidic EO is compromised in the presence of organic material, specifically protein, and optimal activity is exhibited by fresh solutions with an acidic pH (Table 14.7). It is important to note, however, that the activity of acidic EO is highly dependent upon residual chlorine concentration and chlorine is preserved at alkaline pH (Al-Haq, Sugiyama, & Isobe, 2005). As an antimicrobial agent, acidic EO interferes with cell membrane electrons and increases membrane permeability (Jay, 2000).

Alkaline EO (NaOH) is generated by the cathode electrode, contains low levels of sodium hydroxide, and has an oxidation reduction potential of -800 mV (Kim et al., 2005; Venkitanarayanan, Ezeike, Hung, & Doyle, 1999). Alkaline EO is surface-active due to hydrogen, hydrogen peroxide, and NaOH components (Shirahata, 2001; Yamanaka, 1995). Kim et al. (2005) suggested that alkaline EO may function similarly to TSP.

Applications

Acidic and alkaline EO has been applied as antimicrobials with varying degrees of success, although the bulk of publications discuss acidic, rather than alkaline EO applications. The majority of data regarding the success of acid or alkaline EO treatments describe their use as sanitizers or in the decontamination of fruit and vegetables (Chiu, Duan, Liu, & Su, 2006; Deza, Araujo, & Garrido, 2005; Park, Hung, & Kim, 2002; Venkitanarayanan, Ezeike, Hung et al., 1999). In one study, acidic EO treatments (15 s) applied to fresh pork inoculated with *Campylobacter coli*, *L. monocytogenes*, and *S.* Typhimurium were effective only against *C. coli* (Cutter & Fabrizio, 2004). In another study, Kim et al. (2005) found that immersion (40 min) in acidic EO effectively reduced *C. jejuni* counts on poultry skin by 2.5 log CFU/g; these reductions were similar to those achieved during immersion in chlorine-treated (73.1 mg/l) chiller water. Park, Hung, and Brackett (2002) reached similar conclusions with *C. jejuni* counts on chicken skin which were reduced (3 log CFU/g) during immersion treatments. *Campylobacter jejuni* cells on chicken skin were also inactivated during immersion in acidic EO, minimizing the likelihood of cross-contamination of carcasses via immersion chiller water (Park,

Hung, & Brackett, 2002). Fabrizio, Sharma, Demirci, and Cutter (2002) found that acidic EO reduced (1 log) *S.* Typhimurium (3 log) on broiler carcasses during storage (7 days, 4°C), although no immediate reductions were observed. Other treatments (2% acetic acid and 10% TSP) provided greater immediate reductions and were more effective during storage (Fabrizio et al., 2002). West et al. (2001) noted that greater reductions in *E. coli* O157:H7, *L. monocytogenes*, *and Salmonella* were observed on inoculated chicken strips compared to those on inoculated beef strips. Such variation might have been due, in part, to differences in fat content and lean texture (West et al., 2001).

As the intrinsic properties (i.e., pH, oxidation reduction potential) of alkaline EO solutions are similar to those of TSP, a study was designed to compare the ability of alkaline EO and TSP surface treatments to remove, and also prevent the attachment of *C. jejuni* inoculated feces to chicken skin during immersion chilling (Kim et al., 2005). Preinoculation spray-applications of alkaline EO reduced subsequent attachment of *C. jejuni*; however, 10% TSP and alkaline EO were equally effective with tap water in removing fecal material when applied postinoculation (Kim et al., 2005).

Acidic and alkaline EO may be more effective as food animal carcass decontamination solutions when used in combination (Ayebah, Hung, & Frank, 2005), and both forms of EO appeared to be more effective at 25°C than at 4°C (Fabrizio & Cutter, 2003). The reduced cost and fewer environmental hazards associated with EO treatments compared to other interventions may warrant further investigation of EO as an antimicrobial (Fabrizio et al., 2002; Kim et al., 2005). Furthermore, many consumer groups have recently endorsed the use of EO in the food industry as it is generated from sodium chloride, and therefore, may seem less intimidating or chemical-like, compared to other technologies (Guan & Hoover, 2005).

Phosphates

Characteristics and Properties

Treating phosphoric acid with sodium, calcium, or potassium generates two categories of phosphate salts: orthophosphates and condensed phosphates, which are commonly used to improve the color, flavor, and water-holding capacity of cured meat products (Prakash, 2000). Phosphates are polyvalent anions which aggregate at cell surfaces and readily bind the positively charged proteins and divalent cations (e.g., Co^{2+}, Ca^{2+}, Mg^{2+}, Fe^{3+}) required for metabolic purposes and replication (Lueck, 1980; Shelef & Seiter, 2005). While polyphosphates remove metallic ions from the extracellular matrix, metallic ions may also be plucked from the membranes and cytoplasm of cells (Prakash, 2000). Therefore, in the presence of phosphate compounds, microbes are deprived of divalent cations and free available water required for metabolic functions (Leistner et al., 1981; Lueck, 1980). Molds and many species of gram-positive bacteria are sensitive to phosphates (Table 14.4) (Prakash, 2000). Gram-negative bacteria are less susceptible,

due to differences in cell wall composition (Knabel, Walker, & Hartman, 1991), although Somers, Schoeni, and Wong (1994) found that TSP treatments were effective against both gram-negative (i.e., *C. jejuni*, *E. coli* O157:H7, and *S.* Typhimurium) and gram-positive (i.e., *L. monocytogenes*) pathogens.

TSP is a tribasic orthophosphate with a pH of 10.0–12.0 in aqueous solution, and overall alkalinity aids in its interaction with cell membrane lipids, resulting in membrane leakage (Oyarzabal, 2005; Prakash, 2000). TSP may also increase the sensitivity of some microorganisms to nisin and lysozyme (Caneiro de Melo, Cassar, & Miles, 1998; Oyarzabal, 2005). As a general rule of thumb, antimicrobial activity is greater with increasing chain length of polyphosphates; exceptions include acidic polyphosphates and TSP (Prakash, 2000). Long-chain polyphosphates are heat sensitive, and should be applied to foods after thermal treatment. Polyphosphates are also degraded by phosphatases associated with raw muscle (Prakash, 2000). Polyphosphates are typically applied in combination with other antimicrobials; one popular combination is sorbate and a polyphosphate (Nelson, Busta, Sofos, & Wagner, 1983). Hard water sources reduce the activity of polyphosphates as they bind metal anions within the solution prior to application, and subsequent chelation cannot occur (Leistner & Gould, 2002). The human body contains large quantities of phosphorous as a constituent of bones and teeth (Prakash, 2000) and toxic doses of phosphorous are rarely encountered. Acceptable daily intake for persons 9–70 years of age is approximately 4,000 mg and 3,000 mg for those over 70 years of age (Prakash, 2000; Yates, Schlicker, & Suitor, 1998).

Applications

Fresh Meat. TSP is approved for use in the United States and approved levels (Table 14.5) have been shown to reduce bacterial contamination on poultry and beef carcasses by 1–2 log cycles (Doyle, 2005). TSP (8–12%) reduced *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* counts on inoculated beef tissue by 1–1.5 log cycles, and was more effective at higher temperatures (40°C or 50°C compared to 25°C) or when applied as a wash instead of an immersion treatment (Dickson, Nettles Cutter, & Siragusa, 1994). Cutter and Rivera-Betancourt (2000) found that 10% TSP was more effective than lactic or acetic acid treatments (2.0%, 35 ± 2°C, 125 ± 2 psi) against *S.* Typhimurium and *E. coli* O157:H7 on inoculated beef tissue. When applied to lamb breasts inoculated with fecal paste, TSP (12%, 55°C, 9 s dip) reduced *E. coli* O157:H7 and aerobic plate counts by 1.8 and 0.7 log CFU/cm^2, respectively (Ramirez, Acuff, Lucia, & Savell, 2001); lactic acid (2%, 55°C, 9 s) was equally effective against *E. coli* O157:H7 and slightly more effective in reducing aerobic plate counts (1.5 log CFU/cm^2). TSP treatments may also inhibit subsequent bacterial attachment to carcass surfaces, augmenting the removal of such microbes during ensuing carcass washes (Cabedo, Sofos, & Smith, 1996; Gorman et al., 1997; Sofos, Belk et al., 1999; Sofos, Kochevar, Bellinger

et al., 1999; Sofos, Kochevar, Reagan et al., 1999). Capita, Alonso-Calleja, Garcia-Fernandez, and Moreno (2002) and Kim et al. (2005) found that preimmersion chiller TSP treatments removed the thin outermost layer of fat from poultry skin, preventing lipid-smearing and the subsequent entrapment of microorganisms inside de-feathered skin follicles (Capita et al., 2002; Kim et al., 2005). Meager microbial reductions and improved color were observed in ground beef derived from trimmings treated with 10% TSP (Pohlman, Stivarius, McElyea, & Waldroup, 2002). Undegraded phosphatases present in fresh ground meats may account for the inability of polyphosphate treatments to control associated microorganisms (Flores, Sumner, Peters, & Mandigo, 1996).

Processed Meats. Health concerns associated with added salt in cured meat products initiated an interest in the use of polyphosphates as an alternative or adjunct curing agent (Sofos, 1986). While the primary functions of phosphates in cured or otherwise processed meat products include increased tenderness, water-holding capacity, color stabilization, and added flavor (Shelef & Seiter, 2005; Vote et al., 2000), a select group of polyphosphates also possess antimicrobial properties (Sofos 1986). Phosphates with known antimicrobial properties include disodium phosphate (DSP), sodium tripolyphosphate (STPP), sodium acid pyrophosphate (SAPP), tetrasodium pyrophosphate (TSPP), sodium tetrametaphosphate (STMP), and sodium hexametaphosphate (SHMP) (Prakash, 2000; Shelef & Seiter, 2005). SAPP (0.4%) inhibited botulinum toxin production in frankfurter emulsions containing nitrite and sorbate (Wagner & Busta, 1983). SAPP also prolonged the shelf life of low-sodium chloride comminuted meat products inoculated with *Clostridium sporogenes* (Madril & Sofos, 1986). In another study, a 1% sodium polyphosphate solution was sufficient to inhibit growth of *L. monocytogenes*, and increased inhibition was observed when pH or temperature was lowered, or in the presence of sodium chloride (Zaika & Kim, 1993). Polyphosphates also increase the antimicrobial effect of nitrite, sodium chloride, and low pH conditions (Wagner & Busta, 1983).

While meat enhancement has been shown to retard microbial spoilage and may inhibit pathogen growth, the physical displacement of microbes on outer surfaces to the interior region of whole muscle cuts during brine injection and/or the contamination of re-circulated stock brine solutions during enhancement procedures warrant considerable concern (Heller et al., 2007; Wicklund et al., 2005). For these reasons, the development and use of brine solutions which destroy and/or retard the growth of internalized spoilage microorganisms and pathogens should be investigated. In one study, brine solutions containing a combination of 0.4% sodium chloride, 0.4% phosphate, 3.0% sodium lactate, and 0.25% sodium diacetate were more effective against *E. coli* K-12 than solutions which lacked one or more of the aforementioned compounds (Wicklund et al., 2005). Greater microbial reductions (4 vs. 2 log cycles) were achieved when solutions were heated to 70°C instead of 50°C, respectively, indicating that heating re-circulated brine solutions may also minimize cross-contamination events during enhancement processes (Wicklund et al., 2005).

Sodium Chloride

Characteristics and Properties

Sodium chloride (NaCl), or common table salt, was among the primary food preservation tools used by ancient humans and its antimicrobial properties are well documented (Sofos, 1984; Sofos & Busta, 1981; Sofos, Busta, & Allen, 1979a). NaCl is used extensively as a nutrient vehicle (typically supplemented with 0.006–0.01% potassium iodide), flavoring or curing agent, and dough conditioner (IOM, 2003). The antimicrobial action of NaCl involves cellular dehydration resulting in growth inhibition of most pathogenic microorganisms by ≤13% NaCl (Schmidt, Mawson, & Siegel, 1981; Sofos, 1984). Secondary inhibition may follow the extraction of extracellular nutrients and available water (lowering a_w) from the surrounding environment (Schmidt et al., 1981; Sofos, 1984). In one study, McKellar (1993) found that sublethal levels of NaCl in tryptic soy broth interfered with the ability of *L. monocytogenes* to secrete the toxin listeriolysin O.

Advances in food preservation techniques, such as refrigeration, vacuum-packaging, and the use of other antimicrobial ingredients have minimized dependence on NaCl as a preservative. Modern health concerns and the unpleasant sensory effects associated with excessive amounts of NaCl also discourage its use at concentrations sufficient for its autonomous control of microbial populations (Marth, 1998; Sofos, 1984). However, when used in conjunction with other additives, NaCl further reduces a_w and inhibits salt-sensitive microbes (Beuchat, 1983; Troller, 1980). Enhanced activity is observed in the presence of benzoates, nitrite, sorbates, phosphates, spices, liquid smoke, and various antioxidants (Sofos, 1984). Previous heat treatments, storage under refrigeration, and low pH may also increase the sensitivity of microorganisms to acceptable levels of NaCl (Roberts & Ingram, 1966; Sofos, 1984; Troller, 1980).

Applications

The ability of NaCl to control spoilage organisms has been thoroughly investigated and is well accepted (Sofos, 1984; Sofos & Busta, 1980; Sofos et al., 1979a). Most spoilage and pathogenic microorganisms are unable to tolerate the levels of NaCl in fermented meat product formulations, while LAB proliferate in the absence of competition (Sofos, 1984). Levels required for inhibition are generally lower at refrigeration than at ambient temperatures, although the bactericidal effects of NaCl may be enhanced at higher temperatures (Segner, Schmidt, & Boltz, 1966; Sofos, 1984). Pelroy, Peterson, Paranjpye, Almond, and Eklund (1994) found that the effectiveness of sodium nitrite and NaCl combinations against *L. monocytogenes* was affected by inoculum level, packaging conditions, time, temperature and concentration of NaCl (3% vs. 5%). Sodium nitrite plus 5% water-phase NaCl was the most effective combination.

Nitrite

Characteristics and Properties

Nitrite, the main focus of another chapter (Chap. 1), is used as a curing agent to stabilize color, enhance flavor, and inhibit spoilage organisms and pathogens in cured meat products. The antimicrobial action of nitrite against *Clostridium botulinum* involves activity against the iron-containing enzymes ferredoxin and pyruvate oxidoreductase (Carpenter, Reddy, & Cornforth, 1987; Tompkin, Christiansen, & Shaparis, 1978). Various other bacteria, both gram-positive and -negative, are also inhibited by nitrite (Table 14.4) (Tompkin, 2005), although specific modes of action are unclear. In one study, the addition of nitrite inhibited growth and listeriolysin O secretion in *L. monocytogenes* (McKellar, 1993). The antimicrobial activity of nitrite is optimized at an acidic pH and in the presence of NaCl (Buchanan, Stahl, & Whiting, 1989; Duffy, Vanderlinde, & Grau, 1994; Silliker, Greenberg, & Schack, 1958; Sofos et al., 1979a). Anaerobic packaging conditions and refrigeration temperatures also enhance the activity of nitrite, as do nitrates (serve as a source of nitrite), ascorbates, polyphosphates, and sorbates (Buchanan et al., 1989; Duffy et al., 1994; Silliker et al., 1958; Sofos et al., 1979a; Tompkin, 2005). Heating (90°C, 20 min) also increased the efficacy of previously subinhibitory concentrations of nitrite against *C. botulinum*, and similar activity against other pathogens may be possible (Perigo, Whiting, & Bashford, 1967). As a toxic substance at high levels, nitrite use is strictly controlled and regulated. Concerns for potential carcinogenic activity, either directly or indirectly as a substrate for nitrosamine formation under certain conditions, are still controversial (Archer, 2002; Bunin, Kuijten, Buckley, Rorke, & Meadows, 1993; McKnight, Duncan, Leifert, & Golden, 1999; Sarasua & Savitz, 1994; Sofos et al., 1979a; Tompkin, 2005).

Applications

Sodium and potassium nitrite are commonly added to cured meat products including bacon, corned beef, ham, delicatessen meats, frankfurters, and perishable canned cured meats (Sofos et al., 1979a) for the ability of nitrous oxide to stabilize color, enhance flavor, and impede oxidative rancidity. Nitrite is also used for its ability to inhibit botulism toxin production in these products (Christiansen, Johnston, Kautter, Howard, & Aunan, 1973; Christiansen et al., 1974; Sofos et al., 1979a; Sofos, Busta, & Allen, 1979b; Tompkin et al., 1978). The antimicrobial activity of nitrite against *C. botulinum* and botulinum toxin production in cured meat products has been explored extensively, and while nitrite is effective in retarding toxin production, its activity generally decreases as spore load increases (Christiansen et al., 1973; Sofos, Busta, & Allen, 1979c; Sofos et al., 1979b). Evidence indicates that a number of additional pathogens, specifically *L. monocytogenes*, may also be inhibited by nitrite (Gill & Holley, 2003; Grau

& Vanderlinde, 1992; McClure, Beaumont, Sutherland, & Roberts, 1997; Sofos, 1994). Fu, Sebranek, and Murano (1995) reported reductions in *Salmonella* and *L. monocytogenes* populations (7°C) on nitrite-cured ham. In another study, cooked slices of beef, pork, chicken, or turkey were formulated with sodium nitrite (3 μM) or sodium tripolyphosphate (0.3%), inoculated with *L. monocytogenes*, vacuum-packaged, and then stored at 0°C or 5°C (Duffy et al., 1994). Sodium nitrite increased the duration of lag phase, doubling the time required for *L. monocytogenes* populations to increase by 3 log cycles, while sodium tripolyphosphate increased overall growth rate due to elevated product pH (Duffy et al., 1994). Duffy et al. (1994) found that nitrite was more effective in inhibiting *L. monocytogenes* when used in combination with ascorbate (0.042%) although ascorbate alone did not inhibit the growth of *L. monocytogenes*. Buncic, Fitzgerald, Bell, and Hudson (1995) and Rayman, Aris, and Hurst (1981) reported enhanced inhibition of *C. botulinum* and *L. monocytogenes* by nitrite when used in the presence of nisin. Vignolo, Fadda, de Kairuz, de R. Holgado, and Oliver (1998) found that the ability of nitrite to inhibit *L. monocytogenes* in a meat slurry system was also increased in the presence of another bacteriocin, lactocin 705.

Processing treatments (e.g., heat, irradiation) and surviving microorganisms may immediately or gradually deplete nitrite levels in meat products (Grau & Vanderlinde, 1992; Whiting & Masana, 1994). Thus, from a microbiological standpoint, postprocessing residual levels are more critical than initial levels of nitrite (Grau & Vanderlinde, 1992; Whiting & Masana, 1994). Fu et al. (1995) reported greater reductions in *Salmonella* and *L. monocytogenes* populations (7°C) on cured ham treated with nitrite and low dose radiation (0.75–0.90 kGy) applied in combination compared to nitrite alone. In contrast, Ahn, Kim, Jo, Lee, and Byun (2002) found that application of gamma-irradiation (≥10 or 20 kGy in aerobic or vacuum-packaging, respectively) could significantly reduce carcinogenic *N*-nitrosamines and residual nitrite in a model sausage system. The MIC of nitrite and nitrate against foodborne pathogens other than *C. botulinum*, specifically *L. monocytogenes*, within specific products is not known, and should be investigated. Knowledge of the effects of common processing technologies on nitrite depletion would also be particularly useful.

Fermentative and Antagonistic Cultures

Characteristics, Properties, and Applications

In general, acidic foods (pH ≤ 4.6) are of less concern regarding growth of spoilage and pathogenic organisms. The pH of foods differs naturally or, may be lowered with direct addition of organic acids or their production by LAB (Hammes, Bantleon, & Min, 1990; Savic, 1985). Most LAB have GRAS status, and may be

used to alter the chemical, physical, and organoleptic properties of food products, and to create an environment unsuitable for spoilage or pathogenic organisms (Jay et al., 2005). Although competition for available nutrients may be sufficient to inhibit other microorganisms, LAB also metabolize simple carbohydrates to form organic acids (discussed separately), thereby lowering the pH of a product and further inhibiting less acid-tolerant microbes. Other inhibitory compounds, such as hydrogen peroxide, ethanol, diacetyl, and free fatty acids, are also formed during the metabolic processes of LAB (Koutsoumanis & Sofos, 2004; Sofos et al., 1998), and are discussed in other sections of this chapter.

Inhibition by LAB may be due to several factors, including microbial antagonism, production of acid, or presence of inhibitory metabolites. The use of LAB metabolites to inhibit pathogens could be rather practical based upon the following rationale: (1) LAB are already added to raw meat formulations to initiate fermentation and impart desirable product characteristics, (2) the ability of such cultures to produce inhibitory metabolites does not need to be developed or engineered, and (3) many of these metabolites inhibit growth of spoilage organisms as well as pathogens. As an example, the temperature and pH conditions during the production of Spanish dry sausage are similar to those at which *Lactobacillus sakei* produces the highest level of sakacin K, a metabolite with known antilisterial properties (Leroy & De Vuyst, 1999).

Biopreservation of foods using LAB has been, and continues to be, an area of investigation. In one study, Kang and Fung (1999) found that *Pediococcus acidilactici* strains were more effective than other starter cultures in controlling growth of *E. coli* O157:H7 during fermentation. In another study, however, Lahti, Johansson, Honkenen-Buzalski, Hill, and Nurmi (2001) found that *L. monocytogenes* and *E. coli* O157:H7 responded inversely to one another during fermentation, and were not inactivated by the same fermentative cultures. Recently, a composite of LAB including *Lactobacillus acidophilus* (NP35, NP51), *Lactobacillus lactis* (NP7), and *P. acidilactici* (NP3) was approved for addition to nonstandardized comminuted meat products such as ground beef patties (10^6–10^8 CFU/g of product), whole poultry carcasses (10^7 CFU/ml, applied by dipping), and fresh whole muscle cuts or ground poultry products (10^5–10^6 CFU/g of product) (USDA/FSIS, 2007). *Carnobacterium maltaromaticum* CB1 may also be added to RTE comminuted meat products (USDA/FSIS, 2007). In one study, a four strain composite of *L. acidophilus* (NP 51, NP 25, NP 7, NP 3) was added (7 log CFU/ml) to ground beef patties inoculated (5 log CFU/ml) with *E. coli* O157:H7 and *Salmonella* and stored at 5°C (Smith, Mann, Harris, Miller, & Brashears, 2005). After 3 days of storage, untrained panelists (n = 24, 3 replications) did not detect significant differences between treated and control groups, and after 5 days of storage *Salmonella* was inactivated (enrichment negative) and *E. coli* O157:H7 counts were reduced by 3 log CFU/ml (Smith et al., 2005). When added to ground beef, *Lactobacillus reuteri* (3 or 6 log CFU/g) plus glycerol significantly reduced *E. coli* O157:H7 (3 and 6 log CFU/g) during storage (4°C, 25 days) under modified atmospheres (Muthukumarasamy, Han, & Holley, 2003). Additional research examining antimicrobial mechanisms, target organism susceptibility, large-scale

trained panelist evaluation of sensory attributes, and consumer perception of such products is needed to substantiate the use of LAB in fresh ground meat.

Bacteriocins and Antibiotics

Characteristics, Properties, and Applications

Bacteriocins are small secondary metabolites produced by bacteria (generally LAB) as a means of inhibiting other sensitive species or strains (Abee & Delves-Broughton, 2003; Sofos et al., 1998; Thomas, Clarkson, & Delves-Broughton, 2000). These positively charged peptides typically interact with the anionic phospholipids of gram-positive cell membranes instead of a specific protein receptor. Bacteriocin–cell interactions disorder cell membrane lipids and result in the loss of membrane action potential, impaired membrane function, and leakage or lysis (Bhunia, Johnson, & Ray, 1988; Epand & Vogel, 1999; Ray & Miller, 2000). Strains of *L. monocytogenes* are sensitive to numerous bacteriocins, and in one study, 20 of 21 types of LAB produced bacteriocins capable of inhibiting *L. monocytogenes* under optimal growth conditions (Lewus, Kaiser, & Montville, 1991). Many bacteriocins are susceptible to enzymatic proteolysis; thus, antimicrobial activity may be gradually depleted in products which are not heated/cooked during processing as such processes inactivate proteolytic enzymes (Abee & Delves-Broughton, 2003; Ray & Miller, 2000). In general, toxicity is of little concern as bacteriocins are rapidly degraded by proteolytic enzymes in the human digestive tract (Lueck, 1980).

Nisin. Nisin, the only bacteriocin approved for use in foods in the United States (Table 14.6), is a hydrophobic peptide produced by *Lactococcus lactis* subsp. *lactis* which is active against a more diverse spectrum of gram-positive bacteria than other bacteriocins (Table 14.4) (Abee & Delves-Broughton, 2003; Rogers & Whittier, 1928; Thomas et al., 2000). In general, nisin is not effective against yeasts, molds, or gram-negative bacteria (Table 14.4). However, some types of gram-negative bacteria were susceptible to nisin after being exposed to a chelating agent (Delves-Broughton & Gasson, 1994). As a member of the lantibiotic family of bacteriocins, nisin attacks and forms pores on microbial cell membranes (Abee & Delves-Broughton, 2003). A net cationic charge allows the peptide to interact with the large anionic population of cell membrane associated-phospholipids and subsequently disrupt membrane organization and function (Crandall & Montville, 1998). Five nisin derivatives (A, B, C, D, and E) exist, although variant A exhibits the highest level of antimicrobial activity (Smith, 2003). Nisin compounds are effective over a broad pH range (3.5–8.0) and its incorporation into calcium alginate gels may preserve antimicrobial activity during product storage (Cutter & Siragusa, 1996). Synergistic inhibition by nisin and lysozyme combinations have been reported (Chun & Hancock, 2000). Low temperatures (i.e., refrigeration) may decrease antimicrobial activity as the reorganization of cell membrane lipids

at refrigeration temperatures obstructs nisin-membrane lipid interactions (Abee & Delves-Broughton, 2003). Antimicrobial activity may also be reduced in the presence of sodium metabisulfite, titanium dioxide, ethylene diamine tetraacetic acid (EDTA), or cations such as Ca^{2+}, Mg^{2+}, and Gd^{3+}, and in high pH products or those with high levels of microbial contamination (Abee & Delves-Broughton, 2003; Smith, 2003; Thomas et al., 2000; Zhang & Mustapha, 1999). As a lipophilic peptide, nisin tends to be unevenly distributed in high-fat products, resulting in areas which are free of, or contain subinhibitory levels of the peptide (Jung, Bodyfelt, & Daeschel, 1991; Ray & Miller, 2000).

Pediocin. Pediocins are class II bacteriocins produced by LAB of the genus *Pediococcus* and by other gram-positive bacteria (Garvie, 1986). Other class II bacteriocins include carnobacteriocins, sakacins, and leucocins (Holzapfel, Schillinger, Geisen, & Lucke, 2003). Antimicrobial action is similar to that of nisin (Ray & Miller, 2000) and pediocin is most effective against gram-positive bacteria, although some strains of *E. coli* may also be sensitive (Table 14.4) (Osmanagauoglu, Gündüz, Beyatli, & Çökmus, 1998). Others have also observed sensitivity in previously stressed or injured gram-negative bacteria (Kalachayanand, Sikes, Dunne, & Ray, 1998; Ray, 1993; Ray & Daeshel, 1992). Pediocins may trigger suicide mechanisms in susceptible cells by inducing the unregulated transcription of lytic enzymes involved in cell lysis (Bhunia et al., 1988; Bhunia, Johnson, & Kalchayanand, 1991; Ray & Miller, 2000). Pediocins are tolerant to heat (121°C, 15 min) and high hydrostatic pressure (50,000 psi), and are effective at pH values between 3.0 and 9.0 (Kalachayanand et al., 1998; Osmanagauoglu et al., 1998). The presence of some anions (Cl^-, I^-, $P[O_4]^{3-}$) in high-salt formulations may antagonize pediocin–phospholipid interaction (Bhunia et al., 1991). Pediocin is a hydrophobic compound, and therefore, less likely to be homogenously distributed in high-fat formulations (Jung et al., 1991; Ray & Miller, 2000).

Natamycin. Natamycin, also known as pimaracin, belongs to the polyene family of antibiotics; (a group of antifungal agents which target and bind to eukaryotic sterols and specifically ergosterol), and it is a secondary metabolite of *Streptomyces natalensis* (Thomas, 1986). Very low levels (10–20 ppm) are needed to inhibit almost all yeasts and molds (Table 14.4), while no amount of natamycin is sufficient to inhibit most bacteria, as they lack the sterol targeted by natamycin (some gram-positive types may be susceptible) (Stark & Tan, 2003). Thus, natamycin may be used to retard the growth of fungi in meat products to which fermentative cultures are added, and is typically applied as a surface treatment (i.e., dip or spray) (Delves-Broughton, Thomas, Doan, & Davidson, 2005). Resistant organisms are not typically encountered even though natamycin has been used as a food preservative for more than three decades (Stark & Tan, 2003). Unlike most bacteriocins, natamycin is toxic to eukaryotes. Acceptable daily intake of natamycin for humans is 0–0.3 mg/kg of body weight (FAO/WHO, 1976).

The bacteriocin nisin has been approved for use in the United States as an antimicrobial agent (Table 14.6) and a blend of encapsulated nisin, rosemary extract, and sodium chloride is approved for use in frankfurters (500 ppm of product formulation) and similar types of cooked meat and poultry sausages;

a similar blend which also includes maltodextrin and cultured dextrose or sodium diacetate may be added to cooked RTE meat and poultry sausages and to cured meat products (Table 14.6). Nisaplin™ (Aplin & Barrett Ltd., Dorset, England) is a commercially available form of nisin and it is also approved for use in meat products.

Delves-Broughton (2005) found that sausages formulated with (1.25–6.25 mg/kg) or dipped in (5.0–25.0 mg/l) nisin exhibited a longer shelf life (6°C and 12°C) than untreated controls. Nisin was less effective in high-fat sausages and in the presence of orthophosphates (vs. diphosphates) (Delves-Broughton, 2005). Samelis et al. (2005) found that dipping sliced pork bologna in nisin (125 μg/ml) plus sodium diacetate (3 μg/ml) was more effective against *L. monocytogenes* (2–3 log CFU/cm^2) during vacuum-packaged storage (90 days, 4°C, vacuum-packaged) than nisin plus lactic or acetic acid, sodium actetate, sodium diacetate, potassium benzoate, or potassium sorbate, although nisin plus acetic acid (3 and 5 μg/ml) or potassium benzoate (3 μg/ml) also inhibited *L. monocytogenes* for 90–120 days. When used alone, nisin reduced initial populations but did not inhibit *L. monocytogenes* during storage (Samelis et al., 2005). Similar results, regarding the synergistic activity of nisin and organic acids, were reported by Geornaras et al. (2005). Rayman et al. (1981) found that nisin (75 ppm) was more effective than nitrite (125 ppm) against *C. sporogenes* PA3679 within a meat slurry system. Nisin was not affected by high levels of iron (meat component or iron salts), but was gradually depleted to subinhibitory levels during storage (4°C, 56 days) as spore level and/or pH increased. Nisin (75–100 ppm) plus nitrite (40 ppm) inhibited *C. sporogenes* PA3679 spores throughout extended temperature abuse (56 days at 25°C); when added individually and at much higher concentrations, neither nisin nor nitrite was inhibitory (Rayman et al., 1981). Combinations of nisin and lysozyme were shown to effectively inactivate gram-positive bacteria, including *S. aureus*, due to rapid depolymerization of cell membranes which results in increased damage to membranes (Chun & Hancock, 2000). In the presence of sodium lactate, nisin inactivated gram-negative and gram-positive bacteria in fresh pork sausage (Scannell et al., 1997). Previous exposure to ≤5 mM TSP resulted in increased sensitivity of *C. jejuni* to nisin (Caneiro de Melo et al., 1998). Similar observations were made regarding increased sensitivity of *L. monocytogenes* to nisin in the presence of 5% ethanol (Brewer, Adams, & Park, 2002).

Although an outer layer of harmless white mold mycelia on dried sausages may be preferred in some regions of the world, other types of mycotoxin-forming or nonpathogenic mold species with green, gray, or yellow pigmentation are considered unacceptable by almost all consumer groups (Holley, 1981), and may be controlled with natamycin, if approved for use (natamycin is not approved for use in meat products in the United States). The removal of all/unacceptable types of mold can be both time consuming and costly, but the health risks associated with such practices warrant even greater concern. Toxic and allergenic mold metabolites may not be removed from sausages during washing and additional types of food pathogens may be introduced (Holley, 1981). Natamycin inhibits mold but is ineffective against LAB, and thus, is useful in fermented food products (Delves-Broughton

et al., 2005). When approved for use, Thomas and Delves-Broughton (2005) suggest that dried sausages are most effectively treated when sprayed or immersed in natamycin at levels between 1,2501981 and 2,000 ppm. In general, immersion treatments are not effective unless applied to sausages in natural casings (Holley, 1981). In one study, a 2,000 ppm natamycin dip did not inhibit mold during ripening (50 days) of Genoa, Abruzzese or Casalingo salami, while sequential 1,000 ppm spray applications of natamycin (days 0 and 5) inhibited mold during ripening, and were slightly more effective than potassium sorbate (2,500 μg/ml) treatments (Holley,). Mold was not observed on Dutch salami in protein fiber casings sprayed with natamycin (1,000 ppm) after 35 days at 18°C (75% ± 10% relative humidity) (Holley, 1981; Moerman, 1972).

Foegeding, Thomas, Pilkington, and Klaenhammer (1992) measured the production of pediocin by fermentative cultures used during dry fermented sausage production and subsequent antilisterial activity during fermentation and drying. The processing conditions used in the study facilitated production of pediocin which inhibited *L. monocytogenes* during fermentation and drying; inactivation was achieved when pH levels dropped below 4.9 by the end of fermentation (Foegeding et al., 1992). Osmanagauoglu et al. (1998) found that lysozyme catalyzed the antimicrobial activity of pediocin, while papain, a proteolytic enzyme derived from *Carica papaya* (21 CFR 184.1585; CFR, 2007c), was destructive. Lipids and high concentrations of NaCl also appeared to antagonize the activity of pediocin (Bhunia et al., 1991; Jung et al., 1991; Ray & Miller, 2000). Degnan and Luchansky (1992) found that pediocin encapsulated within phosphatidylcholine liposomes provided a greater antilisterial effect in beef muscle and beef tallow slurries (heated 100°C, 3 min; and unheated) than did free pediocin.

Gravesen, Jydegaard Axelsen, Mendes da Silva, Hansen, and Knøchel (2002) assessed the environmental factors involved in the development of resistance to pediocin and nisin by multiple strains of bacteriocin-sensitive *L. monocytogenes*. The development of resistance to pediocin (and other class II bacteriocins) was not affected by environmental factors and would most likely occur in a standardized proportion of a population (Gravesen et al., 2002). In contrast, the development of resistance to nisin was strain- and nisin-concentration dependent, and was also influenced by temperature, pH and NaCl concentration. As one might expect, resistance to nisin occurred less frequently when higher concentrations were applied under nonoptimal growth conditions (Gravesen et al., 2002).

Other Microbial Metabolites

Characteristics, Properties, and Applications

Ethanol is ineffective at concentrations of 95–100%, but inactivates nearly all microorganisms within 60 s when diluted with water at 60–75% (Table 14.4) (Seiler & Russell, 1991; Shelef & Seiter, 1993; Sofos et al., 1998). Shapero,

Nelson, and Labuza (1978) found that ethanol was more effective under low a_w or high sugar conditions. A synergistic inactivation of *L. monocytogenes* (5–37°C, tryptic soy broth) was observed when 5% ethanol was used in combination with nisin (10–50 IU/ml). The addition of ethanol (5.0%) to tryptic soy agar plus yeast extract (TSBYE) dramatically increased the sensitivity of *L. monocytogenes* (5 log) to lactates, formates, and benzoates, and to hyper- and hypoosmotic stresses (Barker & Park, 2001). The most effective combination was 50 mM formate plus 5.0% ethanol in TSBYE lowered to pH 3.0 with HCl, which inactivated 5 log cycles of *L. monocytogenes* in 4 min (Barker & Park, 2001).

Diacetyl is a GRAS substance produced by species of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* (Holzapfel et al., 2003). The intense flavor and aroma of butter associated with diacetyl encourage its use in dairy product formulations and in some wines (Jay, 1982). Gram-negative bacteria, yeasts, and molds are sensitive to diacetyl, while gram-positive bacteria may only be susceptible to very high levels (≤200 mg/kg) (Holzapfel et al., 2003). The activity of diacetyl may be compromised in the presence of nisin, glucose, or acetate (Jay, 1982; Olasupo, Fitzgerald, Gasson, & Narbad, 2003). Kang and Fung (1999) found that diacetyl (50 ppm) inhibited growth of *E. coli* O157:H7 and *S.* Typhimurium during the fermentation of raw salami batter, but did not effect the growth or fermentative capability of *P. acidilactici*. In other studies, however, diacetyl (20–100 or 344 μg/ml) did not control the growth of gram-positive or -negative bacteria in fresh ground beef during storage (7 days) or in a cooked meat matrix (Jay, 1982; Williams-Campbell & Jay, 2002).

Lactoferrin

Characteristics, Properties, and Applications

Lactoferrin, a transferrin protein that binds free iron (Fe^{2+}) and possesses antioxidant, antifungal, antibacterial and antiviral properties, is most commonly recovered from the whey protein fraction of bovine milk, and is also present in saliva, tears, neutrophil granules associated with inflammation, mammalian exocrine secretions, and at trace levels in meat (Naidu, 2000, 2002; Shin et al., 1998). Lactoferrin-sensitive microorganisms include viruses, fungi, and bacteria (Table 14.4) possessing outer membrane proteins which act as lactoferrin receptors, also known as porins in gram-negative bacteria (Bellamy et al., 1992; Naidu, 2000, 2002; Stopforth, Skandamis, Davidson, & Sofos, 2005). Specifically, pathogenic species of *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, *L. monocytogenes*, *Proteus*, *Staphylococcus*, *Vibrio*, *Aeromonas*, *Corynebacterium*, *Enterococcus*, *Lactobacillus*, *Micrococcus*, *Candida*, *Bacillus*, *Pseudomonas*, *Klebsiella*, *Brochothrix*, and *Acinetobacter* are affected by lactoferrin (Meat Industry Services, 2006; Naidu, 2002). Lactoferrin binds to surface receptors and disrupts normal membrane and endotoxin function.

It has been reported that microorganisms attached to surfaces (e.g., carcass tissue) detach upon lactoferrin-binding and subsequent attachment is also blocked (Naidu, 2002, 2003).

Since lactoferrin acts as the ligand in a ligand/receptor-mediated pathway, its activity is highly conformation dependent (Naidu, 2002; Naidu & Arnold, 1994; Testa, 2002). Conformation is both pH- and citrate/bicarbonate ratio-dependent and may also be altered in the presence of excess calcium or phosphates (Naidu, 2000, 2002). Lactoferrin exhibits an affinity for Fe^{2+} , and iron-rich water sources diminish antimicrobial activity and should not be used to dilute lactoferrin decontamination solutions (Naidu, 2000, 2002). Outer membrane porin (OmpC and OmpE) synthesis by gram-negative enteric bacteria is indirectly regulated by extracellular osmolarity; thus, NaCl may indirectly influence the efficacy of lactoferrin in processed meat products (Rombouts, Notermans, & Abee, 2003).

When bound to a GRAS glucosamine, lactoferrin is fixed in its conformationally active form (activated lactoferrin or ALF) (Naidu, 2002). ALF is commercially available as Activin™, a NaCl and citrate/bicarbonate solution containing free lactoferrin and lactoferrin bound to carrageenan (Table 14.5) (Naidu, 2002). Published scientific data regarding the efficacy of lactoferrin as an antimicrobial are limited, especially as a decontamination solution applied to carcasses or processed meat products. In one study, beef subprimals inoculated with *E. coli* O157:H7 (4 log CFU/100 cm^2) were sequentially treated with ALF (2.0%) and lactic acid (5.0%, 55°C), and then moisture enhanced or blade tenderized (Heller et al., 2007). Surface treatment with ALF reduced pathogen counts by 0.9 log CFU/100 cm^2, although *E. coli* O157:H7 was internalized in 99% of samples during tenderization/enhancement as would be expected in the presence of any level of contamination (Heller et al., 2007).

Lysozyme

Characteristics, Properties, and Applications

Lysozyme, a muraminidase recovered from egg white albumin, milk, and other tissues (Nattress, Yost, & Baker, 2001), is best known for its ability to inhibit fermentative butyric acid bacteria responsible for late blowing during ripening of semihard cheeses (Cunningham, Proctor, & Goettsch, 1991). When used alone, lysozyme breaks β-glycoside bonds between the *N*-acetylglucosamine and *N*-acetylmuramic acid groups of peptidoglycan (component of gram-positive bacterial cell walls) (Cagri, Ustunol, & Ryser, 2004; Proctor & Cunningham, 1989). Uncompromised outer membranes protect gram-negative bacteria against lysozyme activity (Table 14.4); however, when used in combination with other antimicrobials, specifically those which damage outer membranes, gram-negative bacteria exhibit increased susceptibility (Malicki et al., 2004). Lysozyme is heat stable and remains active after thermal processing treatments and is most commonly

used in its hydrochloride form (Cunningham et al., 1991). Maximum antimicrobial activity is observed at an acidic to neutral pH (3.5–7.0), although optimal pH depends on NaCl concentration (Johnson & Larson, 2005). Lysozyme becomes increasingly effective in the presence of EDTA, nisin, nitrite, or NaCl (Cunningham et al., 1991; Nattress et al., 2001). In a detailed review of lysozyme as an antimicrobial, Johnson and Larson (2005) reported that TSP, lysolecithin and polyethylene glycol, polylysine, hydrogen peroxide, ascorbic acid, lactic aid, trypsin, carvacrol, and other terpenoids enhanced antimicrobial activity of lysozyme, as did coapplication of heat, alkaline pH, osmotic shock, freeze-thawing, and high-pressure treatments. Antimicrobial activity is not impaired when lysozyme is used in combination with most ingredients, although some surfactants, fatty acids, and alcohols (≥12 carbon chain length), as well as pectin, alginate, iodine, and high concentrations of cobalt, manganese, copper, and mercury may be antagonistic (Procter & Cunningham, 1989; Smith & Stroker, 1949). The bactericidal activity of lysozyme is concentration dependent and may be depressed in the presence of excess iron or calcium (Ellison & Giehl, 1991). As lysozyme is a naturally occurring compound, postconsumption immune response and anaphylactic shock were investigated in human and animal models (Verhamme, Storck, Racchelli, & Lauwers, 1988), and it was determined that egg-white related allergic reactions in humans are not typically caused by lysozyme (Langeland & Aas, 1987).

Nattress et al. (2001) found that nisin and lysozyme were more effective at controlling spoilage organisms (*Brochothrix thermospacta* B2 and *Carnobacterium* sp. 845) on fat and lean pork tissue when used in combination, and overall activity was concentration dependent. Chun and Hancock (2000) also observed synergistic inactivation of gram-positive bacteria by combinations of lysozyme and nisin. Malicki et al. (2004) found that a combination of lysozyme (200 ppm) and sodium lactate (2%) was increasingly lethal for LAB, but did not affect total aerobic bacterial counts. In another study, lactoferrin and lysozyme individually inhibited growth of *Vibrio cholerae* 3083, *S.* Typhimurium SL696, and *E. coli* 5448, while combinations resulted in death (Ellison & Giehl, 1991). Bactericidal activity was concentration dependent and antagonized by the presence of excess iron or calcium (Ellison & Giehl, 1991). Due to the apparent antimicrobial properties of lysozyme, in conjunction with increasing demand for natural additives and fewer chemical additives altogether, potential utilization of lysozyme in meat products should be investigated more extensively.

Plant Extracts and Oils

Characteristics, Properties, and Applications

The antimicrobial activities of plant extracts and essential oils are well established (Sofos et al., 1998). Examples of plants yielding antimicrobial oils and extracts include cinnamon, cloves, thyme, sage, rosemary, oregano, basil, allspice, nutmeg,

and coriander (Søltoft-Jensen & Hansen, 2005). However, a defined spectrum of sensitive microorganisms and an inventory of food components or processing conditions that promote or antagonize the activity of individual oils/extracts are currently unavailable. In general, bacteria are less susceptible to plant extracts/oil than fungi, and gram-negative bacteria are less susceptible than gram-positive types (Zaika, 1988). Many plant extracts are sensitive to other components in food product formulations, and interactions may result in increased/decreased antimicrobial activity. High levels of fat and proteins can antagonize antimicrobial activity, while low a_w, high concentrations of NaCl, organic acids and other preservatives may enhance the antimicrobial properties of plant extracts and oils (Doyle, 1999; Lin, Labbe, & Shetty, 2004; Snyder, 1997). The antimicrobial activity of plant extracts and essential oils may be preserved by dispersing them in sodium citrate or NaCl solutions prior to food applications (Cutter, 2000).

In general, phenols inhibit or destroy microorganisms by damaging cell membranes, altering permeability and/or causing the intracellular components of cells to aggregate (Russell & Chopra, 1996). Rosemary extract (16–24% carnosic acid) is commercially available for use as a natural antioxidant and interacts synergistically with ascorbic acid and ascorbyl palmitate (Shahidi & Wanasundara, 2003). Other phenols isolated from rosemary include borneol, carnosol, rosmanol, isorosmanol, and rosmarinic acid (Lin et al., 2004; Madsen & Bertelsen, 1995). Borneol and carnosol are also recovered from sage and rosmarinic acid from oregano (Madsen & Bertelsen, 1995; Smith-Palmer, Stewart, & Fyfe, 1998). Rosmarinic acid has exhibited antilisterial activity in laboratory media and in meat systems, which is enhanced in the presence of lactic acid (Lin et al., 2004). Overall, the effect of borneol on gram-positive bacteria and fungi is unclear, and gram-negative bacteria do not appear to be susceptible (Mangena & Muyima, 1999; Smith-Palmer et al., 1998).

Eugenol, a phenolic compound recovered from cloves, cinnamon, and sage, has been shown to inhibit the growth of both gram-positive and -negative bacteria (Snyder, 1997). Following exposure to eugenol, production of listeriolysin O by *L. monocytogenes* was dramatically reduced (80–100%) (Filgueiras & Vanetti, 2006); cell membranes were damaged and bacteriostatic vs. bactericidal outcomes were concentration dependent (Filgueiras & Vanetti, 2006). *Bacillus cereus*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica* have also exhibited various levels of susceptibility to eugenol (Bara & Vanetti, 1996; Karapinar & Aktug, 1987; Sofos et al., 1998). Isoeugenol, which is also a component of liquid smoke formulations, has been shown to exhibit antilisterial activity at 50–200 ppm (Faith, Yousef, & Luchansky, 1992; Sofos, 2005b). Clove essential oil extract is composed almost entirely (95%) of eugenol, which may explain the greater degree of microbial inhibition by clove oil compared to other eugenol-containing oils/extracts (Snyder, 1997).

Cinnamaldehyde (cinnaminic aldehyde), a component of cinnamon, has been shown to inhibit growth of gram-positive and -negative bacteria, specifically *C. jejuni, C. botulinum*, *E. coli* O157:H7, *L. monocytogenes*, *S. aureus* and *S.* Typhimurium, *L. sakei*, and some species of mold (*Aspergillus*) (Bowles & Miller, 1993; Bowles, Sackitey, & Williams, 1995; Gill & Holley, 2004; Lis-Balchin & Deans,

1997; Sofos et al., 1998). Gill and Holley (2004) found that six times more cinnamaldehyde, compared to eugenol, was needed to inhibit *L. monocytogenes*, and speculated that the antimicrobial action of each compound must differ (Davidson & Naidu, 2000; Gill & Holley, 2004; Helander et al., 1998; Snyder, 1997). When the antilisterial activity of 93 commercial essential oils was compared, oils which contained the highest level of monoterpenes, eugenol, cinnamaldehyde and/or thymol were the most effective (Lis-Balchin & Deans, 1997).

Terpenes disrupt the cell membranes of susceptible microorganisms (Davidson & Naidu, 2000). Thymol and carvacrol are terpenes, both of which can be recovered from oregano. Thymol is also a constituent of thyme and sage extracts (Snyder, 1997). Hammer, Carson, and Riley (1999) found that the minimum inhibitory concentration (MIC) of thyme extract for *E. coli* was lower than that of 52 other plant oils and extracts. Thymol has also been shown to inhibit growth of *Aspergillus* spp., *Candida albicans*, *Saccharomyces cerevisiae*, *B. cereus, C. botulinum*, other *E. coli* spp. including *E. coli* O157:H7, *S. aureus*, *S.* Typhimurium, *V. parahaemolyticus*, and *Y. enterocolitica* (Cosentino et al., 1999; Olasupo et al., 2003; Snyder, 1997; Sofos et al., 1998). *Escherichia coli* also exhibited sensitivity to a compound recovered from nutmeg oil (β-pinene), with O157 subtypes exhibiting higher levels of sensitivity than non-O157 subtypes (Takikawa et al., 2002).

The antimicrobial nature of thiosulfinates, the antimicrobial compounds associated with *Allium* species of plants (e.g., garlic, onions, horseradish, leeks), has been studied extensively (Whitmore & Naidu, 2000). Allicin is the thiosulfinate which is formed as garlic cloves are crushed; more specifically, allinase hydrolyzes allin and forms allicin, pyruvate, and ammonia (Sofos et al., 1998; Stoll & Seebeck, 1951). The antimicrobial action of allicin involves inhibition of sulfhydryl enzymatic activity in gram-positive and -negative bacteria and fungi (Wills, 1956), including *S.* Typhimurium, *E. coli*, *S. aureus*, *B. cereus*, *B. subtilis*, *L. monocytogenes*, *Aspergillus*, and *C. albicans* (Snyder, 1997; Sofos et al., 1998; Whitmore & Naidu, 2000). Onions contain the thiosulfinate compound allyl isothiocyanate, which also inhibits *Aspergillus* (Snyder, 1997).

Beuchat and Brackett (1996) reported a substantial reduction in *L. monocytogenes* when 1% raw carrot juice (which contains phytoalexins) was added to a broth system; however, the antimicrobial components of carrots and carrot juice are denatured during thermal processing. Tea extracts are commercially available for use as antioxidant agents in deep-fried and extruded snack foods (Shahidi & Wanasundara, 2003), and flavonoids recovered from tea leaves/extracts also exhibit activity against gram-positive and -negative bacteria, viruses and some fungi (Friedman, 2007). Specific flavonoids include, but are not limited to, catechins, theaflavins, and thearubigins. Variability in antimicrobial activity may be due, in part, to different solvents used to extract flavonoids from tea leaves (Lin, Tsai, Tsay, & Lin, 2003). Leaves harvested during the summer may also yield greater concentrations of flavonoids (Freidman, 2007).

While hop vine blossoms are most celebrated for their role in the development of beer flavor, hop flower extracts (α- and β-acids) also exhibit antimicrobial properties to which gram-positive bacteria and some fungi are susceptible (Larson et al.,

1996; Sofos et al., 1998). In general, α-acids (lupulones) impart a more bitter "hoppy" flavor profile than β-acids (humulones), which is fortunate, as humulones are superior as antimicrobials (Chin, Anderson, Alderton, & Lewis, 1949; Hough Howard, & Slater, 1957; Millis & Schendel, 1994).

A 0.2% concentration of rosemary extract (oleoresin) in ground ostrich meat patties did not affect total aerobic bacteria, coliforms, or other spoilage bacteria (Seydim, Guzel-Seydim, Acton, & Dawson, 2006). A mixture of oregano and cranberry extracts (0.1 mg phenolic/ml; pH 7.0), applied to the surfaces of beef slices inoculated with *L. monocytogenes* (8.5 log CFU/g), demonstrated no antilisterial activity after 18 h at 37°C, but when the pH was adjusted to 6.0 with lactic acid, pathogen counts were reduced by almost 3 log cycles (37°C, 18 h) (Lin et al., 2004). Bactericidal activity was not observed when lactic acid was used alone (Lin et al., 2004). In another study, isoeugenol (50–200 ppm) exhibited antilisterial activity, which increased significantly as the pH of the broth system was lowered (7.0 vs. 5.8) with glacial acetic acid (Faith et al., 1992). *Escherichia coli* O157:H7 and *Salmonella* were increasingly radiosensitive after being exposed to trans-cinnamaldehyde (1.5%), thymol (1.15%), and thyme (2.33%) (Lacroix, Chiasson, Borsa, & Ouattara, 2004).

Allyl isothiocyanate (AIT), at 1,300 ppm, reduced *E. coli* O157:H7 (3 or 6 log CFU/g) in ground beef stored at 4°C for 25 days, by 4.5 log CFU/g (Muthukumarasamy et al., 2003). However, it is unlikely that such high concentrations of AIT would be acceptable in fresh meat products. Initial levels of AIT (as a component of horseradish distillate) were gradually depleted during storage (7 days, 12°C) of vacuum-packaged precooked roast beef and reached concentrations of 1.1–21.2% that of original levels (Ward, Delaquis, Holley, & Mazza, 1998). Initial *E. coli* O157:H7 populations in sausage (60.7% pork, 17.6% beef, 17.6% pork fat) were reduced more effectively during fermentation and drying by microencapsulated vs. unencapsulated AIT (500 ppm) (4.8 vs. 1.5 log CFU/g, respectively) (Chacon, Muthukumarasamy, & Holley, 2006). Higher concentrations of microencapsulated AIT were increasingly effective, but not considered acceptable during taste panel evaluation; concentrations of 500 ppm were acceptable, but "slightly spicy" (Chacon et al., 2006).

Friedman (2007) suggested the need to study the efficacy of flavonoids as antimicrobial agents in meat and poultry, and the possible synergism and/or antagonism between flavonoids and other antimicrobials and food ingredients as well as the development of coatings and films containing flavonoids. In one such study, tea extracts were effective against *L. monocytogenes* and *S. aureus* in culture broth but not in ground beef, suggesting that tea extracts may interact with other compounds in a meat matrix instead of the pathogen (Kim, Ruengwilysup, & Fung, 2004). Hop flower β-acids are currently approved for use in the casings of meat and poultry products or for application to cooked meat and poultry products, although Larson et al. (1996) recommended the use of hop extracts be limited to minimally processed, low-fat foods.

While lower a_w, higher NaCl concentrations, and/or the presence of acids or other preservatives may enhance pathogen inhibition by plant extracts and oils, higher levels of fat and proteins tend to impair antimicrobial activity (Doyle, 1999;

Snyder, 1997). The origin and composition of plant extracts/oils depend on species and original growth conditions, leading to inconsistent antimicrobial activities among commercially available products (Hammer et al., 1999). Extract oils are generally hydrophobic and their homogeneous distribution within product formulations can be difficult (Hammer et al., 1999). Finally, many oils and extracts possess strong flavor and aroma profiles which prevent their use in certain product formulations. Purified antimicrobial compounds may be used in smaller doses, minimizing the unfavorable sensory attributes associated with parent extracts/oils. For these reasons, the efficacy of individual plant extract/oil components, alone and in combination with different emulsifiers, requires further investigation.

Smoke

Characteristics, Properties, and Applications

Smoking of meats is discussed in Chap. 10. It is well known that exposure to smoke or its derivatives helps preserve meat products against spoilage and pathogenic microorganisms (Doyle, 1999; Faith et al., 1992). Wood smoking or application of liquid smoke preparations as ingredients, or through atomization, introduce antimicrobial compounds in meat products, which include phenols, alcohols, carbonyls, hydrocarbons, and various gases (Sofos, 2005b).

Boyle, Sofos, and Maga (1988) compared the antimicrobial activity of smoke condensates from 20 different types of wood against *Pseudomonas fragi* ATCC 4973, *S. aureus* A100, and two strains of *Aeromonas hydrophila*. Sapwood of Douglas fir was the most inhibitory, although liquid smoke from aspen, birch and southern yellow pine were also inhibitory. The least inhibitory smoke condensates were those derived from mesquite and lodge pole pine (Boyle et al., 1988). A similar trend was observed when the antimicrobial activity of ether extracts recovered from each of the same 20 types of wood against *S. aureus* and *A. hydrophila* was examined; *S. aureus* was the more sensitive of the two microorganisms (Sofos et al., 1988). The efficacy of different liquid smoke preparations may vary, due in part, to the chemical components of each formulation. Messina, Ahmad, Marchello, Gerba, and Paquette (1988) investigated the efficacy of CharSol liquid smoke and four other types of liquid smoke, all produced by Red Arrow Products Co., LLC (Manitowoc, WI; Red Arrow Products Company LLC, 2007), and found CharSol to be the most effective against *L. monocytogenes*. The ability of liquid smoke (CharSol Supreme) and 11 individual phenolic compounds to inhibit growth of *L. monocytogenes* was also examined (Faith et al., 1992). Of the 11 compounds, isoeugenol (50–200 ppm) was the only phenol which exhibited antilisterial activity, and activity increased significantly as pH of the broth system was lowered (7.0 vs. 5.8) with glacial acetic acid. In one study, *L. monocytogenes* was inactivated at lower temperatures when liquid smoke (10–100% CharSol) was added to the thermal treatment used to process salmon steaks (Poysky et al., 1997); the temperature

required to inactivate *L. monocytogenes* increased as the concentration of liquid smoke or the duration of traditional smoke treatments decreased.

The residual antimicrobial effects of liquid smoke during product storage have also been documented (Faith et al., 1992). In one study, initial reductions in *L. monocytogenes* counts (6 log CFU/cm^2) following liquid smoke treatments (0.4 ml/frankfurter) were negligible, although liquid smoke treatments reduced counts by an additional 2.0 log CFU/cm^2 after 12 days of storage (4.4°C) (Murphy et al., 2005). When followed by a steam pasteurization process (100°C, 1.5 s), lower concentrations of liquid smoke were required for comparable reductions in *L. monocytogenes* populations (Murphy et al., 2005). Traditional smoke treatments tend to be less effective against microbial contaminants when applied post pellicle formation. Pellicles form a protective shiny and slightly tacky outer membrane on the surface of drying lean tissue to which smoke components adhere (Poysky et al., 1997).

Novel and Emerging Antimicrobials

Bacteriophages

Characteristics, Properties, and Applications

While all living organisms are susceptible to viral infection, most viruses are exceptionally host-specific, and therefore, harmless to unrelated organisms (Summers, 2001). Bacteriophages (phages) are bacterial viruses which may be useful in controlling the survival/growth of spoilage and pathogenic organisms associated with meat and other food products (Greer & Dilts, 1990). One specific bacteriophage preparation (bacteriophage P100) cultivated in *Listeria innocua*, a nonpathogenic relative of *L. monocytogenes*, has GRAS status and was recently approved for use as an antimicrobial at levels not to exceed 9 log pfu/g of cheese. Rats used in bacteriophage P100 oral toxicity assays did not exhibit negative changes in histological characteristics, and incidence of illness and death did not increase following treatment (Carlton, Noordman, Biswas, de Messter, & Loessner, 2005). Another bacteriophage preparation which includes six individually purified lytic-type phages, specific against *L. monocytogenes*, has been approved for use as an antimicrobial surface treatment for RTE meat and poultry products (21 CFR 172.785) (CFR, 2007b; USDA/FSIS, 2007).

When applied to the surface of Muenster (red smear) cheese wheels, P100 treatments (7.8 log pfu/cm^2) were highly effective against *L. monocytogenes* (1.3 log CFU/cm^2), and inhibited growth during the ripening process (Carlton et al., 2005). Unfortunately, the use of phages to control spoilage flora has been less successful. Greer and Dilts (1990) found that a composite of seven pseudomonad-specific phage resulted in lower bacterial counts, but did not extend shelf life or reduce the spoilage of fresh beef products. Inability to control growth was not associated with inadequate numbers of viable phage during storage, and was more

likely due to the presence of resistant *Pseudomonas* spp. and other species of bacteria which would not have been susceptible to the phage treatment (Greer & Dilts, 1990; Summers, 2001).

Repeated lysis of bacterial cells by phage has resulted in the development of resistant cultures (Summers, 2001). One mechanism of resistance involves the blocking of phage DNA injection by bacterial cells (McGrath, Fitzgerald, & Van Sinderen, 2002) and such alterations in bacterial cell membranes could potentially decrease the ability of other antimicrobials (specifically those which interact with cell membranes) to inhibit/inactivate pathogens. Future research should examine the impact of food processing conditions on phage viability, as well as the incidence and development of phage-resistant spoilage organisms and pathogens.

Calcium Sulfate

Characteristics, Properties, and Applications

Calcium sulfate ($CaSO_4$), also known as alabaster, gypsum, and plaster of paris, can be obtained by mining natural reservoirs or by chemical synthesis (Peterson, Kaleta, & Kingston, 1992). Naturally occurring calcium sulfate has GRAS status and is most commonly used as a dough conditioner, tofu firming agent, nutrient supplement, pH control agent, or stabilizer/thickener. When acidified with a GRAS acid, calcium sulfate (ACS) has exhibited antimicrobial activity against gram-positive and -negative bacteria, and molds (Nuñez de Gonzalez, Keeton, Acuff, Ringer, & Lucia, 2004; Zhao, Doyle, Kemp, Howell, & Zhoa, 2004). The antilisterial properties of ACS in RTE meat products have been investigated, and in addition to the destruction of existing contamination, ACS may provide residual inhibitory effects against survivors or during future recontamination events (Marshall, 2003).

Aqueous solutions containing ACS and other antimicrobial compounds (sodium phosphate, lactic acid, propionic acid) may be added to various RTE meat products and raw comminuted beef, or to raw whole muscle beef cuts, cooked beef products, and to cooked poultry carcasses/parts (USDA/FSIS, 2007). In 2003, $Safe_2O$ RTE O3, a proprietary blend of ACS (acidified with lactic acid, and propionic acid), was developed by Mionix Corporation (Rocklin, CA) as an antimicrobial food additive for the inhibition of *L. monocytogenes* in RTE meat products (Mionix, n.d.). Luchansky et al. (2005) examined the ability of ACS (acidified with lactic acid; 1:1 or 1:2) to control *L. monocytogenes* on table brown ham (7 log CFU/ham) during storage, when added to shrink-wrap bags just before being sealed. Reductions (1.2–3.1 CFU/ham, respectively) after 24 h (4°C) were concentration dependent, although both concentrations were sufficient to inhibit survivors for 40 days at 4°C (Luchansky et al., 2005). In another study, frankfurters (formulated with or without 2.0% potassium lactate) were surface inoculated with *L. monocytogenes* (8 log CFU/ml), then dipped in ACS (acidified with lactic and propionic acid in

1:2 aqueous solution), 3.0% lactic acid, or 2.0% potassium lactate solution for 30 s (Nuñez de Gonzalez et al., 2004). *Listeria monocytogenes* and aerobic plate counts were at or just above the detection limit throughout storage (4.5°C, 84 days) on frankfurters, with and without potassium lactate, then dipped in ACS or lactic acid (Nuñez de Gonzalez et al., 2004). *Escherichia coli* O157:H7 in fresh ground beef was increasingly sensitive to heat following the addition of ACS and lactic acid (0.4% and 0.2% of final product), although the degree of increased heat-sensitivity was most likely of little biological significance (Zhao et al., 2004).

Epsilon-Polylysine

Characteristics, Properties, and Applications

Epsilon-polylysine (ε-polylysine), a fermentative by-product of *Streptomyces albulus*, is a polyamino acid (not a protein), comprised exclusively of L-lysine residues linked together by amide bonds (Hiraki et al., 2003; Shih, Shen, & Van, 2006; Yoshida & Nagasawa, 2003). ε-Polylysine is used extensively in Japan as an emulsifier, drug carrier, and dietary/anticancer supplement, or as a component of biodegradable fiber and biochip coatings (Shih et al., 2006). ε-Polylysine is also used in Japan as a natural preservative (0.1–0.5%) in surimi products (Hiraki et al., 2003; Ting, Ishizaki, & Tanaka, 1999). It is readily soluble in water and in aqueous solution functions as a surface-active cation, to which both gram-negative and -positive bacteria are highly susceptible (1–8 μg/ml; typical inhibitory level). In general, yeasts and molds are less vulnerable than bacteria (Shima, Matsuoka, Iwamoto, & Sakai, 1984). Shima, Fukahara, and Sakai (1982) found that bacteriophages were susceptible to ε-polylysine and spore germination by some strains of *Bacillus* was also inhibited (2.5–12.5 μg/ml). The antimicrobial action of ε-polylysine involves electrostatic interactions between the cationic compound and cell membranes and results in the sloughing-off of the outer membrane and disruption of cytoplasm (Shima et al., 1984). Antimicrobial activity appears to increase with number of residues within the polymer and at least ten residues are required for optimal antimicrobial activity (Shima et al., 1984). ε-Polylysine polymers produced by *S. albulus* typically contain 25–35 L-lysine residues (Yoshida & Nagasawa, 2003). The polymer is heat-stable (120°C for 20 min or 100°C for 30 min), and remains active at an acidic or alkaline pH (5.0–8.0) (Hiraki, 2000; Ting et al., 1999; Yoshida & Nagasawa, 2003). Antimicrobial activity was enhanced in the presence of glycine, vinegar (acetic acid), ethanol, and thiamine lauryl sulfonate (Hiraki, 2000). ε-Polylysine may be less active in the presence of proteins and acidic polysaccharides, and is therefore most commonly used in starch-based products (Hiraki, 1995).

Geornaras and Sofos (2005a) investigated the antimicrobial activity of ε-polylysine (0.0025–0.05%) against *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 in culture broth (tryptic soy broth plus 0.6% yeast extract;

24°C, 24 h or 4°C, 30 days), and found it to inhibit all three pathogens at 24°C, and to inactivate *S.* Typhimurium and *E. coli* O157:H7 at 4°C. When used in combination with other antimicrobials (0.25% sodium diacetate, 3.0% sodium lactate, 0.1% lactic or acetic acid) a greater level of activity against *E. coli* O157 and *S.* Typhimurium was observed (Geornaras & Sofos, 2005a). Another recent study examined the antimicrobial activity of ε-polylysine (0.005–0.04%) against *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* in various food extracts, including those of beef and bologna (Geornaras, Yoon, Belk, Smith, & Sofos, 2007). The antimicrobial activity of ε-polylysine during storage (12°C, 6 days) was affected by type of pathogen and/or food extract, while no immediate reductions in pathogen populations were observed in protein-dense food extracts. When added to the beef or bologna extract, ε-polylysine generally resulted in lower pathogen counts (compared to controls) by the end of storage, and higher concentrations of ε-polylysine were generally more effective. Unlike many other antimicrobial compounds, ε-polylysine exhibited a greater antimicrobial activity against the pathogens in food extracts than in a broth (Geornaras et al., 2007). The efficacy of ε-polylysine (100 ppm), acidified calcium sulfate (ACS; 1:4 ACS and water), and a combination treatment (1:4 ACS and water plus 100 ppm ε-polylysine) as carcass decontamination washes (50–55°C; 14 ml/s) was also investigated (Keeton, Ricke, Anderson, Miller, & Azefor, 2006). Although no details were provided, it was reported that ACS followed by ε-polylysine reduced pathogen levels by 1.5 log CFU/cm^2 over a 7 day period, and ACS plus ε-polylysine was more effective than individual treatments of water, L-lactic acid, ACS, or ε-polylysine (Keeton et al., 2006).

Chitosan

Characteristics, Properties, and Applications

Chitin (*N*-acetyl-D-glucosamine) is a structural component of crustacean, fungi, insect, annelid, mollusk, and coelenterata exoskeletons (Shepherd, 2003). When deacetylated with a strong alkaline solution, chitin produces chitosan (Weiner, 1992). Chitosan is typically insoluble in water, sucrose or NaCl solutions, ethanol, vegetable oils or propylene glycol, although the absence of acetyl groups allows solubility in aqueous solutions of organic acid (Shepherd, 2003). To date, a specific mode of antimicrobial action remains unclear, although in an anionic state, chitosan interacts with cell membranes and degree of deacetylation and depolymerization appear to influence level of activity (Rudrapatnam & Farooqahmed, 2003; Shahidi, Arachchi, & Jeon, 1999; Søltoft-Jensen & Hansen, 2005). Gram-positive bacteria also appear to be less sensitive to chitosan than gram-negative types (Søltoft-Jensen & Hansen, 2005). A synergistic inhibition of yeast was also observed when chitosan was used in combination with benzoate (Sagoo et al., 2002). NaCl (2%) and proteins appear to antagonize activity; thus, the incorporation of chitosan in meat

products is not appropriate (Søltoft-Jensen & Hansen, 2005). However, pure chitosan films reduced *L. monocytogenes* counts (2 log cycles) on inoculated bologna (10°C, 5 days) (Zivanovic, Chi, & Draughton, 2005).

Xylitol

Characteristics, Properties, and Applications

Xylitol is a sugar alcohol which provides 100% of the sugar sweetness of sucrose and has been shown to inhibit growth of some types of oral bacteria (International Food Information Council Foundation [IFIC Foundation], 2006). Greiner (2005) investigated the use of a 10% xylitol wash against *Salmonella* and *E. coli* O157:H7 on the surface of beef carcasses. At a 10% concentration, xylitol did not inhibit pathogen attachment to beef tissue when applied before inoculation, nor did it increase the efficacy of a 2.5% L-lactic acid or water spray (35°C) in reducing pathogen contamination after inoculation.

Application Methods

The effectiveness of any antimicrobial depends on numerous factors, such as temperature or stage of processing in which it is applied. Spray, cascade, rinse, and deluge applications of chemical solutions such as organic acids (e.g., lactic, acetic) are commonly used to decontaminate carcasses and meat products, and parameters associated with each treatment (temperature, pressure, duration) should be attuned for optimal activity and efficiency (Cutter, Dorsa, & Siragusa, 1997; Sofos, Kochevar, Reagan et al., 1999; Sofos & Smith, 1998). A greater number of nozzles delivering a high-pressure spray (1.4–2.1 kPa) can significantly increase the efficacy of an antimicrobial treatment (Pordesimo, Wilkerson, Womac, & Cutter, 2002; Sofos, Belk et al., 1999). Efficacy may also be enhanced by optimizing the size, range, and angle of spray droplets (Pordesimo et al., 2002; Sofos, Belk et al., 1999). Antimicrobial solutions should be capable of greater microbial reductions when surface contact time is extended (Arrit et al., 2002; Russell & Axtell, 2005; White, 1992), and when applied at, within limits, higher temperatures (Hardin et al., 1995). Immersion and flood treatments are commonly used in the pork and poultry industries during scalding and chilling processes. Organic load and the subsequent renewal schedule of antimicrobial solutions can significantly impact the consistency and degree of microbial reductions (Lillard, 1980; Yang & Johnson, 2001).

The direct addition of antimicrobials to product formulations is an effective way to control microorganisms for extended periods although many factors will affect the resulting level of microbial inactivation and/or inhibition. Measurements (weight vs. volume) and final concentration of each ingredient are important in any

application method (Durán & Sofos, 2006). The characteristics of a product are critical, as pH, water content and availability, fat, and protein content may increase/decrease antimicrobial activity, as can other nonmeat ingredients or antimicrobial compounds (Grau & Vanderlinde, 1992; Padilla-Zakour, 1998). The extent and type of microbial contamination, and processing, packaging, and storage conditions can also impact the efficacy and longevity of an antimicrobial agent (Sofos, 2004).

Multiple Hurdle Technology

A scientifically validated, systematic approach for the control of microbial contaminants is the most efficient way to minimize food safety concerns and human health risks while producing wholesome, high-quality food on a large or small scale. Thus, the systematic application of individually sublethal combinations of antimicrobial agents or interventions, commonly referred to as "multiple hurdles" (Leistner & Gould, 2002), is a logical approach to pathogen reduction. The combined application of multiple inhibitory compounds and/or interventions should elicit greater activity against a more diverse range of microorganisms than if each agent/factor were used alone; for this very reason, the use of various fresh meat decontamination treatments, individually and in combination, has been the topic of extensive investigation (Bacon et al., 2003; Graves-Delmore, Sofos, Schmidt, & Smith, 1998; Hardin et al., 1995; Pohlman, Stivarius, McElyen, Johnson, et al., 2002; Sofos & Smith, 1998; Stopforth & Sofos, 2006). Multiple hurdles may be applied as sequential or simultaneous interventions, and should be designed to result in greater microbial reductions or control than if such antimicrobial factors were applied individually (Geornaras & Sofos, 2005b; Graves-Delmore et al., 1998; Hardin et al., 1995).

Synergistic inhibition/inactivation may be observed following the simultaneous application of nitrite and NaCl, and lactates and sodium diacetate in RTE meat and poultry products (Barmpalia et al., 2005; Blom et al., 1997; Sofos, 1984; Sofos et al., 1979a). Heating (55°C) organic acid solutions used to decontaminate carcasses may also increase microbial reductions (Hardin et al., 1995; Sofos et al., 2006). The sequential application of some compounds may induce microbial sensitivity to otherwise nonlethal agents. Examples of this phenomenon include the sensitization of gram-negative bacteria to the antimicrobial mechanism of bacteriocins following exposures to agents that alter cell membrane permeability, and a greater sensitivity of most microorganisms to irradiation treatments in the presence of nonmeat ingredients (Bricher, 2005; Delves-Broughton & Gasson, 1994). Beef carcass decontamination is optimized through application of multiple sequential interventions including animal hide washing, knife-trimming, steam-vacuuming, pre-evisceration washing, spray washing, thermal pasteurization, organic acid rinsing, chilling, and potentially postchilling chemical sprays (Sofos & Smith, 1998; Stopforth et al., 2004). Antagonism may result when one or more chemical reactions take place between agents, which diminish the antimicrobial activity of one or both compounds (Ahn

et al., 2002). Antagonistic behavior may depend on the concentrations of antimicrobial compounds, level and type of microbial contaminants, and processing and storage conditions.

Previous exposure of bacteria to sublethal stress may alter their ability to overcome additional types or degrees of stress (Gahan, O'Driscoll, & Hill, 1996; Koutsoumanis & Sofos, 2004; Maxcy et al., 1971; Samelis & Sofos, 2003; Whiting, 1993). Microbes subjected to sublethal stress may (1) recover and proliferate in food products or in food processing environments, (2) become increasingly resistance to previously inhibitory or lethal compounds/processes (Davidson & Harrison, 2002; Duffy et al., 2000; Samelis & Sofos, 2003; Sofos & Smith, 1998), and/or (3), be more likely to overcome the low pH conditions of the gastrointestinal tract (Rombouts et al., 2003). As an example, mildly acidic conditions (pH 5.0–6.0) may initiate survival mechanisms that enable microorganisms to overcome increasingly severe acid exposures (Koutsoumanis & Sofos, 2004). In addition to increased acid-tolerance, brief exposure to mild acidity may also result in increased resistance to thermal and osmotic stresses (Gahan et al., 1996). Antimicrobial interventions help assure that contamination levels are minimized and reduce the probability that processing failures will lead to foodborne illness (Sofos et al., 2006). Therefore, it is imperative that these same interventions are indeed reducing such risks, and not compounding existing problems by creating stress-tolerant populations.

While antimicrobial agents exert maximum activity under certain conditions, it is difficult to optimize all parameters for individual antimicrobial agents in complex food systems. In general, antimicrobial activity is maximized in aqueous broth systems and in the absence of lipids (Ray & Miller, 2000; Smith, 2003; Sofos et al., 1998). The pH of a solution or product may influence overall antimicrobial activity and degree of water hardness or presence of excess solute may decrease the efficacy of some agents (Cords et al., 2005). Trying to minimize friction-induced denaturation of muscle proteins when mixing ground meat and nonmeat ingredients may result in nonhomogenous distribution of antimicrobials in processed meat products (Romans, Costello, Carlson, Greaser, & Jones, 2001). Emulsified products are the exception, and a fairly homogenous ingredient distribution should be expected. Thermal processes and irradiation treatments may impair activity or even destroy some antimicrobials (Prakash, 2000). Some antimicrobial agents may also be depleted by surviving microorganisms during storage (Grau & Vanderlinde, 1992; Whiting & Masana, 1994).

Sublethal applications of antimicrobial agents may have two important consequences: (1) foodborne pathogens and/or spoilage organisms may be allowed to grow leading to subsequent foodborne illness or product spoilage and (2) lead to development of resistant organisms. In recent years, the number of published reports indicating some increase in the level of resistance to antimicrobials following exposure to sublethal stresses has risen. In general, three types of resistance are possible and include innate, acquired, and apparent resistance (Davidson & Harrison, 2002). Defense mechanisms such as efflux pumps and the ability to degrade a compound are both examples of innate resistance, and acquired resistance is the result of lateral transfers of genetic material (e.g., plasmid acquisition), genetic mutations, or the deletion of a

specific biochemical target (Souza, Castillo, & Equiarte, 2002). Apparent resistance is generally reversible, and depends on the conditions under which an antimicrobial is applied (Davidson & Harrison, 2002), reinforcing the need for strict and specific guidelines for the application of antimicrobial agents.

Suggested Direction of Future Research Efforts

Additional research is needed to determine the role of various antimicrobial interventions in the development of stress-adapted foodborne pathogens. Decontamination technologies and antimicrobial ingredients which allow and/or facilitate stress-adaptation should be identified, and when possible, modified, or avoided. As part of such efforts, stress-adapted and nonadapted pathogens should be incorporated into studies which examine the effects of immediate vs. gradual stress (e.g., acid, alkaline, osmotic) on subsequent survival and proliferation in meat products during processing, storage, and cooking (when applicable).

Proactive measures to avoid creation of stress-adapted pathogens should also include the optimization of single and multiple hurdle preservation strategies. The order in which antimicrobial agents are applied may impact the efficacy of multiple hurdle programs (Sofos & Smith, 1998; Sofos et al., 2006). As more data from research which validates the use of individual or combinations of antimicrobials are made available the opportunity to select the most ideal sequence of interventions becomes more realistic. In one study, the combined effects and most effective order of acid (acetic), alkaline (sodium hydroxide, NaOH), osmotic (NaCl), and biocide (lauric acid or monolaurin) treatments against *L. monocytogenes* and *Pseudomonas* were examined (Vasseur, Rigaud, Hebraud, & Labadie, 2001). The most effective sequence and combination of treatments was alkaline followed by osmotic, and then biocide shock (Vasseur et al., 2001). Unfortunately little information is available regarding the most effective combination and order of antimicrobials for different pathogen/product scenarios, without stress-adaptation and selection of resistant pathogens, and future research efforts should address this deficit.

Synthetic chemical preservatives have fallen under intense scrutiny by consumer groups and health advocates, and, when possible, naturally occurring ingredients should be investigated as potential replacements. For situations in which chemical preservatives cannot be entirely replaced (i.e., sodium nitrite in cured meats) efforts should be made to minimize concentrations used in product formulations. Modifications which minimize antagonistic interactions and/or prolong the activity of naturally occurring antimicrobials (e.g., encapsulation or constitual activation) that tend to be less effective in the presence of surviving microorganisms, lipids, salts, or other nonmeat ingredients, should be explored. The impact of novel and traditional processing technologies on artificial and naturally occurring preservatives and antimicrobials also requires further investigation.

The antimicrobial mode of action of most antimicrobial ingredients, regarding microbial survival and growth, and the impact of inhibitory agents on the metabolic

and pathogenic mechanisms of pathogens remains unclear or is not well defined, and need exploration. The impact of individual antimicrobials on virulence factors (i.e., toxin production or attachment to and invasion of host cells), subsequent cross-protection against other stresses, and the duration of such effects are of extreme importance, and should also be investigated.

Biofilms are complex communities of bacteria that produce a protective layer of polysaccharides (glycocalyx) and persist in diverse reservoirs for extensive periods of time, by demonstrating increased resistance to suboptimal growth conditions, and to traditional cleaning and sanitation programs (Nychas et al., 2007; Stopforth, Samelis, Sofos, Kendall, & Smith, 2002, 2003a, 2003b; Wong, 1998). The efficacy of most antimicrobial agents against microorganisms within biofilms (as compared to planktonic cells) is generally reduced, but for reasons which are not well defined (Stopforth et al., 2002). Due to this lack of understanding, concerns regarding the development and maintenance of biofilms within the meat and poultry industry are justified and increasing evidence and repercussions associated with such niches are quite sobering. Thus, the types, concentrations, combinations, sequences, and environmental conditions during the application of antimicrobial interventions which facilitate/inhibit the development of biofilms on carcasses, meat products, and equipment surfaces should be at the forefront of research efforts. Improved knowledge in any of the above will lead to better antimicrobial intervention systems and improved food safety.

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Chapter 15
Alternative Curing Systems

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Introduction

Through the years, the meat industry has seen the development and production of foods to meet the demands of health-conscious consumers. Food products with natural, organic, preservative-free, and minimally processed claims are now commonly available to consumers in the marketplace. These claims are also frequently found for fresh and processed meat and poultry products. The association of these claims with healthier and safer foods has resulted in a dramatic increase in consumer demands and availability of these products. This has also created the opportunity for larger profit margins for manufacturers, as higher prices are often sought and paid for these products. For traditionally cured processed meat products, new choices have become available to health-conscious consumers. Thus, uncured, no-nitrate/nitrite-added meat and poultry products have been developed to satisfy consumer demands for "healthier" and "safer" processed meat and poultry products.

According to the United States Department of Agriculture, meat products which permit or require nitrate or nitrite can also be manufactured without nitrates or nitrites, but must be labeled to reflect this. The labeling requirements state the term "uncured" must precede the common, usual, or descriptive name, with additional disclaimers including a statement that no nitrates or nitrites were added (Code of Federal Regulations [CFR], 2007a, 2007b).

Sodium nitrite has been historically utilized for the purpose of altering the color, flavor, safety, and shelf-life characteristics of processed meat and poultry products. A wealth of research has shown that nitrite is responsible for the development of cured color and flavor, prevents lipid oxidation and warmed-over flavors by serving as a strong antioxidant, and perhaps, most importantly, acts as a strong antimicrobial agent to control the outgrowth of *Clostridium botulinum*. Over the past four decades, nitrite has been scrutinized by media, consumers, and the scientific community regarding the formation of potentially carcinogenic nitrosamines. Although a great deal of research has been performed addressing this topic, no conclusive link between nitrite and cancer has ever been established. Perceptions still fresh in consumer minds may result in

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uncertainty and skepticism about this extremely important yet still controversial substance.

Uncured, no-nitrate/nitrite-added meat products have provided consumers with purchasing choices for a growing number of different processed meat and poultry products. One would not expect to find any meat product labeled "uncured" having the appearance, aroma, or flavor characteristics of a nitrite-added cured product. This, however, is not the case as commercially available products labeled "uncured" which exhibit characteristics similar to those of a nitrite-cured product are readily accessible to consumers. This can lead to a great deal of consumer confusion.

There are two types of uncured products commonly available to consumers. The first type is where no intention of replacing nitrate or nitrite was made during product manufacture. These products, as would be expected, exhibit appearance, aroma, and flavor characteristics of a product in which no nitrate- or nitrite-related curing reactions took place. The second type is, where there was an intentional replacement of nitrate or nitrite, resulting in products possessing attributes similar to those of nitrate- or nitrite-cured products.

Benefits of Using Nitrates and Nitrites

The addition of salt and nitrite to meat products, otherwise known as curing, has traditionally been associated with processed meats for the purpose of altering their color, texture, flavor, safety, and shelf-life characteristics. Upon the discovery of nitrate and nitrite, cured meat and poultry products that historically were being heavily spiced and cured for preservation reasons were able to be refined to meet flavor characteristics that were demanded by consumers (Cerveny, 1980). These compounds allowed for the emergence of early ready-to-eat-type meat products. By using significantly less salt and/or other preservation methods due to the introduction and incorporation of nitrate or nitrite, meat products began to move from a state of unsatisfactory quality and short shelf life to improved quality and longer shelf life. Of interest to cured meat and poultry products are the benefits of using nitrate and nitrite from both a microbiological as well as a qualitative standpoint.

Microbiological Benefits

The microorganism *C. botulinum* is derived from the Latin word for sausage, botulus (Archer, 2002). It was discovered and first isolated by Van Ermengem in 1896 in an outbreak associated with raw salted pork (Jay, 2000) and incidences involving this microorganism have occurred worldwide (Sofos, Busta, & Allen, 1979). *C. botulinum* is a gram-positive, anaerobic, spore-forming rod (Jay, 2000). Based on

the serological specificity of *C. botulinum* toxins, seven toxigenic types are recognized. These include types A, B, C, D, E, F, and G. In low-acid foods, such as meat products, spores of *C. botulinum* can germinate, grow, and produce toxin. An important and dangerous characteristic of *C. botulinum* spores is that once present, they are extremely heat-resistant. *C. botulinum* grows in the absence of oxygen, making vacuum packaged meat products an ideal medium. If consumed, the resulting toxin causes the disease known as botulism. Botulism is the most lethal of all food-borne diseases, with a 20–50% mortality rate. Clinical symptoms of the disease include double vision, drooping upper eyelid, difficulty swallowing, nausea, vomiting, and abdominal cramping. Botulism toxins prevent certain nerves from functioning, resulting in muscle paralysis and respiratory impairment.

From 1793 to 1990, 688 outbreaks, 1,784 cases, and 978 deaths have been attributed to botulism in the United States, as reported by the Centers for Disease Control (Tompkin, 1980). From 1990 to 2000, 160 outbreaks, 263 cases, and 11 deaths from botulism occurred in the United States (Sobel, Tucker, Sulka, McLaughlin, & Maslanka, 2004). *C. botulinum* grows well in nutrient-rich meat and is not considered a good competitor if other microorganisms are present. Its optimum growth temperature is near 37°C and specific strains can grow at temperatures as low as 3.3°C or as high as 55°C. The necessary pH range for *C. botulinum* growth lies between 4.6 and 8.3 (Jay, 2000). Although *C. botulinum* is a strict anaerobe most commonly associated with growth in the anaerobic conditions of canned products or vacuum packaged products, the interior portion of sausage and cured meat and poultry products that have a low enough oxidation–reduction environment can also support growth and toxin production (Sofos, Busta, & Allen, 1979).

Nitrite is exceptionally effective in controlling *C. botulinum* (Archer, 2002; Huhtanen, Trenchard, & Milness-McCaffrey, 1985; Hustad et al., 1973; Lövenklev et al. 2004; Pierson & Smooth, 1982; Sofos, Busta, & Allen, 1979). In fact, botulism was considered a serious problem associated with meat and sausages until nitrite was utilized as a curing ingredient. The role nitrate and nitrite have on preventing and controlling microbial growth has been examined by numerous researchers. Sofos, Busta, and Allen (1979) stated that as nitrite levels increase, control of *C. botulinum* growth and toxin production also increases. It is important to mention the importance of salt (NaCl) in *C. botulinum* control, as it works synergistically with nitrite to control spore outgrowth. Salt levels of 5% (wt/vol) were shown to completely inhibit *C. botulinum* under optimal growth conditions (Lövenklev et al., 2004). However, salt at this high level would be deemed too salty by consumers. Thus, the salt and nitrite synergistic interaction should be explored in greater detail.

The properties of nitrite that make it an effective antibotulinal compound are dependent on its interactions with several factors. These interacting factors include salt concentration, pH, heat treatment, spore level, ingoing nitrite level during manufacture, and residual nitrite levels in the finished product (Archer, 2002). Other factors include the nature of the competing flora, availability of iron, and the presence of other additives, such as ascorbate, erythorbate, phosphate, etc. (Roberts & Gibson, 1986). The antibotulinal effect of nitrite in thermally processed meat and

poultry product systems takes place at two different stages in the life cycle of *C. botulinum*. The first *C. botulinum*-controlling effect of nitrite is the inhibition of vegetative cells emerging from surviving spores. The second controlling effect is the prevention of cell division in any vegetative cells that do emerge from surviving spores (Pierson & Smooth, 1982).

Sofos, Busta, Bhothipaksa, and Allen (1979) found that heat-shocked (80°C for 15 min) *C. botulinum* spores added to poultry emulsion that was cooked to an internal temperature of 68.5°C and incubated at 27°C grew to toxic levels during 3 or 4 days of incubation when no nitrite or sorbic acid was added. When 156 ppm nitrite was added, that time doubled to 6 days. However, an increase in the delay of toxin production was found when sorbic acid was added in conjunction with sodium nitrite. Sorbic acid at 0.2% and sodium nitrite at 40 ppm controlled *C. botulinum* growth for up to 10–17 days of incubation. Even longer delays (31 days) were shown when sodium nitrite was added at 156 ppm. The authors summarized that a fourfold to tenfold increase in botulinal safety was shown by the combination of sorbic acid and nitrite. This variation was directly related to the amount of nitrite added (40 or 156 ppm). No sensory evaluation was performed in this experiment to determine the acceptance of these combinations, but results do support the importance of sodium nitrite on the control of *C. botulinum.*

Nitrite-cured bacon was found to be considerably more resistant to *C. botulinum* outgrowth, which was attributed to higher salt content as well as the additive effect of nitrite and sodium chloride (Huhtanen, Talley, Feinberg, & Phillips, 1981). Huhtanen, Feinberg, Trenchard, and Phillips (1983) reported that the use of potassium sorbate alone or when combined with hydrochloric, phosphoric, acetic, citric, lactic, succinic, or sorbic acids was not as effective as 120 ppm sodium nitrite in inhibiting *C. botulinum* in injected bacon. The authors' data did, however, state that the control of *C. botulinum* in ham, chicken, and turkey frankfurters with a combination of sorbic acid or potassium sorbate and phosphoric acid was effective. The levels necessary for this control may be high enough to result in negative acceptance by consumers, although this was not suggested in this study. Nelson, Busta, Sofas, and Wagner (1983) showed that a combination of 0.26% potassium sorbate and 40 ppm nitrite was effective in inhibiting *C. botulinum* spore germination and that sorbic acid plus sodium nitrite treatments offered comparable results, contrary to results by Huhtanen et al. (1983). Product pH differences between studies are believed to be responsible for the discrepancies and support the importance of pH in the control of *C. botulinum*. Sodium acid pyrophosphate, sodium hexametaphosphate, and sodium tripolyphosphate in combination with sorbic acid (0.20%) or potassium sorbate (0.26%) and sodium nitrite (40 ppm) also showed inhibitory effects on botulinal toxin production by extending the delay phase.

Hustad et al. (1973) stated the development of *C. botulinum* toxin in wieners was directly influenced by the level of nitrite added to the meat. They reported that nitrate had little effect on the control of toxin production by *C. botulinum*, yet nitrite added at 50 ppm resulted in only 2 toxic samples out of 110 tested, while nitrite levels higher than 50 ppm ingoing resulted in 0 toxic samples. This work is

in agreement with that of Lövenklev et al. (2004), who noted that 45 ppm sodium nitrite was effective in suppressing *C. botulinum* gene expression.

Bayne and Michener (1975) reported that nitrite concentrations present in commercial meat products are not sufficient to effectively inhibit the growth of *Staphylococcus* or *Salmonella.* This is in disagreement with earlier work performed by Buchanan and Solberg (1972), who demonstrated that, at pH 7.2, sodium nitrite levels ranging between 0 and 2,000 ppm in Brain-Heart Infusion Agar had no inhibiting effect on *Staphylococcus aureus* aerobically. Oxygen pressure and pH influence the bacteriostatic action of sodium nitrite on *S. aureus*. It is reported that nitrite inhibits bacteria more effectively at low pH or acid values (Roberts, 1975). The same results were found under anaerobic conditions, with the exception of a significant extended lag phase found at a level comparable to prepared curing brines of 2,000 ppm. Interestingly, at pH 6.3, increasing ppm of sodium nitrite increased the lag phase and generally reduced the growth phase aerobically and, even more drastically, under anaerobic conditions. The authors suggested their results offer evidence that *S. aureus* could be significantly controlled in cured meats with ingoing sodium nitrite levels of 200 ppm, especially if vacuum packaging is used.

De Giusti and De Vito (1992) reported that results on the inactivation of *Yersinia enterocolitica* by nitrate and nitrite in treated pork versus in vitro can actually be difficult to compare. However, they found that 100, 150, and 250 ppm sodium nitrite mixed with pork mince and inoculated with *Y. enterocolitica* resulted in no colony forming units present after 24 h of incubation. It is important to note that inoculum levels may affect the results of inoculation studies. Further, investigations studied at very high levels may not correlate well with large-scale commercial production scenarios (Roberts, 1975).

Qualitative Benefits

Besides the previously described antibacterial effects, nitrite possesses several characteristics that improve meat and poultry products from a qualitative standpoint, including color, lipid stability, and cured meat flavor development.

Color

Slightly before and at the turn of the twentieth century, E. Polenske, J. Haldane, and K. B. Lehman were among the first scientists to closely examine the curing process and report their scientific findings (Pierson & Smooth, 1982). Polenske examined sterilized and unsterilized solutions of saltpeter (potassium nitrate) for the presence of nitrite. Based on his findings, he was able to show that nitrate was reduced to nitrite and subsequently nitric oxide. This phenomenon was attributed to microorganisms. Polenske and Haldane also showed that subsequent heating of meat products containing nitrite and nitric oxide resulted in the production of cured color as

a result of the modifications of meat pigments during the heating process. Haldane found similar results with the same overall conclusions. Haldane also investigated the pigments that were involved with the red color of cured meats. By adding nitrite to hemoglobin (Hb) to produce nitrosylhemoglobin (NOHb) and then heating these pigments, Haldane was able to generate nitrosylhemochromogen. He then explained that NOHb was the pigment responsible for the red color in cooked cured meat. These early scientists' work established the concept that unpurified salt contaminated with sodium or potassium nitrates was responsible for the color of cured meat. According to Sebranek (1979), in 1899 Lehman showed that nitrite, not nitrate, was responsible for cured color. His findings were later confirmed by R. Hoagland in 1908 and R. H. Kerr and others in 1926. In an investigation by Hoagland, the author noted that nitrates were converted by bacteria or enzymes to nitrites, which subsequently changed to nitric oxide that united with myoglobin in meat to produce a red color (Cerveny, 1980).

Hoagland then continued his investigations and in 1914 presented further proof that saltpeter (potassium nitrate) must be reduced to nitrite to have any functionality as a curing ingredient. He also reported that nitrite needed to have a reducing agent present to produce nitric oxide. His investigation of adding nitrite to alkaline meat, which resulted in no cured color development, supported his theory.

While the importance of nitrite in cured color development is important, considerably less nitrite is needed to provide for color development than to control bacteria (Roberts, 1975). The main portion of nitrite added to cured meats is for *C. botulinum* control, whereas only a small portion (roughly 25 ppm or less) is needed for color development and stability (Sofos, Busta, & Allen, 1979). It has been suggested that as little as 5 ppm is actually needed for satisfactory cured color development, 20 ppm may be needed for cured color stability, and at least 50 ppm is needed for *C. botulinum* control (Sofos, Busta, & Allen, 1979).

Lipid Stability

The addition of nitrite is also extremely effective in controlling lipid oxidation in cooked meat products (Roberts & Gibson, 1986). Nitrite increases the qualitative shelf life of cured meat and poultry products (Erduran & Hotchkiss, 1995; Pearson & Tauber, 1984; Townsend & Olson, 1987) by controlling and stabilizing the oxidative states of lipids in meat and poultry products (Shahidi & Hong, 1991). Lipid oxidation is considered to be a major reason for quality deterioration in meat products, which often results in the development of rancidity and, subsequently, warmed over flavors (Vasavada & Cornforth, 2005; Yun, Shahidi, Rubin, & Diosady, 1987). The rate and degree of lipid oxidation is related to the amount of unsaturated fats present, as well as to oxygen exposure, removal of oxygen, and the addition of antioxidants and/or reducing agents (Ramarathnam, 1998). Salt is known to be a strong pro-oxidant and thus adds difficulty to controlling lipid oxidation. The effect of nitrite on controlling lipid oxidation is explained by Townsend and Olson (1987),

and involves controlling the iron that would otherwise serve as a catalyst for lipid oxidation reactions. As nitrite reactions form cured pigments, the iron present in the meat is retained by these reactions, reduced to the Fe^{+2} form, and thus rendered inactive or unavailable as a catalyst for lipid oxidation reactions. Erduran and Hotchkiss (1995) supported this and stated the importance the above reactions had on preventing Fe^{+2} from being released and made available for lipid oxidation reactions during the heating or thermal processing of meat and poultry products. Once oxidative reactions begin, auto-oxidation normally commences. Vacuum packaging has been shown to be an effective intervention to control these quality problems, by virtue of its aid in preventing or delaying rancidity (Chang & Chen, 1998). However, even when vacuum packaging is utilized, no-nitrite-added bacon has been shown to develop off-flavors rapidly, likely due to the exclusion of nitrite (Gray, Macdonald, Pearson, & Morton, 1981).

Cured Meat Flavor Development

The role nitrite has on meat flavor involves complex stimuli, such as aroma/odor, texture, taste, and temperature (Gray et al., 1981). The chemistry behind the composition and formation of cured meat flavor is not clearly understood (Gray et al., 1981; Ramarathnam, 1998). Ramarathnam (1998) explained that the nature of cured meat flavor is unknown, but the inhibitory effects nitrite has on lipid oxidation aid in the development of the unique flavor. In addition, sensory research has suggested that cured flavor is not solely a result of retarding lipid oxidation, but a combination of a complex cured aroma and flavor in cooperation with a lack of rancid flavors. Many of the same compounds that may contribute to aroma and flavor are present in both uncured and cured, cooked meat products. The volatile compounds 4-methyl-2-pentanone, 2,2,4-trimethylhexane, and 1,3-dimethylbenzene are three components identified as possible direct or indirect contributors to cured meat aroma (Ramarathnam, 1998).

Ramarathnam, Rubin, and Diosady (1991a) studied aroma concentrates from cooked, uncured, and cured pork. The authors identified 50 hydrocarbons, 37 carbonyls, 6 acids, and 2 alcohols present in both the uncured and cured pork that may contribute to cured meat flavor. By utilizing purge-and-trap methods of gas chromatography, they identified 32 new meat flavor compounds for uncured and cured pork (Ramarathnam, Rubin, & Diosady, 1993a) and 12 new meat flavor compounds for beef and chicken (Ramarathnam, Rubin, & Diosady, 1993b). One component of interest, hexanal, was found in both cured and uncured pork, but was reported to be at a considerably lower level in cured pork (0.24% of amount found in uncured).

A similar pattern for hexanal has also been shown for both cooked, uncured and cured beef and chicken (Ramarathnam, Rubin, & Diosady, 1991b). Hexanal is an oxidation product of lipid and may play a role in understanding flavor from a lipid oxidation effect standpoint. Ramarathnam et al. (1991b) also observed many volatiles were either absent or present in lower concentrations in cured compared to uncured pork, possibly providing an initial explanation of cured meat flavor.

Difficulty in identifying specific components involved in cured meat flavor has been attributed to the formation and interference of carbonyls and hydrocarbons during isolation and detection that make it difficult to isolate many minor (in concentration) yet possibly significant components (Ramarathnam et al., 1991b).

In sensory studies, panelists were able to differentiate between samples manufactured with different levels of nitrite (10, 156, and 200 ppm) (Gray et al., 1981). Olesen, Meyer, and Stahnke (2004) reported considerable differences in the production of volatile compounds in fermented dry sausages manufactured with nitrite, nitrate, or nitrite/ascorbate combinations and two different starter culture combinations. The authors state that, besides curing compounds, microbial starter cultures also play significant roles in the generation of important fermented sausage volatile compounds. Also investigating nitrite versus no-nitrite usage in dry sausage, Noel, Briand, and Dumont (1990) concluded that nitrite plays an extremely important role in the development of specific flavor notes as supported by sensory analysis. Dethmers and Rock (1975) stated the addition of nitrite above 50 ppm in Thuringer sausage reduced off-flavor development and improved the flavor quality, whereas treatments with no nitrite added were considered to be the most rancid and had the poorest flavor quality. Investigating the role of nitrite addition in ham, Froehlich, Gullet, and Usborne (1983) reported a significant improvement in trained sensory cured meat flavor intensity scores as ingoing nitrite levels increased from 0, 50, and 100 ppm. As levels of nitrite increased (from 0 to 100 ppm), significant differences were also reported for Hunter a* color values indicating that higher ingoing amounts of nitrite yielded objectively redder (pinker) colored ham.

Human Exposure to Nitrates and Nitrites

Up to this point, the discussion of nitrate and nitrite has been centered on the manufacture and consumption of cured meat and poultry products. However, the whole story of this unique compound has not yet been told. Knowingly or not, all humans consume, synthesize, and utilize nitrate and nitrite on a daily basis. Nitrite is actually excreted in sweat. Nitrate and/or nitrite present in humans can be derived from endogenous or exogenous sources. Where those sources originate is of particular interest. Nitrite is found throughout the environment and is a primary component of the global nitrogen cycle. The major source of human exposure to nitrite is by oral intake of food and water (Abuharfeil, Sarsour, & Hassuneh, 2001; Chung, Chou, & Hwant, 2004; White, 1975). It has been suggested that 80% of ingested nitrate and 40% of ingested nitrite pass through the body unchanged and are excreted in the urine. The remaining 20% of nitrate is absorbed and found in blood plasma, but the fate of the remaining 60% of nitrite is not precisely known.

Nitrite can be synthesized endogenously in the human body by enzymatic and other possible reactions. Nitrite plays profound roles in normal bodily functions. As a product of enzymatic synthesis in humans, nitric oxide from the synthesis of

nitrite controls blood pressure, immune response, wound repair, and neurological functions (Archer, 2002). Nitric oxide has even been believed to act as a defensive weapon by inhibiting key metabolic pathways to block growth or kill disease cells in the body (Cassens, 1995). Thus, nitrite can be viewed as a natural metabolite vital to the repair, function, and survival of human biological system.

The presence of nitrates and nitrites in the human body can also result from exogenous sources. Nitrate is commonly ingested when people consume vegetables (Archer, 2002) and perhaps certain fruits (Hardisson, González Padrón, Frías, & Reguera, 1996). It is well known that leafy green or root vegetables and drinking water are sources of nitrate that humans are exposed to (Cassens, 1997b). Nitrates and nitrites are part of the nitrogen cycle of plants and are by-products of green plant photosynthesis (Bednar & Kies, 1994). Nitrogen-containing fertilizers used on vegetables, plant genetics, maturity, and environmental conditions plants grow in (e.g., lack of water, soil fertility) also can play a role in the amount of nitrate found in these foods (Wolff & Wasserman, 1972). It has been proposed that food sources that contain nitrate are the primary source of human nitrate intake, from which vegetable and water consumption can account for 80–95% of total ingested nitrate. White (1975) estimated that 81.2% of nitrate intake and 1.6% of nitrite intake are derived from vegetable consumption. The National Academy of Sciences (National Academy of Sciences [NAS], 1981) stated that vegetables account for 85% of dietary nitrate, reporting levels of 2,600 ppm nitrate in beets, 1,5001987 ppm in celery, and 1,700 ppm in lettuce. Knight, Forman, Al-Dabbagh, and Doll (1987) found that vegetables in Great Britain contributed over 90% of nitrate intake. The same authors additionally noted that a greater reduction in human exposure of nitrate is more feasible by reducing the intake of vegetables and water than that of cured meat and poultry products. A study by García Roché, García, and Torres (1987), estimating the intake of nitrates and nitrites of 12,000 12–17-year-old students in Havana, Cuba, reported that daily consumed levels of 65–79 mg of nitrate and 2.3–4.8 mg of nitrite were lower than the acceptable maximum daily intake recommended by the Food and Agriculture Organization of United Nations and the World Health Organization. From these findings, the authors stated that there was no health risk associated with these common levels of nitrate and nitrite consumption.

The National Academy of Sciences reported that 39% of dietary nitrite intake was from cured meat, 34% from baked goods and cereals, and 16% from vegetables (NAS, 1981). White (1975) reported four-fifths of dietary nitrate may originate from vegetables and less than one-sixth from cured meats. In an American Meat Institute Foundation report, a panel of scientists concluded that less than 5% of nitrite intake derives from cured meats (American Meat Institute [AMI], 2003). Surprisingly, saliva can easily account for over 90% of ingested nitrite. Previously ingested nitrate, which is absorbed in blood plasma and later re-circulated to the oral cavity, in addition to a portion of dietary nitrate present during consumption, are both reduced to nitrite by the bacteria and acidic conditions present in the oral cavity (Archer, 2002; Cassens, Greaser, & Lee, 1979).

Residual Nitrite

When nitrite is added to meat systems, it reacts chemically or is bound to components such as protein. Heat during thermal processing serves to speed up these reactions. After normal manufacturing processes, the amount of detectable nitrite is usually only approximately 10–20% of the initial added amount when measured analytically (Cassens, 1997a). These levels of nitrite, also known as residual nitrite, decline over the storage life of cured meat products until they are often undetectable (Eakes & Blumer, 1975; Skjelkvåle & Tjaberg, 1974).

In a comprehensive study of nitrite and cure accelerator levels for cooked sausages and dry and semidry sausages, the Nitrite Safety Council (1980) reported "highly variable" residual nitrite and cure accelerator levels, not only within product categories but also between specific types of sausages (beef, pork, poultry, and so forth). They also reported that, generally, 25–50% of added nitrite remained in the product during 24–48 h after processing. Cassens (1997a), after purchasing and analyzing local retail commercial cured bacon, sliced ham, and wieners, reported residual nitrite levels of 1–15 ppm in bacon, 3–9 ppm in sliced ham, and 1–9 ppm in wieners. In a separate study investigating retail commercial cured bacon, bologna, ham, and wieners, Cassens (1997a) reported a range of 0–48 ppm residual nitrite for all four products, with an overall product average of 10 ppm.

In Thuringer sausage, researchers discovered residual nitrite concentrations varied based on ingoing levels (50, 100, 150 ppm), finished product storage time, and storage temperature (Dethmers & Rock, 1975). Initial ingoing nitrite levels compared to raw emulsion levels showed a nitrite decrease of approximately 40%, approximately 80% at 0 weeks of storage, and approximately 86% and 92% at 1 and 4 weeks of storage, respectively, in samples held at 7.5°C. A similar rapid decrease was observed at a storage temperature of 27°C for all ingoing levels.

Residual nitrite has been criticized and associated with cancer formation in humans. Epidemiological data generated by human study participants who were instructed to recall past consumption of cured meat products brought the concern of nitrite usage to a high level of public awareness. Researchers who reviewed these epidemiological reports concluded that (1) methodology problems existed, resulting in misleading conclusions, (2) nitrite is not a carcinogen, (3) nitrosamines are not found in hot dogs, and (4) most of the patients in the studies were from low income groups, not clearly depicting the entire population (Cassens, 1990; NAS, 1981). Nonetheless, residual nitrite is not only important from a qualitative perspective but also from a food safety standpoint.

Szczawinski, Szczawinsia, and Szulc (1989) discussed the importance of residual nitrite in nitrite-cured, pasteurized, and irradiated pork meat, and stated that the extent of *C. botulinum* spore inhibition is related to the rate of nitrite depletion which, if too fast, can allow the germination of spores. These authors maintain that botulinal spores can germinate in the presence of nitrite but vegetative cell growth is clearly inhibited by that same nitrite. These residual nitrite levels can adversely be affected by irradiation. High-dose irradiation (10–50 kGy) can severely deplete nitrite levels and reduce the inhibition of *C. botulinum* spore germination and outgrowth.

Safety of Nitrates and Nitrites

The concern regarding intake of nitrates and nitrites in humans is centered on the possibility that these two compounds may be a source of nitrosating compounds, leading to the subsequent and toxic development of carcinogenic *N*-nitroso compounds such as *N*-nitrosamines (Walker, 1990). Fiddler, Pensabene, Gates, Hale, and Jahncke (1992) stated that the first incident involving a nitrosamine was associated with a fish-derived food product. The presence of carcinogenic *N*-nitrosamines in meat products, particularly in bacon, has caused concern with regard to the use of nitrite in meat and poultry products (Cassens, 1995; Cassens, 1997a; Shahidi, 1988). Carcinogenic volatile *N*-nitrosamines have been suggested to induce tumors in many organs in the human body (Ahn, Kim, Jo, Lee, & Byun, 2002). The formation and presence of these dangerous compounds can be caused by exposing foods containing sodium nitrite to high temperatures (Ahn, Kim et al., 2002). It has also been suggested that nitrates react with amines present in gastric acids to form carcinogenic nitrosamines (Archer, 2002). However, no association or link between nitrates in the presence of gastric acids and carcinogenic nitrosamines has been identified. In fact, no epidemiological study to date has linked nitrate or nitrite consumption to a specific cancer or cancer risk. Eichholzer and Gutzwiller (1998), after investigating dietary nitrates, nitrites, and *N*-nitroso compounds, summarized that no epidemiologic evidence was found linking brain, esophageal, and nasopharyngeal cancers to the intake of these compounds. An association between them, however, was not ruled out. Maekawa et al. (1982) investigated the carcinogenicity of sodium nitrite and sodium nitrate in 240 Fischer-344 rats. Over a 2-year study consisting of the continuous administration of sodium nitrite and sodium nitrate in dietary drinking water, these authors found no carcinogenic activity from these ingredients or nitrosamines generated. Furthermore, two summary reports generated from an immense amount of research and testing, published by the National Academy of Sciences (NAS, 1981; NAS, 1982), conclusively asserted that nitrite-cured meat did not pose a human health risk.

Nitrosamine and Cancer Relationship

Nitrite is a reactive chemical and a controlled restricted ingredient and must therefore be used with caution. It can act as a nitrosating compound under certain conditions, producing nitroso compounds, some of which are classified as carcinogens (Cassens, 1997b). It is suggested that nitrite converted to nitric oxide can react with certain classes of secondary amines to form carcinogenic nitrosamines. This is a particular problem in bacon with the presence of residual nitrite (Wolff & Wasserman, 1972). For this reason, the presence and formation of nitrosamines in meat products containing nitrate or nitrite have been considered important to investigate and understand. A common belief is that nitrosamines present or produced in cured meat products can potentially be carcinogenic (Brown, Hedrick, & Bailey,

1974). Thus, a great deal of research has been done to reduce residual nitrite levels in cured meat products.

Among meat products, bacon has presented the greatest challenge in terms of eliminating the presence and formation of nitrosamines (Pearson & Tauber, 1984). The combination of high cooking temperatures (i.e., frying), the presence of secondary amines, and residual nitrite have made it difficult to address the nitrosamine issue in bacon (Pearson & Tauber, 1984). As a result, special regulations by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) require lower ingoing nitrite levels of 120 ppm and the complete exclusion of nitrate in bacon manufacture. In addition, further reduction of ingoing nitrite levels and other nitrite and nitrosamine reducing technologies have been investigated for their effectiveness.

In an effort to reduce ingoing nitrite in bacon, Tanaka, Meske et al. (1985) investigated the use of lactic acid starter culture and lower sodium nitrite levels. These investigators reported that bacon manufactured with 40 or 80 ppm sodium nitrite, lactic acid starter culture, and sucrose had better antibotulinal protection and less nitrosamine formation than control bacon manufactured with 120 ppm sodium nitrite, no lactic acid starter culture, and no sucrose. In addition, this process, also known as the "Wisconsin Process," resulted in bacon with sensory characteristics similar to traditional manufactured bacon (Tanaka, Gordon et al., 1985).

Ivey, Shaver, Christiansen, and Tompkin (1978) indicated that fried bacon containing potassium sorbate and increasing levels of nitrite did not result in increased formation of the nitrosamine nitrosopyrrolidine. On the contrary, Fiddler et al. (1992) found increased levels of nitrosamine formation in fish-meat frankfurters when heat treated by frying (171°C). The authors investigated nitrosamine formation from boiling, microwaving, roller grilling, broiling, and frying cookery methods and reported that the least amount of nitrosamines was formed by microwaving and the most by frying. Optimal nitrosamine formation temperatures of 100°C, achieved during frying, were suggested to explain their findings. Ahn, Jo et al. (2002) monitored nitrite and *N*-nitrosamine levels in irradiated pork sausage and reported that irradiation effectively reduced residual nitrite levels, as well as the formation of *N*-nitrosamines after a 4-week storage period.

Lowering ingoing nitrite levels has also been considered a possibility for addressing the nitrosamine problem. This approach often affects the quality, consumer acceptability, and, potentially, the food safety of these types of meat products (Pierson & Smooth, 1982). Thuringer sausage manufactured with 50, 100, and 150 ppm nitrite had no nitrosamines detected over 4 weeks of storage at 7.5°C and 27°C (Dethmers & Rock, 1975). Sebranek (1979) supported the effectiveness of reduced levels of ingoing nitrite in product formulations by the use of cure accelerators such as erythorbate and ascorbate, for reducing both residual nitrite as well as potential nitrosamine formation in cured meat products. Rywotycki (2002), investigating ingredient and heat effects on nitrosamine formation, concluded that curing with sodium nitrite can increase nitrosamines, but that these effects can be negated by the addition of sodium ascorbate to cured meat product formulations.

Uncured, No-Nitrite/Nitrate Added Meat Products

Rationale and Concept

Although there are several advantages of using nitrite in meat curing systems, there is one disadvantage that has plagued this additive for over 30 years. The implications surrounding the presence and formation of *N*-nitrosamines in cured meat products, specifically bacon, have continuously held nitrite at the forefront of carcinogen-related concerns (Shahidi, 1988). Negative perceptions of nitrite-cured meat may have, in part, prompted consumers to shift purchasing decisions to uncured, no-nitrate/nitrite-added meat and poultry products. The perception that uncured, no-nitrate/nitrite-added meat and poultry products are "safer" and "healthier" are sensible reasons for the consumer demands of these products. Creative marketing and labeling may also play a role in this perception. Commercial uncured, no-nitrate/nitrite-added meat and poultry products are commonly manufactured with organic or natural raw materials and ingredients. Two types of products currently exist in the marketplace: (1) those with no intent of replacing nitrate and/or nitrite and (2) those with the intent of replacing nitrate/nitrite to simulate typical curing.

Product Labeling

Meat and poultry products in which nitrate or nitrite is permitted or required can also be manufactured without nitrates or nitrites but must be labeled according to the regulations (Code of Federal Regulations Title 9, Parts 317.17 and 319.2), which state:

> Any product … which is required to be labeled by a common or usual name … to which nitrate or nitrite is permitted or required to be added may be prepared without nitrate or nitrite and labeled with such common or usual name or descriptive name when immediately preceded with the term "Uncured" as part of the product name, provided that the product is found by the Administrator to be similar in size, flavor, consistency, and general appearance to such product as commonly prepared with nitrate or nitrite, or both.
> Products … which contain no nitrate or nitrite shall bear the statement "No Nitrate or Nitrite Added."
> Products described … shall also bear the statement "Not Preserved–Keep Refrigerated Below 40 °F. At All Times" unless they have been thermally processed to F_o 3 or more; they have been fermented or pickled to pH of 4.6 or less; or they have been dried to a water activity of 0.92 or less.
> Products … shall not be subject to the above mentioned labeling requirements … if they contain an amount of salt sufficient to achieve a brine concentration of 10 percent or more. (Code of Federal Regulations [CFR], 2007a)

Since no other labeling standard exists, products manufactured without the addition of sodium (or potassium) nitrate or nitrite and with the intent to simulate typical curing must still include the labeling term "Uncured." Controversy has resulted

from this dilemma as technology has surpassed the labeling standard for a category of meat products for which it was not originally intended. Thus, the meat industry is required to label both types (no intention and intention of replacing nitrate/nitrite) "uncured," resulting in a potential for consumer confusion.

The terms organic and natural found on labels can often lead to additional confusion when paired with the term uncured. Products labeled organic or natural are not allowed to contain added nitrates or nitrites by regulatory definition. Regulations for organic and natural serve a distinctly different category of products governed by separate labeling policies. Those policies and differences are beyond the scope of this discussion, but a generalization is that all organic- and natural-labeled products are uncured but not all uncured products are necessarily natural or organic.

Concerns

Uncured, no-nitrate/nitrite-added meat and poultry products can carry higher risks for botulism than nitrite-added products. These products lack the barriers that help ensure the safety associated with these types of products, especially if temperature abused (Miller, Call, & Whiting, 1993). Miller et al. (1993) investigated various organic acid salts (lactate, pyruvate, and citrate) for *C. botulinum* control in uncured, no-nitrate/nitrite-added ground turkey breast and found that inoculated samples became toxic before off-odors and soft textures were apparent to sensory panelists. This fact is alarming in that the traditional method of relying on spoilage microorganisms as early warning indicators of potential pathogen growth is not necessarily applicable to uncured, no-nitrate/nitrite-added meat and poultry products. On the other hand, Bayne and Michener (1975) stated that products manufactured with or without nitrite can become hazardous if contaminated with *Staphylococcus* or *Salmonella*. Temperature abuse may be an important factor in regard to the growth of these two microorganisms. Microorganisms that survive thermal processing or are a result of postlethality contamination did not grow significantly faster in uncured, no-nitrate/nitrite-added frankfurters than conventional ones. Hustad et al. (1973), investigating *C. botulinum* toxin production in wieners, reported that 79 of 220 samples that did not contain nitrate or nitrite were positive for the presence of *C. botulinum* toxin after 56 days of storage at 7°C and 27°C.

Trends in the 1990s regarding the introduction and production of preservative-free, low-salt food products packaged in air-tight containers have sparked concern, since little or no intrinsic control of *C. botulinum* spore germination and growth is present in these products (Sobel et al., 2004). Since the sole control method for *C. botulinum* germination and growth in these consumer-perceived, "healthier" uncured products is refrigeration, concerns about these types of products are relevant to food safety discussions.

It has been stated before that no compound has been found to date that provides all the functions of nitrite in cured meats. In fact, efforts to replace nitrite with a compound(s) that imparts similar characteristics have been difficult and often

unsuccessful. Yun et al. (1987) reported that combinations of chelators and antioxidants can successfully duplicate the action of sodium nitrite in cooked pork. However, microbial safety or consumer visual acceptance was not addressed. Huhtanen and Feinberg (1980) reported that sorbic acid can be used successfully to inhibit *C. botulinum*, but neither lipid oxidation nor consumer acceptance of the uncured, no-nitrate/nitrite-added poultry frankfurters were addressed in their study. Huhtanen et al. (1983) also found success in inhibiting *C. botulinum* in bacon research by replacing nitrite with alkynoic and alkenoic acids and esters, yet these researchers did not address lipid oxidation or consumer acceptance. Wasserman, Kimoto, and Phillips (1977) investigated the consumer acceptance of bacon cured with and without nitrite. The authors reported no significant differences for preference between the two treatments; however this study did not consider the risk of *C. botulinum* growth or the oxidative stability over time of the nitrite-free bacon.

Consumer Acceptance

From a sensory standpoint, consumer preferences for meat and poultry products are influenced by appearance, flavor, tenderness, and juiciness (Resurreccion, 2003). In addition, heath concerns (desire for healthy foods), the need for convenience, and price can often be factors in consumer demand for meat products. Bauermann (1979) stated that uncured, no-nitrate/nitrite-added chicken frankfurters have an extremely undesirable gray-green color under normal daylight. The color of uncured, no-nitrate/nitrite-added turkey frankfurter was described as even less desirable in appearance. Huhtanen et al. (1981) reported that flavor scores for nitrite-cured and uncured, no-nitrate/nitrite-added sorbic acid-containing bacon were not significantly different from one another, but large variations of flavor scores within the treatments were shown. The authors summarized that uncured, no-nitrate/nitrite-added sorbic acid-containing bacon presented acceptable sensory quality attributes and satisfactory *C. botulinum* spore outgrowth prevention. However, results from cured ham research performed by Brown et al. (1974) revealed that hams manufactured without nitrite had lower flavor intensity sensory scores than hams cured with either 91 or 182 ppm nitrite.

Investigating the “plate waste” or how much nitrite-cured and uncured, no-nitrate/nitrite-added bacon remained on the breakfast plates of students in a dining hall, Williams and Greene (1979) reported no significant difference between the two types of bacon served four times over a two and one half-hour testing period. However, 2-thiobarbituric acid (TBA) test numbers for nitrite-cured bacon were 0.4 and 0.6 on the first and last days of testing, respectively, whereas no-nitrate/nitrite-added bacon had TBA numbers of 0.8 and 1.7 on the same days. A TBA number of 0.5 to 1.0 is considered to be the threshold for oxidized odor and 1.0 to 2.0 for oxidized flavor (Tarladgis, Watts, & Younathan, 1960).

Hustad et al. (1973) reported that flavor quality scores for wieners with varying levels of nitrite added (50–300 ppm) were all significantly higher than for others with 0 ppm added (no nitrite added). Color of the wieners between all nitrite levels (50, 100, 150, 200, and 300) was not deemed to be different from each other, but all were considered different compared to wieners with 0 ppm added nitrite. In this study, the uncured, no-nitrate/nitrite-added wieners' internal surface was described as brown and gray. Noel et al. (1990) looked at similar criteria for uncooked dry sausage. They reported objective color differences between nitrite-added and no-nitrite-added sausages. Utilizing sensory triangle tests, the authors reported that flavor properties could be significantly differentiated between nitrite-added and uncured, no-nitrite-added treatments. Another sensory triangle test attempted to use hardwood smoke to mask both cured meat flavors in nitrite cured pork roast and flavors present in uncured, no-nitrite cured pork roast (Cho & Bratzler, 1970). However, this effort to possibly improve the acceptance of uncured, no-nitrite-added cured pork roast was determined unsuccessful.

Gray et al. (1981) noted that acceptable bacon could be manufactured without using nitrite, according to sensory preference testing, but this study did not consider the oxidative stability or possible risks associated with *C. botulinum* in uncured bacon. Contrarily, ham samples with no added nitrite were disliked by a consumer sensory panel, compared to nitrite-added ham samples (Froehlich et al., 1983). Interestingly, 50 or 150 ppm added nitrite had no effect on overall desirability of ham, suggesting that the intensity of cured color in cured meats may not necessarily be as important as the presence of cured color in cured meat products.

Judge and Cioch (1979) produced uncured, no-nitrate/nitrite-added hams injected with a solution of water, sodium chloride, brown sugar, sodium tripolyphosphate, and sodium erythorbate. Sensory panelist scores for palatability were deemed acceptable. Flavor and texture attributes were considered not to resemble those of nitrite-cured hams and were generally described as those of uncured roasted pork. Wasserman et al. (1977) showed no difference in consumer preference between uncured, no-nitrate/nitrite-added bacon and nitrite-cured bacon.

The Future

For uncured, no-nitrate/nitrite-added products to become successful, an acceptable food additive/ingredient must be found that produces the characteristic cured flavor and appearance as well as provides the antibotulinal role of nitrite-added products (Tompkin, 1980). Wolff and Wasserman (1972), commenting on removing nitrate and nitrite completely from cured meat products, stated that "we could be replacing one hazard by another, more serious one." Addressing these issues is critical for the manufacture of safe and consumer-desirable uncured, no-nitrate/nitrite-added meat and poultry products.

Alternatives to Nitrates and Nitrites in Cured Meat Products

In order to identify alternatives to adding nitrite itself to cured meat products, a more thorough understanding of the chemical nature of the inhibitor that is formed by heating nitrite in meat products is necessary (Roberts, 1975). Nitrite use dates back to antiquity; however, regulated use has been in effect since 1925 (Shahidi & Pegg, 1992). To date, no replacement for nitrite has been discovered that effectively produces the characteristic cured meat aroma and flavor of the meat products it is used in (Gray et al., 1981). Shahidi and Pegg (1992) stated that "it is unlikely that a single compound will be found that can perform all the functions of nitrite." Furthermore, the National Academy of Sciences committee on nitrite and alternative curing agents in food stated, from a food safety perspective, that "the committee believes that the degree of protection against botulism is likely to decrease if the essential preservative uses of nitrite are substantially reduced without introducing an efficacious, but safer alternative" (NAS, 1981).

Sources to Replace Nitrite

With the continued demand for "safer" and alternative uncured, no-nitrate/nitrite-added meat and poultry products, research revolving around the replacement of sodium nitrite has been performed in great depth. Results and information gathered from the numerous attempts at nitrite replacement have improved the knowledge about both the importance of sodium nitrite as well as the difficulty of removing it from cured meats. Two approaches can be viewed for removing and/or replacing nitrite. Direct replacement is defined as complete removal of nitrate and nitrite from a curing system, while indirect replacement is the process of removing some or all nitrate and nitrite from the curing system and replacing it with another source.

Direct Replacement

Sorbic acid and its alkaline salts have been used to control spoilage by inhibiting yeasts and molds in food products (Sofos, Busta, Bothipaksa et al., 1979). Investigating canned, uncured, no-nitrate/nitrite-added poultry frankfurters, Huhtanen and Feinberg (1980) stated that sorbic acid can be used to inhibit *C. botulinum* spore outgrowth. Growth and toxin production times, which were measured by the time it took for the cans to swell from gas production, were longer for poultry (chicken and turkey) frankfurters with 0.40% added sorbic acid compared to those with none added. This effective level was suggested to be comparable to commercial products containing 135 ppm nitrite. However, Bauermann (1979) found

that using sorbic acid to replace sodium nitrite at intended usage level in chicken and turkey frankfurters imparted flavor acceptance problems.

Sofos, Busta, Bothipaksa et al. (1979) found similar delays in toxin production (6 or 7 days; incubation at 27°C) in poultry emulsions with 0.2% added sorbic acid. Below this level, no effect on toxin prevention was shown. Furthermore, the antimicrobial activity of sorbic acid is dependent on the pH of the meat product (Sofos, Busta, & Allen, 1979). Since free undissociated acid at low pH (5.0–5.5) is the effective form of sorbic acid, its application to an array of meat products that have large variations in pH may be in question. Huhtanen et al. (1981) speculated that the decrease in pH from the addition of sorbic acid of around 0.14% aided in the control of *C. botulinum* spore outgrowth in uncured, no-nitrate/nitrite-added bacon. Thus, lowering the pH had positive effects on controlling *C. botulinum* spore outgrowth.

Huhtanen et al. (1985) and Huhtanen (1983) investigated the potential replacement of nitrite for controlling *C. botulinum* by the action of several short-chain alkynoic and alkenoic acids and esters added to bacon inoculated with *C. botulinum* spores. Several of these compounds were shown to have effective properties when compared with a control manufactured with 120 ppm sodium nitrite. However, the authors stated that the organoleptic and physical properties of the products would need to be addressed and evaluated to determine product acceptance.

The organic acid salts propionate, citrate, acetate, lactate, and pyruvate were tested for their effectiveness in suppressing *C. botulinum* growth in uncured, no-nitrate/nitrite-added turkey product. Although results varied between them, the general conclusion was that these compounds may contribute to improving the margin of food safety in turkey products by acting as secondary barriers to *C. botulinum* outgrowth (Miller et al., 1993). No reduction in toxin formation was shown at sample pH levels of 5.5, 6.0, or 6.5. These compounds were described as "attractive alternatives" to other potential compounds possessing natural antimicrobial properties.

Sodium acid pyrophosphate, sodium hexametaphosphate, and sodium tripolyphosphates added to uncured, no-nitrate/nitrite-added chicken emulsions were reported to have no effect on controlling toxin production by *C. botulinum* (Nelson et al., 1983).

The utilization of a nitrosylated heme pigment to duplicate the cumulative action of nitrite has also been investigated. Cooked cured-meat pigment (CCMP) is a synthetic, chemically developed pigment for replacing nitrite while resulting in products visually similar to nitrite-cured products. Dinitrosyl ferrohemochrome (DNFH) is generally accepted to be the pigment associated with the pink color of cured meats (O'Boyle et al., 1990). Cooked cured-meat pigment DNFH has been developed and studied as a synthetic replacement for DNFH (O'Boyle, Aladin-Kassam, Rubin, & Diosady, 1992; Shahidi, Rubin, Diosady, & Wood, 1985). Shahidi and Pegg (1990, 1991) demonstrated that CCMP prepared from hemin and nitric oxide and added to ground pork could replace sodium nitrite for the development of cured color. In their research, the authors reported that Hunter *L*, *a*, *b* color values were not significantly different between treatments containing cooked

cured-meat pigment and those containing sodium nitrite. Shahidi and Pegg (1992) were also successful in reproducing nitrite-cured color by substitution of nitrite with CCMP prepared from beef red blood cells and a nitrosating agent in the presence of a reductant. The authors noted that cured color development as a result of CCMP addition was dependent upon the myoglobin content of the raw materials into which the CCMP was incorporated. Stevanović, Čadež, Žlender, and Filipič (2000) support the substitution of sodium nitrite with CCMP as an effective replacement for nitrite. Shahidi and Pegg (1994) also investigated nitrosamine formation in nitrite free pork systems with added CCMP and demonstrated that none were present or formed. Work by O'Boyle et al. (1992) demonstrated that CCMP could be successfully incorporated into hams. From these findings, the authors concluded that marketable nitrite-free hams and other similar cured-meat products could be successfully manufactured with CCMP. Wood, Collins-Thompson, Usborne, and Picard (1986) utilized several antioxidants and antimicrobials with CCMP to study the success of uncured, no-nitrate/nitrite-added products from an antibotulinal aspect. The authors reported that a treatment containing ascorbate, sodium tripolyphosphate, tertiary butyl hydroquinone, sodium hypophosphite, and DNFH possessed antibotulinal activity equivalent to the control (150 ppm nitrite and ascorbate), noting the significant accomplishment for developing a nitrite-free curing system.

Indirect Replacement

Skjelkvåle and Tjaberg (1974) investigated salami sausage produced with and without sodium nitrite or nitrate and showed that a nitrite or nitrate source must be used to generate cured product characteristics. Morita, Sakata, and Nagata (1998) reported that salami manufacture with no nitrate or nitrite but containing a starter culture (*Staphylococcus xylosus*) resulted in product color similar to salami manufactured with nitrite. A possible cause was theorized to be the possible presence of nitric oxide-producing bacteria present in the product. This theory, however, is in disagreement with Morita, Niu, Sakata, and Nagata (1996), who investigated the bacterial influence on pigment in parma ham and found no association.

Studies by Tanaka, Gordon et al. (1985) and Tanaka, Meske et al. (1985) have demonstrated that lactic acid starter cultures could be utilized to replace a portion of ingoing nitrite in bacon. The next question to be answered is whether lactic acid starter culture can be incorporated into an uncured, no-nitrate/nitrite-added manufacturing system to completely replace sodium nitrite. The opportunity exists for utilizing nitrate from indirect sources in combination with the addition of nitrate-reducing microorganisms found in certain strains of lactic acid bacteria that could successfully accomplish complete replacement of sodium nitrite.

The possibility of replacing nitrite indirectly through use of naturally occurring nitrate-containing ingredients is an interesting point of discussion. Ingredients that are commonly found on labels of uncured meat and poultry products manufactured

with the intent of simulating typical curing include sea salt, raw sugar (most often shown as turbinado sugar), natural flavorings or spices, and celery (juice, concentrate, or powder). Sea salt and raw sugar may contain small amounts of nitrate, although limited analytical information suggests that the nitrate content of these ingredients is relatively low.

Vegetables are well known to contain reasonably significant amounts of nitrate. Celery, lettuce, and beets, for example, have been reported to contain concentrations as high as 1,500–2,800 ppm (NAS, 1981). Wide ranges in levels of nitrate for a specific vegetable are not uncommon and can partially be explained by fertilizing, maturity, and climate variations. Similar nitrate levels in these and many other vegetables have been reported by Walker (1990), Fujihara, Kasuga, and Aoyagi (2001), Santamaría, Elia, Serio, and Todazo (1999), White (1975), and Cieslik and Sikora (1998).

Vegetables high in naturally occurring nitrates in the form of juices or powders can therefore offer opportunities to replace nitrite in indirectly cured products. In a concentrated and controlled form, juices and powders provide the greatest opportunity to introduce natural sources of nitrate into processed meats. Although a variety of vegetable sources could be used in processed meats, celery is primarily used since it is the most compatible with processed meat and poultry products and is most commercially available. Celery possesses a relatively high concentration of naturally occurring nitrates coupled with a mild flavor profile compatible with spices already used in processed meat products, with little pigment to alter the color of the finished product. Other nitrate-containing vegetable sources are available but few offer the complete compatibility in processed meat products that celery achieves. Sindelar, Cordray, Sebranek, Love, and Ahn (2007a) analyzed commercial celery juice powder and reported a nitrate content of over 27,000 ppm, or about 2.75%, showing a marked increase in concentration as a result of drying. Current practices in commercial celery powder and juice manufacture are now allowing standardized forms at concentrations of 30,000 ppm or higher.

Since nitrate is a nonreactive compound, a nitrate-reducing bacterial culture must be included in the product formulation to chemically reduce it to reactive nitrite. Once this conversion/reduction has occurred, normal nitrite-curing reactions can take place. It is important to note that lactic acid starter cultures typically used for fermenting sausages (e.g., *Lacticobacillus plantarum* and *Pediococcus acidilactici*) do not have the capability to reduce nitrate to nitrite. Therefore, cultures of coagulase-negative cocci, such as *Kocuria* (formerly *Micrococcus*) *varians*, *Staphylococcus xylosus*, *Staphylococcus carnosus* and others that contain the enzyme nitrate reductase and are thus able to reduce nitrate to nitrite must be used. Since nitrate-to-nitrite reduction is a time/temperature dependent reaction, an additional step termed “incubation” prior to the cooking-smoking steps of thermal processing is often needed. The nitrate reduction reactions occur between 10°C and 45°C with 30°C being optimum. Depending on product diameter (the smaller the longer length of incubation time), incubation temperatures may be up to 2 h in length to provide enough time for sufficient nitrate-to-nitrite reduction. As product

diameter increases, incubation times can be reduced, due to the slow rise of internal temperatures remaining in the 10–45°C window.

When using celery juice powder added at 0.2%, 0.35%, or 0.4% (on a total formulation basis), and assuming 100% nitrate-to-nitrite conversion, ingoing nitrite concentrations of approximately 69, 120, and 139 ppm (based on meat block), respectively, could be expected. As the amount of celery juice powder in the formulation increases, higher amounts of generated nitrite can be expected. However, the use of excessively high of levels of celery juice powder can result in strong vegetable-type flavors and aromas in the finished product. This is especially a concern with products that possess more delicate flavor profiles, such as ham or turkey.

Sindelar et al. (2007a) investigated emulsified, frankfurter-type sausages manufactured with a nitrate-reducing (*Staphylococcus carnosus*) starter culture and 0.2% or 0.4% added celery juice powder. Treatments were held (incubated) at 38°C for either 30 or 120 min prior to cooking. The authors reported nitrite levels of 5.6 and 7.7 ppm for the 0.2% and 0.4% celery powder levels, respectively, after 30 min of incubation and 24.5 and 46.0 ppm, respectively, after 120 min of incubation. After thermal processing (day 0), all treatment combinations had less residual nitrite than a 156 ppm nitrite-added control, except for the treatment with 0.4% celery juice powder and 120-min incubation. In fact, this treatment contained a higher level of residual nitrite compared to the control throughout a 90-day vacuum-packaged refrigerated storage period. At day 90, residual nitrite levels in all treatments were not different from the control. From these results it was determined an uncured product with nitrite replaced with a source containing naturally occurring nitrate could result in a product with higher levels of residual nitrite than one in which nitrite was originally and intentionally added. No differences in lipid oxidation were observed between treatments and the control throughout a 90-day vacuum-packaged and refrigerated storage period. Though not always significant, color measurements (cured pigment concentrations, CIE a*, and reflectance ratios) revealed treatments with a short incubation time, regardless of celery juice powder level, were lower than the nitrite-cured control while treatments with a longer incubation time (regardless of celery juice powder level) resulted in higher values and were similar to the nitrite-cured control. Few differences were identified by trained sensory panel analysis, indicating acceptability of all treatments; however, the control received the highest numeric score for all sensory attributes. The authors concluded celery juice powder and starter culture were an effective replacement for direct addition of nitrite. Incubation time was determined to be a more important factor than celery juice powder level for obtaining product quality characteristics similar to a nitrite-added control.

Sindelar, Cordray, Sebranek, Love, and Ahn (2007b) also conducted a similar experiment with hams using either 0.2% or 0.35% celery juice powder and incubation times of 0 or 120 min. Since no incubation was administered to the 0-min treatments, only nitrite levels following the incubation of 120 min were reported. After incubation and before cooking, 19.5 and 36.1 ppm of nitrite were found for the 0.2% and 0.35% celery juice powder additions, respectively. At day 0, ham

treatment residual nitrite levels ranged from 19.3 to 36.0 ppm compared to the nitrite-added (200 ppm) control level of 63.4 ppm. All treatments were similar to the nitrite-added control for color (CIE L*, a*, b*) and cured pigment measurements, indicating sufficient curing reactions occurred in all celery juice powder/incubation combinations. Treatments with long incubation times had lower 2-thiobarbituric acid (TBA) values than the nitrite-added control, although all levels were below 0.3 mg malonadehyde/kg, indicating little lipid oxidation actually occurred. Favorable conditions during the long incubation may have contributed to this finding.

A trained sensory panel evaluation indicated that celery juice powder addition at 0.35%, regardless of incubation time, resulted in less ham aroma and flavor and greater vegetable aroma and flavor compared to treatments with 0.20% addition. Furthermore, no differences were shown for vegetable aroma or vegetable flavor between the 0.20% celery juice powder treatment and the control. Based on these reported results, a consumer sensory testing would be necessary to confirm the acceptability of these treatments.

The results of this study indicate the challenge and success of manufacturing ham with celery juice powder and a starter culture, resulting in both acceptable quality and sensory properties. The authors concluded the level of celery juice powder used was a more critical factor than the length of incubation to yield attributes similar to a nitrite-added control. The slow temperature increase of the larger diameter ham product during cooking appeared to have provided sufficient time for nitrate-to-nitrite reactions to occur and thus partially negated the incubation effect.

Summary

Alternative curing systems are not a new technology in the meat industry. The rapid growth of “natural” and “organic” processed meat products has resulted in a revisiting of this technology from a fresh new approach. This new approach has allowed “uncured” products improved quality and consumer acceptability. However, these products are not without a level of confusion. The word “uncured” found on labels, in reality, describes products manufactured with and without the intent of having nitrite-cured characteristics. This can lead to an abundance of confusion to consumers regarding what types of products they are purchasing or even what might be in them. The labeling of many current commercially available uncured meat products may be more accurate if categorized to depict the manufacturer’s intentions as “no-nitrite added” or “naturally cured.”

It is clear that nitrite plays an essential role in the quality and safety of cured meat and poultry products. Although either the quality or the safety of meat and poultry products manufactured by direct or indirect replacement of nitrite may be successfully addressed individually, at this point no ingredient or technology has demonstrated the combined effect of addressing both the quality and safety of these products.

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Index

A
Aaron, D.K., 71
Acidulants
  functions, 17–18
  regulations, 18
Ackerman, S.-B., 76
Actinidin, 179
Ahmad, H.A., 346
Ahn, D.U., 309, 397, 398
Ahn, H.J., 334
Ahn, J., 7
Ahn, J.Y., 390
Al-Dabbagh, S.A., 387
Älender, B., 396
Aleson-Carbonell, L., 92
Alginate
  description and origin, 69
  functional characteristics and properties
    calcium solution, 69–70
    gelling mechanism, 71, 72
    sodium alginate, 70
    structured meat, 70–71
  G-block and M-block components, 69
  regulatory status, 73
Allen, C.E., 381, 382
Allicin, 344
Allyl isothiocyanate (AIT), 345
Almond J., 332
Alonso-Calleja, C., 331
Alternative protein product (APP), 117
Altunakar, B., 259
Ando, H., 185
Antimicrobial agents
  meat and meat products
    acetic acid, 309–310
    antimicrobial spectrum, 309
    bacteriocins and antibiotics, 336–339
    bacteriophages, 347–348
    benzoic acid and salt, 313
    calcium sulfate ($CaSO_4$), 348–349
    chitosan, 350–351
    chlorine compounds, 322–325
    citric acid, 312
    electrolyzed oxidizing water, 328–329
    Epsilon-polylysine, 349–350
    fatty acids and esters, 318–320
    fumaric and malic acid, 314
    lactic acid, 312–313
    lactoferrin, 340–341
    lysozyme, 341–342
    nitrite, 333–334
    ozone (O_3), 325–326
    parabens, 317–318
    peroxides and peroxyacids, 326–327
    phosphates, 329–331
    plant extracts and essential oils, 342–346
    propionic acid, 314
    quaternary ammonium compounds (QAC), 320–322
    smoke, 346–347
    sodium chloride (NaCl), 332
    sorbic acid and salts, 313–314
    xylitol, 351–354
Antioxidants, meat and meat products
  endogenous antioxidants, 292
  exogenous antioxidants, 292–293
  natural
    grape seed extract, 296
    nonfood based antioxidant systems, 297
    oregano oil and vitamin E, 294–295
    plant extracts, 296–297
    rosemary extracts and chitosan, 295
    rosmanol and rosmarinic acid, 297–298
    spice and herb extracts, 296
  synthetic, 293
Antonova, I., 278
Aoyagi, Y., 397

Aris, B., 334
Ashie, I., 180
Axtell, S.P., 324

B
Bacon, 15
Bacteriocins
  dried sausages, 338–339
  dry fermented sausage, 339
  high-fat sausages, 338
  natamycin, 337–338
  nisin, 336–337
  pediocin, 337
Bacteriophages
  applications, 348
  characteristics, 347
  properties, 347–348
Baixauli, R., 275, 277, 280
Baker, R.C., 256, 271
Bal'a, F.A., 309
Barbut, S., 51, 138
Barreto, G., 41, 50
Bater, B., 61, 63, 64
Batter
  adhesion, 275–277
  adhesion coating/ interface batter, 254–255
  coating adhesion control, 279–280
  color control, 280–281
  final texture control, 277–279
  ingredients and components
    dextrins, 272
    fibers, 272
    gums and hydrocolloids, 260–271
    nonwheat flours and starches, 258–260
    proteins, 271–272
    seasonings, 273–274
    wheat flour, 256–258
  predust, 255–256
  tempura-type/leavened batter, 255
  viscosity control, 275–276
Battista, O.A., 91
Bauermann, J.F., 374, 392
Baumanis, P.J., 43
Bayne, H.G., 383, 391
Beilken, S.L., 74
Bell, H., 259
Bell, R.G., 334
Bergsma, J., 25
Bernal, V.M., 64, 72
Bertelsen, G., 5
Beuchat, L.R., 344
Bhardwaj, S.C., 271
Bhothipaksa, K., 375, 382
Bisht, G.S., 274
Biswas, A.K., 258, 274
Blanchard, S.P., 72, 75
Blended seasonings. *See* Seasonings
Blood plasma protein (BPP)
  plasma protein fractions, 159–160
  properties and applications
    emulsification and solubility, 159
    gelation, 158–159
  regulatory status, 160
Bogaert, J-C., 309
Botsoglou, N.A., 4
Bouchon, P., 281
Boyle, D.L., 261, 346
Boyle, E.A., 71
Brackett, R.E., 328, 344
Breadings, 281–282
Brewer, M.S., 309
Briand, E., 386
Bromelain, 177
Brown, C.L., 392
Brown, C.R., 43
Buchanan, R.L., 312, 383
Buckley, D.J., 135, 139
Bullens, C.W., 67
Buncic, S., 334
Busta, F.F., 375, 381, 382
Butylated hydroxyl anisole (BHA), 293
Butylated hydroxyl toluene (BHT), 293
Byrne, G.A., 211
Byun, M.W., 334

C
Cadeδ, P., 396
Calcium sulfate ($CaSO_4$)
  applications, 349
  characteristics, 348
  properties, 348–349
Calkins, C.R., 179
Calvo, C., 280
Capita, R., 331
Carballo, J., 41, 50
Carpenter, J.A., 65, 92, 97
Carrageenan
  application
    ham applications, 66
    seafood products, 67
    turkey breast and roast beef, 66
  functional characteristics and properties
    concentration effect, 64, 65
    firmness, 65, 66
    gelling characteristics, 62, 63
    Kappa carrageenans and KCl, 62

locust bean gum (LBG), 61
muscle food system, microscopic view, 63
PSE meat, 65
type and composition in, 61
origin and manufacture
extraction steps, 60
Mu and nu carrageenans structures, 59
potassium chloride (KCl) role, 59
processed Euchema seaweed (PES), 59–61
seaweeds species used, 58, 59
regulatory status and toxicological safety, 67–68
Carr, J.M., 62, 65
Carrot fiber, 88
Carson, C.F., 344
Caseins. *See also* Dairy proteins
characteristics, 131, 132
functional ingredients, 134–135
Cassens, R.G., 388
Castillo, A., 327
Ceylan, E., 316
Chang, H.-C., 92, 97
Chan, K.C., 72
Chenneour, R., 39
Chen, T.C., 325
Chinnan, M.S., 270
Chipley, J.R., 309
Chitosan
applications, 350–351
characteristics and properties, 350
Chlorine compounds
applications
chilling application, 323–324
disinfection, 324–325
characteristics, 322
properties, 323
Chondrus crispus, 58, 59
Choy, V., 138
Christiansen, L.N., 390
Chun, W., 342
Cieslik, E., 397
Cinnamaldehyde, 343–344
Cioch, J.J., 393
Citrus and fruit fibers, 88–89
Claus, J.R., 43, 92, 140
COLFLO®, 43
Collagen
added ingredient
enzyme-modified collagen, 151–152
factors in, 149
heat-modified collagen, 151
low-temperature rendering systems, 150
physical extraction and/or concentration, 150
pork collagen and myofibrillar proteins, 150, 151
intra-and intermolecular covalent crosslinks, 148
raw collagen, 149
regulatory status, 152
structure and types, 148
Collins-Thompson, D.L., 396
Colloidal fibers, 91
Colmenero, F., 41, 50
Conjugated linoleic acid (CLA), 238
Conklin, J.R., 271
Conley, A.J., 318
Connective tissue (CT), 150
Cordain, L., 83
Cordray, J., 309
Cordray, J.C., 397, 398
Cotton, L.N., 309
Cummings, T., 193
Curdlan, 78–79
Curing process, meat and meat products
cure accelerators
acidulants, 17–18
reductants, 16–17
definitions, 2
nitrates and nitrites
cancer, 389–390
direct replacement, 395–397
functions, 9–15
human exposure, 386–387
indirect replacement, 397–400
microbiological benefits, 380–383
qualitative benefits, 383–386
regulations, 10, 15–16
toxic properties, 9–11
phosphates
functions, 19–21
properties, 18–19
regulations, 21
residual nitrite, 388
salt
functions, 6–8
properties, 6
regulations, 8
water
functions, 3–5
properties, 2–3
regulations, 5–6
Cutter, C.N., 314, 329, 330

D
Daigle, K., 261
Dairy proteins
  antioxidant property, 140
  applications in, 133
  caseins, 131
  functional ingredients
    caseins, 134–135
    chicken meat batters, 138, 139
    commercial meat products, 134
    comparison of, 138–140
    skim milk powder, 133–134
    transglutaminase, 137
    whey proteins, 135–137
  milk protein products, 132–133
  physicochemical properties, 132
  poultry meat, pink color defects inhibition, 141–142
  regulatory issues, 141
  whey protein isolate (WPI), 132
  whey proteins, 131, 132
Darfler, J.M., 256
Decker, E.A., 2
DeFreitas, Z., 65
De Giusti, M., 383
Degnan, A.J., 339
de Kairuz, M.N., 334
Delves-Broughton, J., 338, 339
Demirci, A., 329
de R. Holgado, A.P., 334
Descamps, O., 61, 63
Desmond, E.M., 135
Dethmers, A.E., 386
Deumier, F., 315
Devatkal, S., 71
De Vito, E., 383
Dewettinck, K., 65
Dexter, D.R., 41
Dexter, L.B., 86
Diacetyl, 340
Dianova, V., 49
Dilts, B.D., 347
Diosady, L.L., 385
Dogan, S.F., 275
Doll, R., 387
Doores, S., 309
Dransfield, E., 183
Drosinos, E.H., 309
Duffy, L.L., 334
Du, M., 309
Dumont, J.P., 386
Duncan, S.E., 278

E
Earle, R.D., 73, 268
Eichholzer, M., 389
Eitenmiller, R.R., 270
Eklund, M., 332
Eklund, T., 309
Electrolyzed oxidizing water
  applications, 328–329
  characteristics and properties, 328
Elia, A.V., 397
Ellebracht, J.W., 327
Ensor, S.A., 71, 72, 138
Epsilon-polylysine
  applications, 350
  characteristics, 349
  properties, 349–350
Erduran, S., 385
Erickson, M.C., 270
Ernst, E.A., 71
Ethanol, 339–340
Euchema species, 58, 59
Eugenol, 343
Extract oils, 345–346
Eyles, M.J., 317

F
Fabrizio, K.A., 329
Fadda, S., 334
Fawcett, S.L., 182
Feinberg, J., 392, 394
Feinberg, J.I., 382
Fengel, D., 218
Fermentation and acidification ingredients
  chemical acidulants
    disadvantages, 241
    glucono-delta-lactone (GdL), 240
    lactic acid, 240–241
    role of, 239–240
  classical starter cultures
    composed of, 228
    fungi, 230
    lactic acid bacteria, 229
    limitations of, 230–232
  functional starter cultures
    bacteriocins, 234–236
    definition, 232
    flavor production, 233, 234
    healthier sausages production, 237–239
    micronutrients and nutraceuticals, 238
    probiotic LAB, 237
    reuterin and reutericyclin, 236
    safer sausages production, 234–237
  lactic acid bacteria (LAB), 227

Fernández-Martín, F., 63
Fernández, P., 41, 50
Fiber
  application
    allergenicity and GMO, 95
    fiber grades, 94
    goals, 93
    oat fiber, 92
    regional meat quality, 93, 95
    remixes and compound mixes, 102
  cooked sausages
    fibers and hydrocolloids in, 104–105
    formulation, 103, 104
  dry sausage, 106–107
  ground meat products, 105–106
  nutritional characteristics, 83
  nutritive fiber usage
    bile acids in, 99
    fat reduction, 96–98
    fiber enrichment, 96
    fiber:water ratio, 100, 101
    inulin, 97, 98
    nutritionally enhanced processed meat products, 99–100
    process implementation, 100–102
    product line, 98
    salt/ phosphates, 99
    water-holding capacity, 97
  restructured and injected ham, 106
  types of
    carrot fiber, 88
    citrus and fruit fibers, 88–89
    colloidal fibers, 91
    definition, 84
    inulin and hydrolyzed oat flour, 90
    oat fiber, 86–87
    potato fiber, 89
    powdered cellulose, 85–86
    properties, 85
    soybeans and peas, 87–88
    sugar beet fiber, 89
    wheat bran and corn bran, 84, 85
    wheat fiber, 87
Fibrimex®, 162
Ficin, 177
Fiddler, W., 389, 390
Filipi, I., 65
Filipi., M., 396
FIRMTEX®, 45
Fiszman, S.M., 262, 275, 277, 278, 280
Fitzgerald, C.M., 334
Flavonoids, 345
Flavourzyme®, 140
Foegeding, E.A., 76
Foegeding, P.M., 339
Fogle, D.R., 182
Forman, D., 387
Fox, J.-B., Jr., 76
Friedman, M., 345
Froehlich, D.A., 386
Fu, A.-H., 334
Fujihara, S., 397
Fukuhara, Y., 349
Funami, T., 78
Functional soy protein concentrates (FSPCs), 115, 120
Fung, D.Y.C., 335, 340

G

García, A., 387
Garcia-Fernandez, M.C., 331
García Roché, M.O., 387
Gardener, D., 212
Gates, R.A., 389
Gelatin and gelatin hydrolysates
  properties and applications, 153
  regulatory status, 154
  type A and type B gelatins, 152, 153
Gellan gum, 77
Georgantelis, D., 5
Geornaras, I., 316, 338, 349
Gerba, C.P., 346
Gigartina species, 58, 59
Gill, A.O., 344
Ginger rhizome protease, 179
Glass, K., 309
Glucono-delta-lactone (GdL), 240
Golden, M.H., 312
Gordon, N.M., 396
Gorman, B.M., 326
Gould, J.M., 86
Graham, R.G., 217
Gram-positive, catalase-positive cocci (GCC), 228, 230
Granberg, D., 309
Gravesen, A., 339
Gray, J.I., 393
Greene, B.E., 392
Greer, G.G., 347
Greiner, S.T., 351
Gullett, E.A., 386
Gutzwiller, F., 389

H

Hadorn, R., 93, 96, 98
Hajmeer, M.N., 316

Halden, J.P., 35
Hale, M., 389
Hall, C.J., 308
Hammer, K.A., 344
Hancock, R.E., 342
Han, J., 7
Hansen, T.B., 339
Hayes, J.E., 135
Hemoglobin and red blood cells
  properties and applications, 163–164
  regulatory status, 164
Herald, T.J., 261
Herbs, 200
Hermansson, A.-M.I., 49
Hernando, I., 278
Hill, D., 91
Hill, P., 335
Hoffman, L.C., 43
Holley, R.A., 344
Holmes, F.H., 212
Holownia, K.I., 270
Honkenen-Buzalski, T., 335
Hop flower extracts, 344–345
Hotchkiss, J.H., 385
Hsia, H.-Y., 75
Huber, W., 107
Hudson, J.A., 334
Huhtanen, C.N., 374, 375, 382, 392
Hung, S.C., 138
Hung, Y.-C., 278, 328
Hunt, M.C., 43, 92
Hurst, A., 334
Hurtado, J.L., 63
Hustad, G.O., 382, 391, 393

I
Imeson, A.P., 71
Inglett, G.E., 90
Inulin, 90, 97
Isolated soy proteins (ISPs), 115, 119, 120
Ivey, F.J., 390

J
Jahncke, M., 389
Jenkins, R.H., 76
Jo, C., 334, 390
Johansson, T., 335
Johnson, E., 309
Johnson, E.A., 319, 342
Joly, G., 45
Judge, M.D., 393
Juneja, V.K., 316
Jydegaard Axelsen, A.-M., 339

K
Kabara, J.J., 318
Kampani, A., 309
Kang, D.-H., 335, 340
Kao, W.T., 43
Kasuga, A., 397
Keeton, J.T., 72, 74
Kerr, W.L., 282
Kerry, J.F., 139
Keshri, R.C., 258, 274
Kim, C., 328, 331
Kim, J.H., 334
Kim, J.M., 63
Kimoto, W., 392
King, A.J., 7
Klaenhammer, T.R., 339
Knight, T.M., 387
Konjac
  advantage, 74
  regulatory status, 75
Kritikos, D., 309
Kuraishi, C., 188
Kurt, L., 137

L
Labuza, T.P., 340
Lactic acid bacteria (LAB), 227, 229
Lactoferrin
  characteristics, 340–341
  properties and applications, 341
β-Lactoglobulin, 136
Lahti, E., 335
Langan, R.E., 269
Larson, A.E., 342, 345
Lau, D.W., 7
Ledward, D.A., 71
Lee, C.H., 334
Lee, C.M., 65
Lee, C.-M., 63
Lee, M.-J., 259, 260, 277
Le Mintier, Y., 107
Lenchin, J.M., 259
Lewis, Y.S., 201–202
Li, J.-Y., 35
Lima, I., 277
Lim, S.-T., 259, 260
Lin, K.W., 43, 72
Llanto, M.G., 67
Llorca, E., 278

Lluch, M.A., 278
Locust bean gum (LBG), 76–77
Loewe, R., 271
Love, J.A., 397, 398
Lövenklev, M., 383
Luchansky, J.B., 339, 348
Lyon, C.E., 65
Lysozyme
  characteristics, 341–342
  properties and applications, 342

M
Madsen, H.L., 5
Maekawa, A., 389
Maga, J.A., 202, 346
Malicki, A., 324
Mallikarjunan, P., 278
Marchelo, J.A., 346
Marmur, A., 276
Marsden, J.L., 316
Marshall, D.L., 309
Maskat, M.Y., 282
Mataragas, M., 309
Mathiesen, C.-H., 35
Maurer, A.J., 61, 63
Maxcy, R.B., 320
McCarthy, T.L., 6
McDowell, D.A, 182
McKee, D.H., 73, 268
McKellar, R.C., 332
Means, W.J., 71, 72
Meat and meat products
  antimicrobial agents
    acetic acid, 309–310
    antimicrobial spectrum, 309
    bacteriocins and antibiotics, 336–339
    bacteriophages, 347–348
    benzoic acid and salt, 313
    calcium sulfate ($CaSO_4$), 348–349
    chitosan, 350–351
    chlorine compounds, 322–325
    citric acid, 312
    electrolyzed oxidizing water, 328–329
    Epsilon-polylysine, 349–350
    fatty acids and esters, 318–320
    fumaric and malic acid, 314
    lactic acid, 312–313
    lactoferrin, 340–341
    lysozyme, 341–342
    nitrite, 333–334
    ozone (O_3), 325–326
    parabens, 317–318
    peroxides and peroxyacids, 326–327
    phosphates, 329–331
    plant extracts and essential oils, 342–346
    propionic acid, 314
    quaternary ammonium compounds (QAC), 320–322
    smoke, 346–347
    sodium chloride (NaCl), 332
    sorbic acid and salts, 313–314
    xylitol, 351–354
  antioxidants
    endogenous antioxidants, 292
    exogenous antioxidants, 292–293
    grape seed extract, 296
    nonfood based antioxidant systems, 297
    oregano oil and vitamin E, 294–295
    plant extracts, 296–297
    rosemary extracts and chitosan, 295
    rosmanol and rosmarinic acid, 297–298
    spice and herb extracts, 296
    synthetic, 293
  blood-derived protein ingredients
    blood plasma protein (BPP), 158–160
    collection, 157
    composition of, 157, 158
    hemoglobin and red blood cells, 163–164
    transglutaminases, 161–163
  classification, 147
  collagen
    amino acid composition and types, 148
    enzyme-modified collagen, 151–152
    heat-modified collagen, 151
    intra-and intermolecular covalent crosslinks, 148
    low-temperature rendering systems, 150
    physical extraction and/or concentration, 150
    pork collagen and myofibrillar proteins, 150, 151
    raw collagen, 149
    regulatory status, 152
  curing systems, nitrates and nitrites
    cancer, 389–390
    direct replacement, 395–397
    human exposure, 386–387
    indirect replacement, 397–400
    microbiological benefits, 380–383
    qualitative benefits, 383–386
  edible animal by-products, 146
  gelatin and gelatin hydrolysates
    properties and applications, 153
    regulatory status, 154
    type A and type B gelatins, 152, 153

Meat and meat products (*cont.*)
hydrolysates and flavors
degree of hydrolysis (DH), 155
flavor enhancers, 155, 156
primary and secondary hydrolysates, 156
regulatory status, 156–157
meat definitions of, 145
mechanically recovered meat, 146
microorganisms, 301–302
processors, 199–200
basic ingredients, 205
characteristic spice profiles, 205–206
description, 199–200
regulations, 206–207
stocks and broths
applications, 154
regulatory status, 154–155
tenderizing enzymes
bacterial and microbial proteases, 180
fungal enzymes, 179–180
labeling, 181
meat tenderization, 181–183
plants, 174–179
transglutaminase
binding applications, 191–194
enzyme reaction, 185
sources, 184–185
substrate protein sources, 185–187
texture applications, 188–191
Mechanically deboned meat (MDM), 93, 95
Medium and long-chain-length fatty acids
applications, 319–320
characteristics, 318
properties, 318–319
Mehra, R., 135
Mei, L., 2
Mei, M.Y., 72
Meiron, T.S., 276
Mellett, L.D., 43
Mendes da Silva, J., 339
Mendiratta, S.K., 71
Meske, L., 390
Messina, M.C., 346
Metaxopoulos, I., 309
Meyer, A.S., 386
Meyers, M.A., 271
Michener, H.D., 383, 391
Milk protein concentrate (MPC), 132
Miller, A.J., 391
Mills, E.W., 61, 64
Mitchell, J.R., 71
Modliszewski, J.J., 67
Mohamed, S., 271
Moir, C.J., 317
Montero, P., 63
Moody, W.G., 61, 71
Moreno, B., 331
Morita, H., 396
Motzer, E.A., 65
Mueller, W.-D., 96, 107
Mukprasirt, A., 261
Münch, S., 93, 96
Murano, E.A., 334
MyoGel®, 150

N
Nagata, Y., 396
Naidu, A.S., 309
Nakao, Y., 78
NATIONAL FRIGEX HV®, 45
Nattress, F.M., 342
Neirinck, N., 65
Nelson, D.A., 340
Nelson, K.A., 382
N-HANCE®, 43, 49, 50
Nicolalde, C.L., 309
Nielsen, P.M., 180
Nitrates
functions, 9–10
properties, 9
regulations, 10
Nitrites
applications
meat curing, 333–334
processing treatments, 334
characteristics and properties, 333
functions
antimicrobial agent, 14
antioxidant function, 13, 14
flavor production, 13
heme pigment in, 12, 13
meat color, 11
pH and reductants, 12
regulations, 15–16
toxic properties, 10–11
Nitsch, P., 105
Noel, D.C., 61
Noel, P., 386, 393
Nonfat dry milk (NFDM), 151, 152
Nonfat milk solid (NFMS), 132–134, 138
Nonstarch hydrocolloids
alginate
description and origin, 69
functional characteristics and properties, 69–73
G-block and M-block components, 69
regulatory status, 73

carrageenan
functional characteristics and properties, 61–66
ham applications, 66
origin and manufacture, 58–61
regulatory status and toxicological safety, 67–68
seafood products, 67
turkey breast and roast beef, 66
curdlan, 78–79
gellan gum, 77
ingredients selection for, 57, 58
locust bean gum (LBG), 76–77
xanthan gum, 75–76
Norton, I.T., 43
NOVATION®, 33, 45, 49
Nurmi, E., 335
Nutrition Labeling and Education Act of 1990, 83

O
Oat fiber, 86–87
Oblinger, J.L., 73
O'Boyle, A.R., 396
Offer, G., 19
Olesen, P.T., 386
Oliver, G., 334
Olson, D.G., 65, 384
Organic acids and salts
acetic acid, 309–310
benzoic acid, 313
characteristics, 307
citric acid, 312
fumaric and malic acid, 314
lactic acid, 312–313
properties, 307–309
sorbic acid, 313–314
Osmanagauoglu, O., 339
Ozone (O_3)
applications, 325–326
characteristics and properties, 325

P
Pale, soft, exudative (PSE) pork meat, 51, 65
Papain, 174, 177
Paquette, M.W., 346
Parabens
applications, 318
characteristics and properties, 317–318
Paranjpye, R., 332
Park, H., 328
Pea proteins, 122
Pegg, R.B., 394–396
Pelroy, G., 332
Pensabene, J.W., 389
Pérez-Mateos, M., 63
Peroxides and peroxyacids
applications, 327
characteristics and properties, 326–327
Peterson, M., 332
Phillips, J.G., 382, 392
Phosphates
applications
fresh meat, 330–331
processed meats, 331
characteristics, 329–330
functions
antioxidant activity, 20
water retention and meat proteins, 19
properties, 330
pH, 18
types, 19
regulations, 21
Picard, B., 396
Piccinali, P., 93
Pickford, K.G., 269
Pilkington, D.H., 339
Plant proteins
coarse ground meat products, 126
dry and semidry fermented sausages, 126, 127
emulsified meat products, 123–124
functional properties
fat emulsification, 113–114
lean meat component, 112
product storage and handling, 116
soluble plant proteins hydration, 112–113
structural and textural integrity, 114–116
water binding, 114
injection and marination applications
boneless ham products, 125
brine solutions in, 124
non-meat ingredient addition levels, 126
nutritional properties, 116–117
pea proteins, 122
soy proteins
functional characteristics, 120
jet-cooking and flash-cooling, 119, 120
processing schematic, 119
SPCs and ISPs, 118, 119
wheat proteins, 121–122
Poligeenan, 67
Potato fiber, 89
Poultry product, 146

Pérez-Munuera, I., 278
Prabhu, G.A., 47, 61, 63
Processed Euchema seaweed (PES), 59–61
Proctor, M.S., 35
Protamex®, 140
Protein digestibility corrected amino acid score (PDCAAS), 116, 117
Protein digestibility index (PDI), 118
Protein efficiency ratio (PER), 116
Puolanne, E., 19
Pyle, D.L., 281

Q
Quaternary ammonium compounds (QAC)
  applications, 321–322
  characteristics and properties, 320

R
Raharjo, S., 71
Ramarathnam, N., 385
Ramaswamy, S.R., 86
Ramsey, S.R., 76
Ransom, J.R., 321
Rausch, K.D., 261
Rayman, M.K., 334, 338
Reductants
  functions and regulations, 17
  properties, 16–17
Resins, 200
Reynolds, A.E., 65
Rhee, K.S., 4, 7
Rhodes, D.N., 183
Riley, T.V., 344
Rivera-Betancourt, M., 314, 330
Rock, H., 386
Rogers, P.J., 137
Rogov, I., 49
Roth, D., 107
Rozum, J.J., 218, 221
Rubin, L.J., 385
Russell, S.M., 324
Rust, R.E., 62, 65
Ruusunen, M., 19
Rywotycki, R., 390

S
Saguy, I.S., 276
Sahin, S., 259
Sakai, H., 349
Sakata, R., 396
Salt
  functions
    flavor enhancer, 7
    ionic strength, 6–7
  properties, 6
  regulations, 8
Salvador, A., 262, 275, 277, 280
Samelis, J., 338
Sammel, L.M., 140
SantamarØa, P, 397
Sanz, T., 262, 275, 277
Savage, J.P., 39
Schaake, S.L., 71, 72
Schmidt, G.R., 41, 71, 72, 77
Schoeni, J.L., 330
Schwarz, S.J., 140
Scott-Kline, D., 271
Seasonings
  definition, 200
  formulations, 203–204
Sebranek, J.G., 47, 61–63, 65, 334, 384, 390, 397, 398
Serio, F., 397
Shahidi, F., 7, 394–396
Shand, P.J., 77
Shapero, M., 340
Sharma, R.K., 309
Sharma, R.R., 329
Shaver, K.J., 390
Shay, B.J, 43
Shih, F., 261
Shima, S., 349
Sikora, E., 397
Simpson, C.A., 306
Sindelar, J.J., 397, 398
Singh, R.P., 277
Skjelkvåle, R., 396
Smajda, C.H., 64
Smith, A., 309
Smith, C.D, 325
Smith, D.-M., 75
Smith, J.L., 64
Smith, P.A., 91
Smoke
  applications, 347
  browning reactions, food
    carbonyls, 216
    enzymatic and nonenzymatic reactions, 215
    Maillard reaction, 215
  characteristics, 346
  cold smoking, 217
  evolution, 220–221
  natural vaporous *Vs.* natural smoke condensates
    meat smoking, 218–220
    polycyclic aromatic hydrocarbons, 218

properties, 346–347
smoke condensate
antimicrobial and antifungal effects, 221–222
atomization, 223–224
drenching/showering, 224
internal/topical application, 224–225
phenolics, 223
smoke creation, 216–217
wood components pyrolysis
cellulose and hemicellulose, 212
lignin, 212–214
Sodium chloride (NaCl), 332
Sofos, J.N., 19, 41, 71, 72, 77, 309, 346, 349, 375, 381, 382
Solas, M.T., 41
Solberg, M., 383
Soluble fibers, 90
Somers, E.B., 330
Sorensen, T., 180
Soy proteins
functional characteristics, 120
jet-cooking and flash-cooling, 119, 120
processing schematic, 119
SPCs and ISPs, 118, 119
Spices
advantages and disadvantages, 201
definition, 200
primary components, 202
Stack, F., 139
Stahnke, L.H., 386
Stanly, D.W., 64
Starches
in brine systems
cold-water swelling starches, 40
cook-up starches, 39–40
and carrageenan, 47–48
correct starch selection, 35, 36
egg proteins, 50–51
emulsification, 42
in emulsified meats
cold-water swelling starches, 41
cook-up starches, 40–41
fat mimicry, 42–43
in manufacturing scenario
cold swelling starches, 39
glatinization temperatures, 36
high amylose corn, 36, 38
potato starches, 38
tapioca starch, 38–39
waxy maize starch, 38
milk proteins, 48–50
minced/ground meat products
cold-water swelling starches, 41
cook-up starches, 41
modified structure
chemical modifications, 31
cross-linking, 32
E numbers for, 30
regulations, 29
substitution or stabilization, 31
native structure
glucose polymer structures, 27, 28
refined starch, 26
performance
starch cooking curve, 37
viscosity curve, 36, 37
phosphate replacement, 43–45
physical modifications
chemical and biochemical modifications, 33, 34
dextrinization, 32
pregelatinization, 29, 30, 32
thermal inhibition, 33
plant troubleshooting
cook-up starch sedimentation, 52
distribution and cook status, 52
PSE meat, 51
soft texture, 51–52
starch pockets/tiger stripes, 51
regulation
Europe, 33
United States, 33–34
sources and unique characteristics, 26, 27
soy proteins, 49–50
starches comparison
modified food starches, 45–46
native *vs.* native functional, 46–47
use of, 25–26
Steenblock, R.L., 92
Steffe, J.F., 75
Stetzer, A.J., 309
Stevanovic´, M., 396
Stratford, M., 309
Sugar beet fiber, 89
Sullivan, G., 179
Sumnu, G., 259
Suter, M., 93
Su, Y.K., 138
Szczawinsia, M., 388
Szczawinski, J., 388
Szulc, M., 388

T

Tahnpoonsuk, P., 278
Tanaka, N., 390, 396
Tang, J., 77
Tea extracts, 344

Tenderizing enzymes, meat products
bacterial and microbial proteases, 180
fungal enzymes, 179–180
labeling, 181
meat tenderization
delivery method, 182–183
enzyme strength and specificity, 181–182
worker safety, 183–184
plants
actinidin and rhizome protease, 179
bromelain and ficin, 177
papain, 174, 177
Tertiary butylhydroquinone (TBHQ), 293
Textured vegetable proteins (TVP®), 118, 126
Thippareddi, H., 316
Thomas, A.B., 339
Thomas, L.V., 339
Thymol, 344
Tjaberg, T.B., 396
Todazo, E., 397
Tokusoglu, O., 93
Tompkin, R.B., 14, 390
Torres, O., 387
Townsend, W.E., 384
Transglutaminase, meat products
binding applications
chunked and formed portions, 192–193
factors affecting reactions, 193–194
muscle profiling, 193
scallops, 192
sodium caseinate and gelatin, 191–192
blood-derived protein ingredients
cross-linking reaction, 161
food protein substrate specificity, 161
properties and applications, 162–163
regulatory status, 163
dairy proteins, 137
enzyme reaction, 185
sources, 184–185
substrate protein sources, 185–187
texture applications
emulsion and sausage applications, 188
injection systems and muscle products, 191
surimi-based products, 188, 191
Trenchard, H., 382
Trinick, J., 19
Trius, A., 62, 65
Trout, G.R., 43, 71, 74
Troy, D.J., 135
Tucker, E.M., 309
Tung, M., 77

U
Uhl, S.R., 199–202
Ünal, M.K., 93
Underdown, J., 43
Underwood, G.L., 217, 218, 221
Usborne, W.R., 386, 396

V
Vadehra, D.V., 256
Vail, W.J., 88
Van der Meeren, P., 65
Verbeken, D., 65
Vignolo, G., 334
Voesgen, W., 107

W
Wade, M.A., 48
Wagner, M.K., 382
Walker, R., 397
Wanasundara, P.K.J.P.D., 7
Wang, L.-L., 319
Wasserman, A.E., 392
Wasserman, A.E., 393
Water
functions
available water (Aw), 4–5
brine strength, 4
protein solubility, 3
functions regulations
moisture:protein (M:P) ratios, 6
protein-fat-free (PFF), 5
properties, 2–3
Wegener, F., 218
Wendorff, W.L., 221, 223
West, P., 329
West, R.L., 73
Wheat fiber, 87
Wheat flour
water binders, 257
wheat starch
composition, 257
gelatinization, 257–258
Wheat glutens, 121–122
Whey protein concentrate (WPC), 132, 133
Whey proteins
characteristics, 131, 132
enhanced meats, 136
frankfurters, 135
myofibrillar protein, 136
texturization, 137
WPC in, 135, 136
White, J.W., 387, 397

Wicklund, S.E., 309
Williams, J.C., 392
Williams, S.K., 73
Wolff, I.A., 393
Wong, A.C.L., 330
Wood, D.S., 396
Wotherspoon, C., 72

X
Xanthan gum
  regulatory status, 76
  structure of, 75
Xiong, Y.L., 61, 72, 75

Y
Yada, H., 78
Yang, A., 43, 74
Yang, P.P.W., 325
Yanyin, Z., 77
Yeh, A.-I., 35
Yotsuzuka, F., 78
Yun, J., 392

Z
Zayas, J.F., 138
Zhang, L., 51
Zhu, M., 309