Handbook of MEAT Processing

Fidel Toldrá EDITOR

WILEY-BLACKWELL

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Contents

Preface

For centuries, meat and its derived products have constituted some of the most important foods consumed in many countries around the world. Despite this important role, there are few books dealing with meat and its processing technologies. This book provides the reader with an extensive description of meat processing, giving the latest advances in technologies, manufacturing processes, and tools for the effective control of safety and quality during processing.

To achieve this goal, the book contains 31 chapters distributed in three parts. The first part deals with the description of meat chemistry, its quality for further processing, and the main technologies used in meat processing, such as decontamination, aging, freezing, curing, emulsification, thermal processing, fermentation, starter cultures, drying, smoking, packaging, novel technologies, and cleaning. The second part describes the manufacture and main characteristics of worldwide meat products such as cooked ham and sausages, bacon, canned products and pâté, dry-cured ham, mold-ripened sausages, semidry and dry fermented sausages, restructured meats, and functional meat products. The third part presents efficient strategies to control the sensory and safety quality of meat and meat products, including physical sensors, sensory evaluation, chemical and microbial hazards, detection of GMOs, HACCP, and quality assurance.

The chapters have been written by distinguished international experts from fifteen countries. The editor wishes to thank all the contributors for their hard work and for sharing their valuable experience, as well as to thank the production team at Wiley-Blackwell. I also want to express my appreciation to Ms. Susan Engelken for her kind support and coordination of this book.

Fidel Toldrá

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About the Editor

Fidel Toldrá, Ph.D., is a research professor at the Department of Food Science, Instituto de Agroquímica y Tecnología de Alimentos (CSIC), and serves as European editor of *Trends in Food Science & Technology*, editor in chief of *Current Nutrition & Food Science,* and as section editor of the *Journal of Muscle Foods*. He is also serving on the editorial board of the journals *Food Chemistry*, *Meat Science*, *Open Nutrition Journal*, *Food Analytical Methods*, *Open Enzyme Inhibition Journal* and *Journal of Food and Nutrition Research*. He is a member of the European Food Safety Authority panel on flavorings, enzymes, processing aids, and materials in contact with foods.

Professor Toldrá has acted as editor or associate editor of several books in recent years, including *Handbook of Muscle Foods Analysis* and *Handbook of Processed Meats and Poultry Analysis* (2009), *Meat Biotechnology* and *Safety of Meat and Processed Meat* (2008, 2009), *Handbook of Food Product Manufacturing* (2007), *Advances in Food Diagnostics*, and *Handbook of Fermented Meat and Poultry* (2007, 2008). Professor Toldrá also wrote the book *Dry-Cured Meat Products* (2002).

Professor Toldrá was awarded the 2002 International Prize for meat science and technology by the International Meat Secretariat and was elected in 2008 as Fellow of the International Academy of Food Science & Technology (IAFOST) and in 2009 as Fellow of the Institute of Food Technologists (IFT).

Handbook of Meat Processing

Part I

Technologies

Chapter 1

Chemistry and Biochemistry of Meat

Elisabeth Huff-Lonergan

Introduction

Muscle cells are among the most highly organized cells in the animal body and perform a varied array of mechanical functions. They are required for the movement of limbs, for locomotion and other gross movements, and they must also perform finer tasks such as maintaining balance and coordination. Muscle movement and metabolism are associated with other diverse functions such as aiding in movement of blood and lymph and also in maintaining body temperature. All of these functions are dependent on cellular metabolism and the ability of the cell to maintain energy supplies. Few cells are required to generate as much force and undergo as dramatic shifts in rate of metabolism as muscle cells. The ability of living skeletal muscle to undergo relatively large intracellular changes also influences its response to the drastic alterations that occur during the first few hours following exsanguination. Thus the organization, structure, and metabolism of the muscle are key to its function and to the maintenance of its integrity both during contraction and during the early postmortem period. Ultimately, these postmortem changes will influence the suitability of meat for further processing.

Muscle Composition

The largest constituent of muscle is water (Table 1.1; U.S. Department of Agriculture 2008). In living tissue, the average water content is 75% of the weight of the muscle; however, can vary, particularly in postmortem muscle (range of 65–80%). Within the muscle, it is the primary component of extracellular fluid. Within the muscle cell, water is the primary component of sarcoplasmic (cytoplasmic) fluid. It is important in thermoregulation; as a medium for many cellular processes; and for transport of nutrients within the cell, between cells, and between the muscle and the vascular system.

The second largest component of muscle is protein (U.S. Department of Agriculture 2008). Protein makes up an average of 18.5% of the weight of the muscle, though that figure can range from 16 to 22%. Proteins serve myriad functions and are the primary solid component in muscle. The functions of proteins are quite varied. Muscle proteins are involved in maintaining the structure and organization of the muscle and muscle cells (the role of highly insoluble stromal proteins). They are also important in the contractile process. These proteins primarily are associated with the contractile organelles, the myofibril, and are thus termed myofibrillar proteins. In general, the myofibrillar proteins are not soluble at low ionic strengths found in skeletal muscle (ionic strength ≤ 0.15), but can be solubilized at higher ionic strengths (≥ 0.3). This class of proteins includes both the proteins directly involved in movement (contractile proteins) and proteins that regulate the interactions between the contractile proteins (regulatory proteins). There are also many soluble proteins (sarcoplasmic pro-

Table 1.1. Composition of Mammalian Muscle

Component	% of Muscle Weight
Water	75% (65–80%)
Protein	18.5% (16–22%)
Lipid	3% (1–13%)
Carbohydrate	1% (0.5–1.5%)
Non-Protein Nitrogenous Substances	1.7% (1–2%)
Other Non-Protein Substances (minerals, vitamins, etc.)	0.85% (0.5–1%)

Numbers in parentheses indicate the average range of that component.(U.S. Department of Agriculture, 2008)

teins) that include proteins involved in cellular signaling processes and enzymes important in metabolism and protein degradation/cellular remodeling.

The lipid content of the muscle can vary greatly due to many factors, including animal age, nutritional level of the animal, and muscle type. It is important to note that the lipid content varies inversely with the water content (Callow 1948). Some lipid is stored inside the muscle cell; however, within a muscle, the bulk of the lipid is found between muscle bundles (groupings of muscle cells). Average lipid content of skeletal muscle is about 3% of the muscle weight, but the range can be as much as 1–13% (U.S. Department of Agriculture 2008). In skeletal muscle, lipid plays roles in energy storage, membrane structure, and in various other processes in the organ, including immune responses and cellular recognition pathways.

The two major types of lipid found in skeletal muscle are triglycerides and phospholipids. Triglycerides make up the greatest proportion of lipid associated with muscle. Triglycerides (triacylglycerides) consist of a glycerol molecule in which the hydroxyl groups are esterified with three fatty acids. The melting point and the iodine number of lipid that is associated with the muscle is determined by the chain length and the degree of saturation of the fatty acids. Phospholipids (phosphoglycerides) are another type of complex lipid found in muscle. In this class of lipids, one of the hydroxyl groups of glycerol is esterified to a phosphate group, while the other constituents are fatty acids. The fatty acids associated with phospholipids are typically unsaturated. Phospholipids in skeletal muscle are commonly associated with membranes. The relative high degree of unsaturation of the fatty acids associated with the phospholipids is a contributing factor to the fluidity of the cell membranes.

Carbohydrates make up a relatively small percentage of muscle tissue, making up about 1% of the total muscle weight (range of 0.5–1.5%). The carbohydrate that makes up the largest percentage is glycogen. Other carbohydrates include glucose, intermediates of glycogen metabolism, and other mono- and disaccharides. Glycosoaminoglycans are also found in muscle and are associated with the connective tissue.

There are numerous non-protein nitrogenous compounds in skeletal muscle. They include substances such as creatine and creatine phosphate, nucleotides (ATP, ADP), free amino acids, peptides (anserine, carnosine), and other non-protein substances.

Muscle Structure

Skeletal muscle has a very complex organization, in part to allow muscle to efficiently transmit force originating in the myofibrils to the entire muscle and ultimately, to the limb or structure that is moved. A relatively thick sheath of connective tissue, the epimysium, encloses the entire muscle. In most muscles, the epimysium is continuous, with tendons that link muscles to bones. The muscle is subdivided into bundles or groupings of muscle cells. These bundles (also known as fasciculi) are surrounded by another sheath of connective tissue, the perimysium. A thin layer of connective tissue, the endomysium, surrounds the muscle cells themselves. The endomysium lies above the muscle cell membrane (sarcolemma) and consists of a base-

ment membrane that is associated with an outer layer (reticular layer) that is surrounded by a layer of fine collagen fibrils imbedded in a matrix (Bailey and Light 1989).

Skeletal muscles are highly diverse, in part because of the diversity of actions they are asked to perform. Much of this diversity occurs not only at the gross level, but also at the muscle cell (fiber) level. First, not only do muscles vary in size, they can also vary in the number of cells. For example, the muscle that is responsible for adjusting the tension of the eardrum (tensor tympani) has only a few hundred muscle cells, while the medial gastrocnemius (used in humans for walking) has over a million muscle cells (Feinstein et al. 1955). Not only does the number of cells influence muscle function and ultimately, meat quality, but also the structure of the muscle cells themselves has a profound effect on the function of living muscle and on the functionality of meat.

Muscle cells are striated, meaning that when viewed under a polarized light microscope, distinct banding patterns or striations are observed. This appearance is due to specialized organelles, myofibrils, found in muscle cells. The myofibrils have a striated, or banded, appearance because different regions have different refractive properties. The light bands have a consistent index of refraction (isotropic). Therefore, these bands are called I-bands in reference to this isotropic property. The dark band appears dark because it is anisotropic and is thus called the A-band.

The myofibrils are abundant in skeletal muscle cells, making up nearly 80–90% of the volume of the cell. Myofibrillar proteins are relatively insoluble at physiological ionic strength, requiring an ionic strength greater than 0.3 to be extracted from muscle. For this reason, they are often referred to as "salt-soluble" proteins. Myofibrillar proteins make up approximately 50–60% of the total extractable muscle proteins. On a whole muscle basis, they make up approximately 10–12% of the total weight of fresh skeletal muscle. Therefore, they are very important in meat chemistry and in determining the functionality of meat proteins.

Myofibrils are the contractile "machinery" of the cell and, like the cells where they reside, are very highly organized. When examining a myofibril, one of the first observations that can be made is that the cylindrical organelle is made up of repeating units. These repeating units are known as sarcomeres. Contained in each sarcomere are all the structural elements needed to perform the physical act of contraction at the molecular level. Current proteomic analysis estimates that over 65 proteins make up the structure of the sarcomere (Fraterman et al. 2007). Given that the sarcomere is the most basic unit of the cell and that the number quoted in this analysis did not take into account the multiple isoforms of the proteins, this number is quite high. Many of the proteins interact with each other in a highly coordinated fashion, and some of the interactions are just now being discovered.

The structure of the sarcomere is responsible for the striated appearance of the muscle cell. The striations arise from the alternating, protein dense A-bands and less dense I-bands within the myofibril. Bisecting the I-bands are dark lines known as Z-lines. The structure between two Z-lines is the sarcomere. In a relaxed muscle cell, the distance between two Z-lines (and thus the length of the sarcomere) is approximately 2.2 μm. A single myofibril is made up of a large number of sarcomeres in series. The length of the myofibril and also the muscle cell is dependent on the number of sarcomeres. For example, the semitendinosus, a long muscle, has been estimated to have somewhere in the neighborhood of 5.8×10^4 to 6.6×10^4 sarcomeres per muscle fiber, while the soleus has been estimated to have approximately 1.4×10^4 (Wickiewicz et al. 1983). Adjacent myofibrils are attached to each other at the Z-line

by proteinacious filaments, known as intermediate filaments. Outermost myofibrils are attached to the cell membrane (sarcolemma) by intermediate filaments that interact not only with the Z-line, but also with structures at the sarcolemma known as costameres (Robson et al. 2004).

Myofibrils are made up of many myofilaments, of which there are two major types, classified as thick and thin filaments. There is also a third filament system composed primarily of the protein titin (Wang et al. 1979; Wang 1984; Wang et al. 1984; Wang and Wright 1988; Wang et al. 1991; Ma et al. 2006;). With respect to contraction and rigor development in postmortem muscle, it is the interdigitating thick and thin filaments that supply the "machinery" needed for these processes and give skeletal muscle cells their characteristic appearance (Squire 1981). Within the myofibril, the less dense I-band is made up primarily of thin filaments, while the A-band is made up of thick filaments and some overlapping thin filaments (Goll et al. 1984). The backbone of the thin filaments is made up primarily of the protein actin, while the largest component of the thick filament is the protein myosin. Together, these two proteins make up nearly 70% of the proteins in the myofibril of the skeletal muscle cell.

Myosin is the most abundant myofibrillar protein in skeletal muscle, making up approximately 50% of the total protein in this organelle. Myosin is a negatively charged protein with an isoelectric point of 5.3. Myosin is a large protein (approximately 500,000 daltons) that contains six polypeptides. Myosin consists of an alpha helical tail (or rod) region that forms the backbone of the thick filament and a globular head region that extends from the thick filament and interacts with actin in the thin filament. The head region of myosin also has ATPase activity, which is important in the regulation of contraction. Each myosin molecule contains two heavy chains (approximately 220,000 daltons each) and two sets of light chains (14,000–20,000 daltons). One of the light chains is required for enzymatic activity, and the other has regulatory functions.

Actin is the second-most abundant protein in the myofibril, accounting for approximately 20% of the total protein in the myofibril. Actin is a globular protein (G-actin) that polymerizes to form filaments (F-actin). G-actin has a molecular weight of approximately 42,000. There are approximately 400 actin molecules per thin filament. Thus the molecular weight of each thin filament is approximately 1.7×10^7 (Squire 1981). The thin filaments (F-actin polymers) are 1 μm in length and are anchored in the Z-line.

Two other proteins that are important in muscle contraction and are associated with the thin filament are tropomyosin and troponin. Tropomyosin is the second-most abundant protein in the thin filament and makes up about 7% of the total myofibrillar protein. Tropomyosin is made up of two polypeptide chains (alpha and beta) The alpha chain has an approximate molecular weight of 34,000, and the beta chain has a molecular weight of approximately 36,000. These two chains interact with each other to form a helix. The native tropomyosin molecule interacts with the troponin molecule to regulate contraction. Native troponin is a complex that consists of three subunits. These are termed troponin I (MW 23,000), troponin C (MW 18,000), and troponin T (MW 37,000). Troponin C has the ability to bind calcium released from the sarcoplasmic reticulum, troponin I can inhibit the interaction between actin and myosin, and troponin T binds very strongly to tropomyosin. The cooperative action of troponin and tropomyosin in response to calcium increases in the sarcoplasm regulates the interaction between actin and myosin and thus is a major regulator of contraction. Calcium that is released from the sarcoplasmic reticulum is bound to the tropo-

nin complex and the resulting conformational changes within troponin cause tropomyosin to move away from sites on actin to which myosin binds and allows myosin and actin to interact.

For contraction to occur, the thick and thin filaments interact via the head region of myosin. The complex formed by the interaction of myosin and actin is often referred to as actomyosin. In electron micrograph images of contracted muscle or of postrigor muscle, the actomyosin looks very much like cross-bridges between the thick and thin filaments; indeed, it is often referred to as such. In postmortem muscle, these bonds are irreversible and are also known as rigor bonds, as they are the genesis of the stiffness (rigor) that develops in postmortem muscle. The globular head of myosin also has enzymatic activity; it can hydrolyze ATP and liberate energy. In living muscle during contraction, the ATPase activity of myosin provides energy for myosin bound to actin to swivel and ultimately pull the thin filaments toward the center of the sarcomere. This produces contraction by shortening the myofibril, the muscle cell, and eventually, the muscle. The myosin and actin can disassociate when a new molecule of ATP is bound to the myosin head (Goll et al. 1984). In postrigor muscle, the supply of ATP is depleted, resulting in the actomyosin bonds becoming essentially permanent.

Muscle Metabolism

From a metabolic point of view, energy use and production in skeletal muscle is simply nothing short of amazing in its range and responsiveness. In an actively exercising animal, muscle can account for as much as 90% of the oxygen consumption in the body. This can represent an increase in the muscle's metabolic rate of as much as 200% from the resting state (Hargreaves and Thompson 1999).

Central to the existence of the muscle cell is the production of adenosine triphosphate (ATP), the energy currency of the cell. ATP consists of adenosine (an adenine ring and a ribose sugar) and three phosphate groups (triphosphate). Cleavage of the bonds between the phosphates (P_i) and the rest of the molecule provides energy for many cellular functions, including muscle contraction and the control of the concentrations of key ions (like calcium) in the muscle cell. Cleavage of P_i from ATP produces adenosine diphosphate (ADP), and cleavage of pyorphosphate (PP_i) from ATP produces adenosine monophosphate (AMP). Since the availability of ATP is central to survival of the cell, there is a highly coordinated effort by the cell to maintain its production in both living tissue and in the very early postmortem period.

Muscular activity is dependent on ample supplies of ATP within the muscle. Since it is so vital, muscle cells have developed several ways of producing/regenerating ATP. Muscle can use energy precursors stored in the muscle cell, such as glycogen, lipids, and phosphagens (phosphocreatine, ATP), and it can use energy sources recruited from the blood stream (blood glucose and circulating lipids). Which of these reserves (intracellular or circulating) the muscle cell uses depends on the activity the muscle is undergoing. When the activity is of lower intensity, the muscle will utilize a higher proportion of energy sources from the blood stream and lipid stored in the muscle cell. These will be metabolized to produce ATP using aerobic pathways. Obviously, ample oxygen is required for this process to proceed. During high intensity activity, during which ATP is used very rapidly, the muscle uses intracellular stores of phosphagens or glycogen. These two sources, however, are utilized very quickly and their depletion leads to fatigue. This is not a trivial point. Concentration of ATP in skeletal muscle is critical; available ATP must remain above

approximately 30% of the resting stores, or relaxation cannot occur. This is because relaxation of contraction is dependent on ATP, which is especially important because removal of calcium from the sarcoplasm is an ATP-dependent process (Hargreaves and Thompson 1999).

The primary fuels for muscle cells include phosphocreatine, glycogen, glucose lactate, free fatty acids, and triglycerides. Glucose and glycogen are the preferred substrates for muscle metabolism and can be utilized either aerobically (oxidative phosphorylation) or anaerobically (anaearobic glycolysis). Lipid and lactate utilization require oxygen. Lipids are a very energy-dense storage system and are very efficient with respect to the high amount of ATP that can be generated per unit of substrate. However, the rate of synthesis of ATP is much slower than when glycogen is used (1.5 mmol/kg/sec for free fatty acids compared with 3 mmol/kg/sec for glycogen utilized aerobically and 5 mmol/kg/sec when glycogen is used in anaerobic glycolysis) (Joanisse 2004).

Aerobic metabolism, the most efficient energy system, requires oxygen to operate, and that oxygen is supplied by the blood supply to the muscle and by the oxygen transporter, myoglobin. It has been estimated that in working muscle, the myoglobin is somewhere in the neighborhood of 50% saturated. Under conditions of extreme hypoxia (as found in postmortem muscle), oxygen supplies are depleted because blood flow is not sufficient (or does not exist), and myoglobin oxygen reserves are depleted if this state continues long enough. Prior to exsanguination, the oxidation of glycogen or other substrates to form water and carbon dioxide via oxidative phosphorylation is a very efficient way for the cell to regenerate ATP. However, after exsanguination, the muscle cell must turn solely to anaerobic pathways for energy production.

Phosphocreatine in living, rested muscle is available in moderate abundance compared with ATP (100 mmol/kg dry muscle weight for phosphocreatine compared with 25 mmol/kg dry muscle weight for ATP) but very low abundance compared with glycogen (500 mmol/kg dry muscle weight for glycogen). Phosphocreatine can easily transfer a phosphate group to ADP in a reaction catalyzed by creatine kinase. This reaction is easily reversible and phosphocreatine supplies can be readily restored when ATP demand is low. In living muscle, when activity is intense, this system can be advantageous, as it consumes H^+ and thus can reduce the muscle cell acidosis that is associated with anaerobic glycolysis. Another advantage of the system is that the catalyzing enzyme is located very close to the actomyosin ATPase and also at the sarcoplasmic reticulum (where calcium is actively taken up from the sarcoplasm to regulate contraction) and at the sarcolemma. However, this system is not a major contributor to postmortem metabolism, as the supplies are depleted fairly rapidly.

In general, glycogen is the preferred substrate for the generation of ATP, either through the oxidative phosphorylation or through anaerobic glycolysis (Fig. 1.1). One of the key steps in the fate of glycogen is whether or not an intermediate to the process, pyruvate, enters the mitochondria to be completely broken down to CO_2 and H_2O (yielding 38 mol of ATP per mole of oxidized glucose-1-P produced from glycogen or 36 mol if the initial substrate is glucose), or if it ends in lactate via the anaerobic glycolysis pathway. The anaerobic pathway, while comparatively less efficient (yielding 3 mol of ATP per mole of glucose-1-P produced from glycogen or 2 mol if the initial substrate is glucose), is much better at producing ATP at a higher rate. Early postmortem muscle obviously uses the anaerobic pathway, as oxygen supplies are rapidly depleted. This results in the buildup of the end product, lactate (lactic acid), resulting in pH decline.

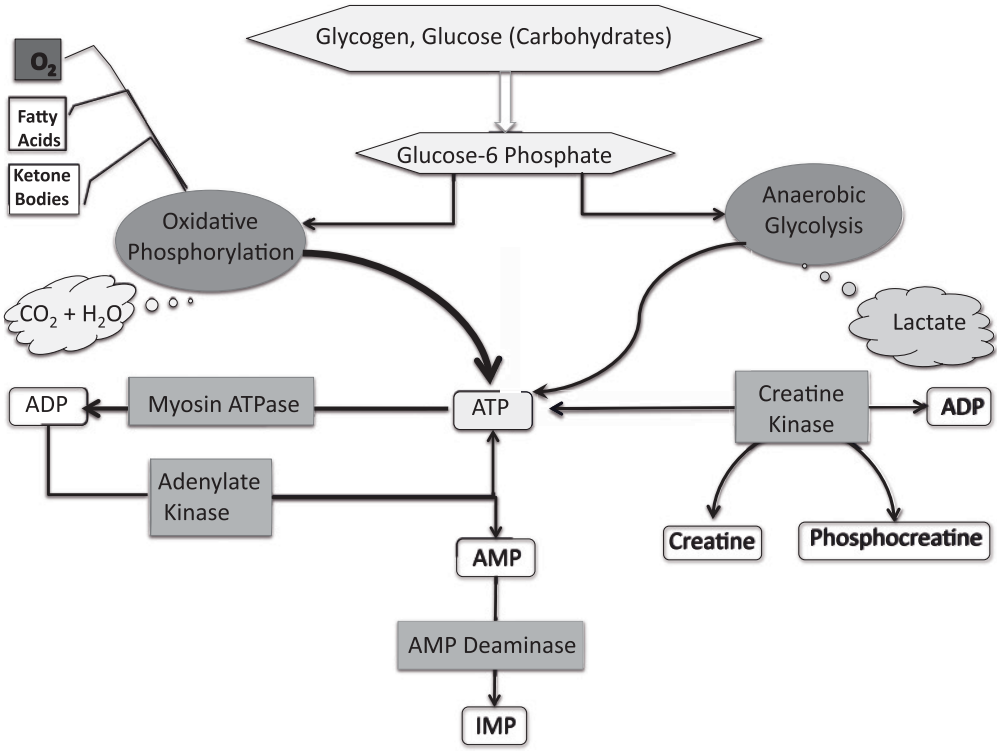

Figure 1.1. ATP production in muscle.

Major Postmortem Changes in Muscle

Tenderization

During refrigerated storage, it is well known that meat becomes more tender. It is commonly accepted that the product becomes more tender because of proteolytic changes occurring in the architecture of the myofibril and its associated proteins. There are several key proteins that are degraded during postmortem aging.

Titin

Titin (aka connectin) is a megaprotein that is approximately 3 megadaltons in size. In addition to being the largest protein found in mammalian tissues, it is also the third-most abundant. A single titin molecule is estimated to be between 2 and 2.5 μM in length. In striated muscle, titin thus spans fully half of a sarcomere, with its C-terminal end localizing in the M-line at the center of the sarcomere and the N-terminal forming an integral part of the Z-line. Titin aids in maintaining sarcomeric alignment of the myofibril during contraction. Titin integrates the Z-line and the thick filaments, maintaining the location of the thick filaments between the Z-lines. Titin is also hypothesized to play a role in generating at least a portion of the passive tension that is present in skeletal muscle cells. During development of the myofibril, titin is one of the earliest proteins expressed, and it is thought to act as a "molecular ruler" by providing a scaffolding or template for the developing myofibril (Clark et al. 2002).

Due to the aforementioned roles of titin in living cells, it is quite conceivable that

its degradation in postmortem muscle would lead to weakening of the longitudinal structure of the myofibrillar sarcomere and integrity of muscle. This weakening, in conjunction with other changes in postmortem muscle, could lead to enhanced tenderness. The degradation of titin has been observed in several studies (Lusby et al. 1983; Zeece et al. 1986; Astier et al. 1993; Huff-Lonergan et al. 1995; Melody et al. 2004; Rowe et al. 2004a, b). When titin is degraded, a major degradation product, termed T_2, is observed that migrates only slightly faster under SDS-PAGE conditions than intact titin. This product migrates at approximately 2,400 kDa (Kurzban and Wang 1988, 1987; Huff-Lonergan et al. 1995). Another titin degradation product that has been observed by SDS-PAGE analysis migrates at approximately 1,200 kDa (Matsuura et al. 1991; Huff-Lonergan et al. 1995). This latter polypeptide has been shown to contain the portion of titin that extends from the Z-line to near the N_2 line in the I-band (Kimura et al. 1992), although the exact position that the 1200 kDa polypeptide reaches in the sarcomere is still not certain. The 1,200-kDa polypeptide has been documented to appear earlier postmortem in myofibrils from aged beef that had lower shear force (and more desirable tenderness scores) than in samples from product that had higher shear force and/or less favorable tenderness scores (Huff-Lonergan et al. 1995, 1996a, b). The T2 polypeptide can also be subsequently degraded or altered during normal postmortem aging. Studies that have used antibodies against titin have been shown to cease to recognize T2 after prolonged periods of postmortem storage or μ-calpain digestion (Ho et al. 1994; Huff-Lonergan et al. 1996a)

Nebulin

Nebulin is another mega-protein (Mr 600–900 kDa) in the sarcomere. This protein extends from the Z-line to the pointed ends of the thin filament. The C-terminal end of nebulin is embedded into the Z-line. Nebulin is highly nonextensible and has been referred to as a molecular ruler that during development may serve to define the length of the thin filaments (Kruger et al. 1991). Nebulin, via its intimate association with the thin filament (Lukoyanova et al. 2002), has been hypothesized to constitute part of a composite nebulin/thin filament (Pfuhl et al. 1994; Robson et al. 1995) and may aid in anchoring the thin filament to the Z-line (Wang and Wright 1988; Komiyama et al. 1992). Degradation of nebulin postmortem could weaken the thin filament linkages at the Z-line, and/or of the thin filaments in the nearby I-band regions (Taylor et al. 1995), and thereby weaken the structure of the muscle cell. Nebulin has also been shown to be capable of linking actin and myosin (Root and Wang 1994a, b). It has been hypothesized that nebulin may also have a regulatory function in skeletal muscle contraction (Root and Wang 1994a, b; Bang et al. 2006). Portions of nebulin that span the A-I junction have the ability to bind to actin, myosin, and calmodulin (Root and Wang 2001). More interesting, this portion of nebulin (spanning the A-I junction) has been shown to inhibit actomyosin ATPase activity (Root and Wang, 2001; Lukoyanova et al. 2002). This region of nebulin also has been suggested to inhibit the sliding velocities of actin filaments over myosin. If the latter role is confirmed, then it is also possible that nebulin's postmortem degradation may alter actin-myosin interactions in such a way that the alignment and interactions of thick and thin filaments in postmortem muscle is disrupted. This, too, could lead to an increase in postmortem tenderization. Nebulin degradation does seem to be correlated to postmortem tenderization, although the exact cause-and-effect relationship remains to be substantiated (Huff-Lonergan et al. 1995; Taylor et al. 1995;

Huff-Lonergan et al. 1996a; Melody et al. 2004).

Troponin-T

For many years it has been recognized that the degradation of troponin-T and the appearance of polypeptides migrating at approximately 30 kDa are strongly related to, or correlated with, the tenderness of beef (Penny et al. 1974; MacBride and Parrish 1977; Olson and Parrish 1977; Olson et al. 1977). It has been shown that purified bovine troponin-T can be degraded by μ-calpain in vitro to produce polypeptides in the 30-kDa region (Olson et al. 1977). In addition, polypeptides in the 30-kDa region found in aged bovine muscle specifically have been shown to be products of troponin-T by using Western blotting techniques (Ho et al. 1994). Often, more than one fragment of troponin-T can be identified in postmortem muscle. Increasing postmortem time has been shown to be associated with the appearance of two major bands (each is likely a closely spaced doublet of polypeptides) of approximately 30 and 28 kDa, which label with monoclonal antibodies to troponin-T (Huff-Lonergan et al. 1996a). In addition, the increasing postmortem aging time was also associated with a loss of troponin-T, as has been reported in numerous studies (Olson et al. 1977; Koohmaraie et al. 1984a, b; Ho et al. 1994). It has recently been shown that troponin-T is cleaved in its glutamic acid-rich amino-terminal region (Muroya et al. 2007). Some studies have shown labeling of two very closely spaced bands corresponding to intact troponin-T. This is likely due to isoforms of troponin-T that are known to exist in skeletal muscle (Briggs et al. 1990; Malhotra 1994; Muroya et al. 2007), including specifically bovine skeletal muscle (Muroya et al. 2007). Both the appearance of the 30- and 28-kDa bands and the disappearance of the intact troponin-T in the myofibril are very strongly related to the shear force (Penny 1976; Huff-Lonergan et al. 1996b; Huff-Lonergan and Lonergan, 1999; Lonergan et al. 2001; Rowe et al. 2003; Rowe et al. 2004a). Troponin-T is a substrate for μ-calpain, and it is hypothesized that μ-calpain is at least partly responsible for the postmortem degradation of troponin-T and the concomitant production of the 28- and 30-kDa polypeptides. Degradation of troponin-T may simply be an indicator of overall postmortem proteolysis (i.e., it occurs as meat becomes more tender). However, because troponin-T is an integral part of skeletal muscle thin filaments (Greaser and Gergely 1971), its role in postmortem tenderization may warrant more careful examination as has been suggested (Ho et al. 1994; Uytterhaegen et al. 1994; Taylor et al. 1995; Huff-Lonergan et al. 1996b). Indeed, the troponin-T subunit makes up the elongated portion of the troponin molecule and through its interaction with tropomyosin aids in regulating the thin filament during skeletal muscle contraction (Greaser and Gergely 1971; Hitchcock 1975; McKay et al. 1997; Lehman et al. 2001). It is conceivable that postmortem degradation of troponin-T and disruption of its interactions with other thin filament proteins aids in the disruption of the thin filaments in the I-band, possibly leading to fragmentation of the myofibril and overall muscle integrity. During postmortem aging, the myofibrils in postmortem bovine muscle are broken in the I-band region (Taylor et al. 1995). Because troponin-T is part of the regulatory complex that mediates actin-myosin interactions (Greaser and Gergely, 1971; Hitchcock, 1975; McKay et al. 1997; Lehman et al. 2001), it is also conceivable that its postmortem degradation may lead to changes involving thick and thin filament interactions. Regardless of whether or not troponin--T aids in disruption of the thin filament in the I-band, alters thick and thin filament interactions, or simply reflects overall protein degradation, its degradation and appearance

of polypeptides in the 30-kDa region seem to be a valuable indicator of beef tenderness (Olson et al. 1977; Olson and Parrish, 1977; Koohmaraie et al. 1984a, b; Koohmaraie 1992; Huff-Lonergan et al. 1995; Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan 1999).

Desmin

It has been suggested that desmin, an intermediate filament protein (O'Shea et al. 1979; Robson 1989) localized at the periphery of the myofibrillar Z-disk in skeletal muscle (Richardson et al. 1981), plays a role in the development of tenderness (Taylor et al. 1995; Huff-Lonergan et al. 1996a; Boehm et al. 1998; Melody et al. 2004). The desmin intermediate filaments surround the Z-lines of myofibrils. They connect adjacent myofibrils at the level of their Z-lines, and the myofibrils to other cellular structures, including the sarcolemma (Robson, 1989; Robson et al. 1995). Desmin may be important in maintaining the structural integrity of muscle cells (Robson et al. 1981, 1991). It is possible that degradation of structural elements that connect the major components (i.e., the myofibrils) of a muscle cell together, as well as the peripheral layer of myofibrils to the cell membrane, could affect the development of tenderness. Desmin is degraded during postmortem storage (Hwan and Bandman 1989; Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan, 1999; Melody et al. 2004; Rowe et al. 2004b; Zhang et al. 2006). Furthermore, it has been documented that desmin is degraded more rapidly in myofibrils from samples with low shear force and higher water-holding capacity (Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan, 1999; Melody et al. 2004; Rowe et al. 2004b; Zhang et al. 2006). A major degradation product that is often seen in beef is a polypeptide of approximately 38 kDa. This degradation product also has been shown to be present in μ-calpain-digested myofibrils (Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan, 1999; Carlin et al. 2006). Thus, the proteolytic enzyme μ-calpain may be, at least in part, responsible for desmin degradation under normal postmortem aging conditions. Whether or not this degradation is truly directly linked to tenderization or is simply an indicator of overall postmortem proteolysis remains to be determined.

Filamin

Filamin is a large (Mr = 245,000 in skeletal and cardiac muscle) actin-binding protein that exists in numerous cell types (Loo et al. 1998; Thompson et al. 2000; van der Flier et al. 2002). There are several different isoforms of filamin (Hock et al. 1990). The amount of filamin in skeletal and cardiac muscle is very low (approximately ≤0.1% of the total muscle protein). In skeletal and cardiac muscle, filamin is localized at the periphery of the myofibrillar Z-disk, and it may be associated with intermediate filaments in these regions (Loo et al. 1998; Thompson et al. 2000; van der Flier et al. 2002). Thus, postmortem degradation of filamin conceivably could disrupt key linkages that serve to help hold myofibrils in lateral register. Degradation of filamin may also alter linkages connecting the peripheral layer of myofibrils in muscle cells to the sarcolemma by weakening interactions between peripheral myofibrillar Z-disks and the sarcolemma via intermediate filament associations or costameres (Robson et al. 1995). A study using myofibrils from beef showed that some filamin was degraded to form an approximately 240-kDa degradation product that migrated as a doublet in both myofibrils from naturally aged muscle and in μ-calpain-digested myofibrils (Huff-Lonergan et al. 1996a). This same doublet formation (composed of intact and degraded filamin) has been seen in cultured embryonic skeletal muscle cells and was attributed to calpain

activity (Robson et al. 1995). Uytterhaegen et al. (1994) have shown increased degradation of filamin in muscle samples injected with $CaCl_2$, a process that has been shown to stimulate proteolysis and postmortem tenderization (Wheeler et al. 1992; Harris et al. 2001). Compared with other skeletal muscle proteins, relatively little has been done to fully characterize the role of this protein in postmortem tenderization of beef. Further studies that employ a combination of sensitive detection methods (e.g., one- and two-dimensional gels, Western blotting, immunomicroscopy) are needed to determine the role of filamin in skeletal muscle systems and postmortem tenderization.

Water-Holding Capacity/Drip Loss Evolution

Lean muscle contains approximately 75% water. The other main components include protein (approximately 18.5%), lipids or fat (approximately 3%), carbohydrates (approximately 1%), and vitamins and minerals (often analyzed as ash, approximately 1%). The majority of water in muscle is held within the structure of the muscle and muscle cells. Specifically, within the muscle cell, water is found within the myofibrils, between the myofibrils themselves and between the myofibrils and the cell membrane (sarcolemma), between muscle cells, and between muscle bundles (groups of muscle cells) (Offer and Cousins 1992).

Water is a dipolar molecule and as such is attracted to charged species like proteins. In fact, some of the water in muscle cells is very closely bound to protein. By definition, bound water is water that exists in the vicinity of nonaqueous constituents (like proteins) and has reduced mobility (i.e., does not easily move to other compartments). This water is very resistant to freezing and to being driven off by conventional heating (Fennema 1985). True bound water is a very small fraction of the total water in muscle cells; depending on the measurement system used, approximately 0.5 g of water per gram of protein is estimated to be tightly bound to proteins. Since the total concentration of protein in muscle is approximately 200 mg/g, this bound water only makes up less than a tenth of the total water in muscle. The amount of bound water changes very little if at all in postrigor muscle (Offer and Knight 1988b).

Another fraction of water that can be found in muscles and in meat is termed entrapped (also referred to as immobilized) water (Fennema 1985). The water molecules in this fraction may be held either by steric (space) effects and/or by attraction to the bound water. This water is held within the structure of the muscle but is not bound per se to protein. In early postmortem tissue, this water does not flow freely from the tissue, yet it can be removed by drying and can be easily converted to ice during freezing. Entrapped or immobilized water is most affected by the rigor process and the conversion of muscle to meat. Upon alteration of muscle cell structure and lowering of the pH, this water can also eventually escape as purge (Offer and Knight 1988b).

Free water is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat. Free water is not readily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structures where it is found (Fennema 1985).

The majority of the water that is affected by the process of converting muscle to meat is the entrapped (immobilized) water. Maintaining as much of this water as possible in meat is the goal of many processors. Some of the factors that can influence the retention of entrapped water include manipulation of the net charge of myofibrillar proteins and the structure of the muscle cell and its components (myofibrils, cytoskeletal linkages, and membrane permeability), as well as the

amount of extracellular space within the muscle itself.

Physical/Biochemical Factors in Muscles That Affect Water-Holding Capacity

During the conversion of muscle to meat, anaerobic glycolysis is the primary source of ATP production. As a result, lactic acid builds up in the tissue, leading to a reduction in pH of the meat. Once the pH has reached the isoelectric point (pI) of the major proteins, especially myosin (pI = 5.3), the net charge of the protein is zero, meaning the numbers of positive and negative charges on the proteins are essentially equal. These positive and negative groups within the protein are attracted to each other and result in a reduction in the amount of water that can be attracted and held by that protein. Additionally, since like charges repel, as the net charge of the proteins that make up the myofibril approaches zero (diminished net negative or positive charge), repulsion of structures within the myofibril is reduced, allowing those structures to pack more closely together. The end result of this is a reduction of space within the myofibril. Partial denaturation of the myosin head at low pH (especially if the temperature is still high) is also thought to be responsible for a large part of the shrinkage in myofibrillar lattice spacing (Offer 1991).

Myofibrils make up a large proportion of the muscle cell. These organelles constitute as much as 80–90% of the volume of the muscle cell. As mentioned previously, much of the water inside living muscle cells is located within the myofibril. In fact, it is estimated that as much as 85% of the water in a muscle cell is held in the myofibrils. Much of that water is held by capillary forces arising from the arrangement of the thick and thin filaments within the myofibril. In living muscle, it has been shown that sarcomeres remain isovolumetric during contraction and relaxation (Millman et al. 1981; Millman et al. 1983). This would indicate that in living muscle the amount of water within the filamentous structure of the cell would not necessarily change. However, the location of this water can be affected by changes in volume as muscle undergoes rigor. As muscle goes into rigor, cross-bridges form between the thick and thin filaments, thus reducing available space for water to reside (Offer and Trinick 1983). It has been shown that as the pH of porcine muscle is reduced from physiological values to 5.2–5.6 (near the isoelectric point of myosin), the distance between the thick filaments declines an average of 2.5 nm (Diesbourg et al. 1988). This decline in filament spacing may force sarcoplasmic fluid from between the myofilaments to the extramyofibrillar space. Indeed, it has been hypothesized that enough fluid may be lost from the intramyofibrillar space to increase the extramyofibrillar volume by as much as 1.6 times more than its pre-rigor volume (Bendall and Swatland 1988).

During the development of rigor, the diameter of muscle cells decreases (Hegarty 1970; Swatland and Belfry 1985) and is likely the result of transmittal of the lateral shrinkage of the myofibrils to the entire cell (Diesbourg et al. 1988). Additionally, during rigor development, sarcomeres can shorten; this also reduces the space available for water within the myofibril. In fact, it has been shown that drip loss can increase linearly with a decrease in the length of the sarcomeres in muscle cells (Honikel et al. 1986). More recently, highly sensitive low-field nuclear magnetic resonance (NMR) studies have been used to gain a more complete understanding of the relationship between muscle cell structure and water distribution (Bertram et al. 2002). These studies have suggested that within the myofibril, a higher proportion of water is held in the I-band than in the more protein-dense A-band. This observation may help explain why shorter sarcomeres (especially in cold-shortened

muscle) are often associated with increased drip losses. As the myofibril shortens and rigor sets in, the shortening of the sarcomere would lead to shortening and subsequent lowering of the volume of the I-band region in myofibril. Loss of volume in this myofibrillar region (where much water may reside), combined with the pH-induced lateral shrinkage of the myofibril, could lead to expulsion of water from the myofibrillar structure into the extramyofibrillar spaces within the muscle cell (Bendall and Swatland 1988). In fact, recent NMR studies support this hypothesis (Bertram et al. 2002). It is thus likely that the gradual mobilization of water from the intramyofibrillar spaces to the extramyofibrillar spaces may be key in providing a source of drip.

All the previously mentioned processes influence the amount of water in the myofibril. It is important to note that shrinkage of the myofibrillar lattice alone could not be responsible for the movement of fluid to the extracellular space and ultimately out of the muscle. The myofibrils are linked to each other and to the cell membrane via proteinacious connections (Wang and Ramirez-Mitchell 1983). These connections, if they are maintained intact in postmortem muscle, would transfer the reduction in diameter of the myofibrils to the muscle cell (Diesbourg et al. 1988; Morrison et al. 1998; Kristensen and Purslow 2001; Melody et al. 2004). Myofibril shrinkage can be translated into constriction of the entire muscle cell, thus creating channels between cells and between bundles of cells that can funnel drip out of the product (Offer and Knight 1988). Extracellular space around muscle fibers continually increases up to 24 hours postmortem, but gaps between muscle fiber bundles decrease slightly between nine and 24 hours postmortem, perhaps due to fluid outflow from these major channels (Schafer et al. 2002). These linkages between adjacent myofibrils and myofibrils and the cell membrane are made up of several proteins that are associated with intermediate filament structures and structures known as costameres. Costameres provide the structural framework responsible for attaching the myofibrils to the sarcolemma. Proteins that make up or are associated with the intermediate filaments and costameres include (among others) desmin, filamin, synemin, dystrophin, talin, and vinculin (Greaser 1991). If costameric linkages remain intact during the conversion of muscle to meat, shrinkage of the myofibrils as the muscle goes into rigor would be transmitted to the entire cell via these proteinacious linkages and would ultimately reduce volume of the muscle cell itself (Offer and Knight 1988b; Kristensen and Purslow 2001; Melody et al. 2004). Thus, the rigor process could result in mobilization of water not only out of the myofibril, but also out of the extramyofibril spaces as the overall volume of the cell is constricted. In fact, reduction in the diameter of muscle cells has been observed in postmortem muscle (Offer and Cousins 1992). This water that is expelled from the myofibril and ultimately the muscle cell eventually collects in the extracellular space. Several studies have shown that gaps develop between muscle cells and between muscle bundles during the postrigor period (Offer et al. 1989; Offer and Cousins 1992). These gaps between muscle bundles are the primary channels by which purge is allowed to flow from the meat; some investigators have actually termed them "drip channels."

Postmortem Changes in Muscle That Influence Quality

As muscle is converted to meat, many changes occur, including: (1) a gradual depletion of available energy; (2) a shift from aerobic to anaerobic metabolism favoring the production of lactic acid, resulting in the pH of the tissue declining from near neutrality to 5.4–5.8; (3) a rise in ionic strength, in part, because of the inability of ATP-dependent

calcium, sodium, and potassium pumps to function; and (4) an increasing inability of the cell to maintain reducing conditions. All these changes can have a profound effect on numerous proteins in the muscle cell. The role of energy depletion and pH change have been covered in this chapter and in other reviews (Offer and Trinick 1983; Offer and Knight 1988a). What has not been as thoroughly considered is the impact of other changes on muscle proteins, such as oxidation and nitration.

Protein Oxidation

Another change that occurs in postmortem muscle during aging of whole muscle products is increased oxidation of myofibrillar and sarcoplasmic proteins (Martinaud et al. 1997; Rowe et al. 2004a, b). This results in the conversion of some amino acid residues, including histidine, to carbonyl derivatives (Levine et al. 1994; Martinaud et al. 1997) and can cause the formation of intra- and/or inter-protein disulfide cross-links (Stadtman 1990; Martinaud et al. 1997). In general, both these changes reduce the functionality of proteins in postmortem muscle (Xiong and Decker 1995). In living muscle, the redox state of muscle can influence carbohydrate metabolism by directly affecting enzymes in the glycolytic pathway. Oxidizing agents can also influence glucose transport. Hydrogen peroxide (H_2O_2) can mimic insulin and stimulate glucose transport in exercising muscle. H_2O_2 is increased after exercise, and thus oxidation systems may play a role in signaling in skeletal muscle (Balon and Yerneni 2001). Alterations in glucose metabolism in the ante- and perimortem time period do have the potential to cause changes in postmortem muscle metabolism and thus represent an important avenue of future research.

In postmortem muscle, these redox systems may also play a role in influencing meat quality. The proteolytic enzymes, the calpains, are implicated in the proteolysis that is involved in increasing the tenderness of fresh meat and in influencing fresh meat water-holding capacity (Huff-Lonergan and Lonergan 2005). Because μ-calpain and m-calpain enzymes contain both histidine and SH-containing cysteine residues at their active sites, they are particularly susceptible to inactivation by oxidation (Lametsch et al. 2008). Therefore, oxidizing conditions in postmortem muscle lead to inactivation or modification of calpain activity (Harris et al. 2001; Rowe et al. 2004a, b; Maddock et al. 2006). In fact, evidence suggests oxidizing conditions inhibit proteolysis by μ-calpain, but might not completely inhibit autolysis (Guttmann et al. 1997; Guttmann and Johnson 1998; Maddock et al. 2006). In postmortem muscle, there are differences between muscles in the rate that postmortem oxidation processes occur (Martinaud et al. 1997). It has been noted that differences in the rate of oxidation in muscle tissue are seen when comparing the same muscles between animals and/or carcasses that have been handled differently (Juncher et al. 2001). These differences may arise because of differences in diet, breed, antemortem stress, postmortem handling of carcasses, etc. In fact, there have been reports of differences between animals and between muscles in the activity of some enzymes involved in the oxidative defense system of muscle (Daun et al. 2001). Therefore, there may be genetic differences in susceptibility to oxidation that could be capitalized on to improve meat quality. It is reasonable to hypothesize that differences in the antioxidant defense system between animals and/or muscles would influence calpain activity, proteolysis, and thus tenderization.

Exposure to oxidizing conditions (H_2O_2) under postmortem-like conditions inhibits calpain activity (Carlin et al. 2006). In a series of in vitro assays using either a fluorescent peptide or purified myofibrils as the substrate it was shown that the presence of oxidizing species does significantly impede

the ability of calpains to degrade their substrates. Oxidation with H_2O_2 significantly limits proteolytic activity of μ- and m-calpain against the fluorescent peptide Suc-Leu-Leu-Val-Tyr-AMC, regardless of the pH or ionic strength. Similar results were seen when using purified myofibrils as the substrate. This inhibition was reversible, as addition of reducing agent (DTT) to the oxidized samples restored activity. Oxidation also has been shown to slow the rate of μ-calpain autolysis and could be part of the mechanism underlying some of the retardation of activity (Guttmann et al. 1997; Carlin et al. 2006).

Oxidation does occur early in postmortem meat, and it does influence proteolysis (Harris et al. 2001; Rowe et al. 2004b). Rowe et al. (2004) showed that there was a significant increase in proteolysis of troponin-T in steaks from alpha-tocopherol-fed steers after 2 days of postmortem aging compared with steers fed a conventional feedlot diet. This indicates that very low levels of oxidation can influence proteolysis and that increasing the level of antioxidants in meat may have merit in improving tenderness in future studies. In fact, low levels of oxidation may be the cause of some heretofore-unexplained variations in proteolysis and tenderness that have been observed in meat.

Nitric Oxide and S-Nitrosylation

Nitric oxide (NO) is often used as a general term that includes NO and reactive nitrogen species (RNS), like S-nitrosothyols, peroxynitrate, and metal NO complexes. In living tissue, NO is involved in arteriole dilation that increases blood flow to muscles, resulting in increased delivery of nutrients and oxygen to the muscle (Kobzik et al. 1994; Stamler et al. 2001). NO species are also implicated in glucose homeostasis and excitation-contraction coupling. The gas NO is produced in biological systems by a family of enzymes known as nitric oxide synthases (NOS). There are three major isoforms of NOS: neural, inducible, and endothelial. Skeletal muscle expresses all three isoforms; however, the neural form, nNOS, is thought to be the predominant isoform (Kaminski and Andrade 2001). These enzymes utilize arginine as a substrate and catalyze the following reaction: L-arginine+NADPH+O_2 forming L-citrulline+$^{\bullet}$NO+$NADPH^{+}$. NO is important in biological systems, particularly because of its role as a second messenger. However, while NO rapidly diffuses through tissues, NO itself is a relatively short-lived species. It does have the ability to combine with other biomolecules that also have physiological importance.

One example of this is its ability to combine with superoxide to form the highly oxidizing molecule peroxynitrite. Proteins are important biological targets of peroxynitrite, particularly proteins containing cysteine, motioning, and/or tryptophan (Radi et al. 2000). Several enzymes are known to be inactivated by peroxynitrite. Among these is the sarcoplasmic reticulum Ca^{2+}-ATPase (Klebl et al. 1998). One indirect effect of NO is S-nitrosylation. In most cases, S-nitrosylation events involve amines and thiols. Nitric oxide can interact with cysteines to form nitrosothiols that can alter the activity of the protein. Because of this, it has been suggested that S-nitrosylation may function as a post-translational modification much like phosphorylation (Jaffrey et al. 2001). Some proteins, such as the ryanodine receptor and the cysteine protease caspase-3, have been shown to be endogenously nitrosylated, further supporting the suggestion that formation of nitrosothiols may be an important regulatory step (Hess et al. 2001; Hess et al. 2005). μ-Calpain is also a cysteine protease that could be influenced by S-nitrosylation. Small thiol peptides like glutathione can be impacted by nitrosative stress to form compounds like S-nitrosoglutathione (GSNO). These compounds can, in turn, influence other proteins

by transnitrosating other reduced thiols (Miranda et al. 2000).

Aspects of skeletal muscle function that can be affected by increased NO production include inhibition of excitation-contraction coupling, increased glucose uptake, decreased mitochondrial respiration, and decreased force production. The decrease in force is apparently because of an inhibitory effect that NO has on actomyosin ATPase activity, which leads to less cross-bridge cycling. S-nitroslyation of the ryanodine receptor (calcium release channel in the sarcoplasmic reticulum) may also play a role on modulating contraction. This protein is responsible for releasing calcium from the sarcoplasmic reticulum into the sarcoplasm. S-nitrosylation of a cysteine in the ryanodine receptor will increase its activity. This effect is reversible (Kobzik et al. 1994). Because muscle contains all the compounds needed to form these intermediates, it stands to reason that they could be important in the conversion of muscle to meat.

It is clear that the composition, structure, and metabolic properties of skeletal muscle have enormous impacts on the quality of fresh meat and, in turn, its suitability as a raw material for further processed meat. Continued attention to factors that regulate changes in early postmortem muscle will improve the quality and consistency of fresh meat. This, in turn, will improve the consistency of the quality of further processed products.

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Chapter 2

Technological Quality of Meat for Processing

Susan Brewer

Introduction

For the purposes of this discussion, technological quality of meat for processing includes the factors that affect meat quality in general, whether endogenous or exogenous. Factors that contribute to the quality of the meat for processing include the breed of the animal and its associated characteristics, gene status within breed, diet and plane of nutrition, fatness/leanness, rate of postmortem pH and temperature decline, and postmortem handling such as aging. Ultimately, meat quality is defined in terms of consumer acceptability, which include tenderness, juiciness and flavor, and appearance characteristics such as color, amount of fat, amount of visible water, and textural appearance, which have a significant impact on consumer expected satisfaction (Brewer et al. 1998, 2001). Because they are the most important traits defining consumer acceptance, tenderness and flavor consistency are important (Robbins et al. 2003). Factors contributing to the sensory quality characteristics of meat include breed (Cameron et al. 1990; Lan et al. 1993), intramuscular fat content (Brewer et al. 2001; Rincker et al. 2008), calpastatin and μ-calpain gene status (Casas et al. 2006), Halothane gene status (Sather et al. 1990; Leach et al. 1998), ryanodine receptor gene status (Fujii et al. 1991), diet, antemortem handling (Ohene-Adjai et al. 2003), and ultimate pH (Zhu and Brewer 1998).

Breed Effects on Quality of Meat

Livestock breed can affect the quality characteristics of the meat produced, either because the breed has naturally adapted to stressful environmental conditions or because two or more breeds have been purposefully crossbred to increase prevalence of desirable qualities. Often these modifications improve one set of attributes at the expense of another.

For example, Brahman cattle are used extensively in the southwestern United States because of their tolerance to adverse environmental conditions; however, Brahman carcasses have tenderness issues. Toughness of meat from Brahman cattle has been associated with high levels of calpastatin in the muscle (Ibrahim et al. 2008). The Japanese Wagyu breed produces highly marbled, tender meat. Cross breeding Brahman with Wagyu cattle to produce Waguli cattle, which have a high degree of marbling and low calpastatin activity in the tissue, results in more tender meat immediately after slaughter. Tenderness of meat from Brahman cattle does catch up with sufficient aging (14 d). Schone et al. (2006) reported initial tenderness differences in beef from Holstein and Simmental cattle, in addition to different responses to aging. Some breed differences (Nelore, Simmental, Simbrasil) in initial postmortem beef tenderness are lost after 7 days of aging (Bianchini et al. 2007). According to Hocquette et al. (2006), cattle

of different breeds or different genotypes of the same breed differ primarily in their connective tissue characteristics (collagen cross-linking and solubility), content, and composition of intramuscular fat and/or the characteristics of their muscle fibers (slow-oxidative, fast-oxidoglycolytic, fast glycolytic). Mutations in the myostatin gene result in muscle hypertrophy, producing cattle with enlarged muscles. However, this mutation favors glycolytic muscle fiber metabolism and decreases collagen and intramuscular fat contents, favoring tenderness.

Collagen constitutes 20–25% of the protein in mammals, and connective tissues are composed mainly of collagen. It occurs in muscle tissue, binding the fibers together in bundles. However, collagen is not distributed uniformly among muscle groups. Generally, the collagen content parallels the level of physical activity of the particular muscle. Increasing intermolecular cross-linking among collagen molecules decreases their extensibility and their solubility (Forrest et al. 1975). Those muscles that are used extensively have higher amounts of collagen and are generally tougher.

Smith et al. (2007a) reported that weight at slaughter, hot carcass weight, loin muscle area, yield grade, calpastatin enzyme activity, and carcass quality grade were relatively highly heritable. They found moderate heritability estimates for marbling score, back fat thickness, and feedlot average daily gain. MacNeil et al. (2001) reported that Limousin-sired calves grew more rapidly than Hereford-sired calves. By the finishing phase, Limousin- and Hereford-sired calves had greater average daily gains than Piedmontese-sired calves. A clear stratification of USDA yield grade, based on differences in carcass weight, longissimus muscle area, fat depth, and percentage kidney, pelvic, and heart fat, existed, depending on sire breed. Hereford-sired calves had more marbling than progeny of Limousin or Piedmontese sires. Schenkel et al. (2005) reported associations between polymorphisms within the gene for bovine leptin, a chemical messenger that affects feed intake, fatness (fat yield and subcutaneous fat), and tenderness.

Thomas et al. (2008) reported that beef from medium-framed, early maturing animals had the highest marbling scores, and had the highest concentration of total n-3 fatty acids, and the lowest n-6/n-3 ratio. Lynch et al. (2002) reported that meat from Hereford cattle had higher levels of C14:0, C16:1, and C18:0 in the phospholipid fraction than that from Friesian and Charolais cattle.

Breed can also have significant effects on beef flavor. Nitrogen- and sulfur-compounds, free amino acids, alcohols, aldehydes, and ketones in the flavor volatiles differ in the meat from different breeds of cattle (Sato et al. 1995; Insausti et al. 2005). Beef from Friesian cattle has a stronger fatty flavor and aftertaste, and a different volatile profile than that from Pirenaica cattle (Gorraiz et al. 2002). Enzymes, such as μ- and m-calpain, known primarily for textural changes, can influence flavor by producing peptides that make significant flavor contributions. Meat from *Bos taurus* and *Bos indicus* cattle inheriting the CC genotype at the calpastatin gene and the TT genotype at the μ-calpain gene produce steaks with more intense flavor (Casas et al. 2006). These genes correlate with increased rancid, sour, and salty flavors, and decreased umami flavor (Toldrá and Flores 2000). In addition, content of several volatile compounds, such as hexane and 2,2,4,6,6-pentamethylheptane, differs between Friesian and Pirenaica cattle (Gorraiz et al. 2002). Breed also affects beef color. Frickh and Solkner (1997) reported that beef from Holstein cattle had higher a* values (redness) than did Simmental and Simmental x Limousin cattle.

Genetic differences in swine have also resulted in pork with different quality characteristics. Since 1990, producers have dramatically improved the nutritional profile of pork, producing a product that is 31% lower

in fat, 10% lower in cholesterol and 17% lower in calories (USDA 2007). However, genetic selection for leanness has not been without unintended consequences. Pigs homozygous for the Halothane gene (nn) have higher gain: feed ratios, and their carcasses are leaner than those from Halothane negative (NN) and heterozygotic (Nn) pigs (Leach et al. 1996). While pigs carrying one or two copies of the Halothane gene have higher lean content, they are likely to produce pale, soft, and exudative (PSE) meat that has excessive drip loss because of rapid pH decline while the carcass is still hot (Sather et al. 1990). Fernandez et al. (2004) reported that NN and Nn pigs exhibited postmortem changes at the same rate, as evidenced by similar glycogen, lactate, creatine phosphate and ATP levels, and pH values at 40 minutes postmortem. Raw meat (longissimus lumborum) from nn pigs had lower visual color intensity and homogeneity scores than meat from NN and Nn pigs. Meat from nn pigs was less tender than that from NN pigs; the Nn pigs were intermediate.

Meat from pigs (Swedish Hampshire x Finnish Landrace) that are homozygous and heterozygous for the rendement napole (RN-; acid meat) allele has been shown to be juicier than that from noncarriers. The RN- allele also contributes to tenderness (Josell et al. 2003). Emnett (1999) reported that Berkshire and Chester White pigs had lower glycolytic potential (thought to be an indicator of the RN- allele) than Hampshire or Hampshire crossbred pigs. High glycolytic potential values were associated with lower pH, poorer WHC, higher cooking loss, and paler color.

Meat derived from pigs of these very different genetic backgrounds does differ in quality characteristics (Brewer et al. 2002). Ellis et al. (1996) reported that Duroc pigs produce meat that is highly marbled and has good eating quality. Brewer et al. (2004) reported that meat from Duroc/Landrace- and Large White-sired pigs was higher in fat, while that from Duroc and Duroc/Hampshire was lower in fat. Pork from Danish Landrace Duroc pigs was more tender than that from Landrace, Duroc, and various crosses with Yorkshire pigs. Blanchard et al. (1999) reported that meat from crossbred pigs that were at least half Duroc were more tender than that from Large White and British Landrace crosses. Brewer et al. (2002) also reported that pork from Duroc-sired pigs is more tender than that from Duroc/Landrace- and Pietrain-sired pigs.

Wood et al. (2004) reported that breed affected the fatty acid composition of intramuscular neutral lipid. Pork from Berkshire and Tamworth pigs (fatter carcasses) had more 14:0 and 16:0, while that from Duroc and Large White (leaner) contained more polyunsaturated fatty acids. Meat from Duroc pigs had high concentrations of 20:5n-3 and 22:6n-3.

Genetic markers for tenderness have been identified for Duroc-Landrace pigs (Rohrer et al. 2006). Chromosome 2 region 60–66 cM appears to be associated with all measures of pork tenderness and the region on chromosome 17 (32–39 cM) was associated with measures of intramuscular fat and loineye area.

Diet Effects on Meat Quality

Diet can contribute to meat quality directly (compounds from the feed source deposit in the meat) or indirectly (primarily by increasing fatness). Feeding fish byproducts, raw soybeans, canola oil, and meal can result in undesirable flavors in meat (Melton 1990). Pork fat is more likely to be affected by alteration of dietary fat source than is beef fat because pigs have little capacity to biohydrogenate unsaturated fats, depositing them in tissues in much the same form as they were consumed. Feeding pigs high levels of PUFA decreases saturation of carcass fat and has detrimental effects on pork quality (Whitney et al. 2006). Unsaturated fatty acids result in

carcass fat that is soft and oily. In addition, carcass fat that is higher in PUFA content is more susceptible to oxidation during storage than fat that contains more saturated fat. Palm oil and whole linseed supplements increase muscle levels of alpha-linolenic (C18:3) and EPA (eicosapentaenoic acid [C20:5]); fish oil increases EPA and DHA (docosahexaenoic acid [C22:6]; Elmore et al. 2004). The effects of changes in dietary fat source on pork fat are more apparent if they occur during the last few weeks before slaughter than if they occur 1 to 2 months before slaughter.

Lampe et al. (2006) reported that while finishing diet (yellow corn, white corn, 1/3 yellow corn and 2/3 white corn, 2/3 yellow corn and 1/3 white corn, or barley) altered saturated, mono- and poly-unsaturated fatty acid content in the subcutaneous fat of pigs, energy source had little effect on the eating quality of pork. However Wood et al. (2004) reported that a low-protein finishing diet increased tenderness and juiciness but decreased flavor quality of pork.

Rosenvold et al. (2001) reported that feeding finishing diets low in digestible carbohydrate can reduce muscle glycogen stores in slaughter pigs without compromising growth rate. This diet reduced μ-calpain activity and increased calpastatin activity, indicating less muscle protein degradation in the muscles compared to muscles of control animals. In an effort to improve the nutritional profile of pork, Janz et al. (2008) fed pigs a plant-based diet containing conjugated linoleic acid, selenium, and vitamin E. The dietary treatments had some effects on meat quality, but the overall effects on appearance and palatability were small.

Diet can shift the bone/muscle/fat ratio of beef carcasses. Grain feeding (high-energy diet) usually increases carcass weight and intramuscular fat content, and produces more intense flavor in red meats than do low-energy forage and grass diets (Melton 1990). The longer the animal is in the feedlot, the higher the phospholipid concentration (Larick et al. 1989). Feedlot-finished cattle have a different fatty acid profile from forage-fed cattle. Meat from forage-fed beef contains more linolenic acid, and less oleic and linoleic acids than that from concentrate-fed beef (Elmore et al. 2004). Intense pasture rotation systems of millet and grain have been shown to alter concentrations of diterpenoids and lactones (Maruri and Larick 1992). Lactones correlate positively with roasted beef flavor and negatively with gamey/stale off-flavor; diterpenoids positively correlate with gamey/stale off-flavor. Differences in oleic, linoleic and linolenic acids, diterpenoids, and lactones may be responsible for flavor differences. Nelson et al. (2004) found that adding restaurant grease to cattle diets to increase energy intake increased initial tenderness and had no effect on drip or cook loss, sustained tenderness, juiciness, and beef flavor.

Feeding antioxidants has been of significant interest with respect to maintaining postharvest meat quality (Guo et al. 2006). Vitamin E locates in the cell membrane in proximity to phospholipids. It can prevent development of free radicals in membranes ante- and postmortem (Onibi et al. 2000). Garber et al. (1996) reported that vitamin E supplementation increased muscle alpha-tocopherol levels, delaying metmyoglobin formation (beef) and lipid oxidation in a dose-dependent manner. Boler et al. (2009) found that feeding natural sources of vitamin E to finishing pigs was more effective in reducing lipid oxidation of pork during subsequent storage and display than were artificial sources. Yang et al. (2002) found that meat from pasture-fed cattle contained as much alpha-tocopherol as grain-fed cattle supplemented with 2500 IU vitamin E. It contained a higher percentage of linolenic acid, a lower percentage of linoleic acid, and was less prone to lipid oxidation and development of warmed-over flavor. Diet can also affect color of the resultant meat. Vitamin E

supplemented into swine diets has been shown to stabilize meat color and decrease fluid loss when fed at >200 mg/kd of diet during finishing (Asghar et al. 1980).

Shifting carcass bone/muscle/fat ratio can also be accomplished with steroid-like drugs. Feeding beta-agonists can have significant effects on feedlot performance and/or carcass characteristics. Quinn et al. (2008) reported that feeding ractopamine-hydrochloride to finishing heifers generally improved the efficiency of carcass gain with minimal effect on marbling score, yield grade, loin muscle area, or percentages of carcasses grading USDA Choice. Avendano-Reyes et al. (2006) reported that feeding either zilpaterol- or ractopamine-hydrochloride considerably improved gain-to-feed ratio, hot carcass weight, and carcass yield. Zilpaterol increased loin muscle area. Both beta-agonists decreased meat tenderness compared with controls. Smith et al. (2007b) reported that implanting anabolic steroids increased hot carcass weight and loin muscle area for both heifers and steers. However, implants had no effect on dressing percent, fat thickness, yield grade, marbling score, intramuscular lipid content, or concentrations of major fatty acids.

Montgomery et al. (2004) reported that supplementation of three biological types of cattle (Bos indicus, Bos Taurus-Continental, Bos Taurus-English) with vitamin D3 (0.5 million IU/d) for 8 days prior to slaughter improved tenderness by affecting muscle Ca^{++} concentrations, calpain activities, and muscle proteolysis.

Marbling Effects on Meat Quality

A high plane of nutrition, especially during the finishing phase, can increase intramuscular fat to a greater or lesser degree depending on species, breed, animal age, and a variety of other factors. The fatness and marbling associated with a high plane of nutrition have been used as indicators of meat quality. Highly marbled meat has traditionally been thought to be the ideal because of the effects of fat on flavor and tenderness. However, Rincker et al. (2008) reported that intramuscular fat (0.8–8.0%) explained less than 15% of the variance in pork flavor scores. Consumers could tell no difference in pork flavor scores until the fat content reached 4.5%. In addition, visible fat content in pork is a major determinant of purchase intent with consumers preferring leaner products (Brewer et al. 2001; Rincker et al. 2008). Fernandez et al. (1999) reported that pork texture and taste are enhanced at intramuscular fat levels up to 3.25%, but inconsistent effects occurred with respect to tenderness/toughness.

Ellis et al. (1996) reported that longissimus muscle from pig genotypes selected for the propensity to increase marbling are more tender and juicy, and have lower shear values. The Duroc breed produces pork that is highly marbled with good eating quality (Ellis et al. 1996). Brewer et al. (2002) reported that chops from Duroc and Pietrain pigs had the most visible marbling, while those from Duroc/Landrace and Large White had the least. Chops from Duroc, Duroc/Hampshire, and Pietrain pigs had the highest fat content. Meat from these breeds, however, differs from other breeds with regard to muscle fiber type and the incidence of PSE (Chang et al. 2003).

Cattle breeds with different growth rates but the same degree of marbling differ substantially in tenderness and Warner Bratzler shear value (Chambaz et al. 2003). Historically, selection of beef breeds has been based on marbling, irrespective of growth rate and simultaneous selection pressure for reduced overall fat deposition.

Postmortem pH Decline

Postmortem biochemical changes dramatically affect tenderness and flavor. The loss of

circulatory competency after harvest requires that the tissues shift to anaerobic metabolism, resulting in the accumulation of metabolic byproducts, including lactic acid, in the muscle. The pH declines from about 6.8 to 5.7. Endogenous thiol proteinases (cathepsins B and L) become activated near pH 5.4. They are redistributed (intracellularly) during aging (Spanier et al. 1990; Spanier and Miller 1993). Proteolytic enzyme activity is temperature-dependent; some (cathepsins B and L) retain high activity levels even at cooking temperatures (70°C). Pigs with defects in the ryanodine receptor gene (rn+) undergo excessive (not necessarily rapid) pH decline, resulting in abnormally acidic conditions in the meat, which affects water-holding capacity, tenderness, and color (Leach et al. 1996; Bidner et al. 2004).

Water-holding capacity (WHC) is the ability of meat to hold onto its own or added water when force (heat, pressure) is applied. Water is the major component (about 75%) of muscle tissue. Most exists in layers around polar molecules and between layers of cellular materials. The majority is located in the intermolecular spaces between the salt-soluble proteins (actin, myosin) of muscle tissue, which varies depending on various intrinsic and extrinsic factors (Offer and Knight 1988). Its movement is restricted in a number of ways that are dependent primarily on the myofilaments. Some of the factors that alter the spatial arrangement of the myofilaments include alterations in net charge induced by pH changes, screening of charges by anions/cations, presence of divalent cations (Mg^{++}, Ca^{++}), denaturing conditions that alter protein conformation (rapid pH decline while the carcass temperature is still high), and presence of plasticizing agents such as ATP and enzymes (ATPase).

In pre-rigor meat, Mg-$ATP^{=}$ serves to prevent cross-linking between the contractile proteins, actin and myosin (Fig. 2.1). This maintains the interfilamental space such that water can move in (Siegel and Schmidt 1979). During the immediate postmortem period, tissues metabolize glycogen via anaerobic pathways, lowering pH. ATP is rapidly consumed, but as reducing equivalents are consumed, it is not regenerated. Without the plasticizing effect of ATP, actin and myosin cross-link, the sarcomere shortens, fibers contract, and rigor results. During the rigor process, muscle cells undergo both longitudinal and lateral contraction, usually within 24 hours. WHC decreases during the postmortem period. Rigor mortis occurs in beef when the pH drops to 5.9 (Honikel et al. 1981). Factors that affect the rate of pH decline, such as Halothane gene status of pigs and residual glycogen in the tissues, affect tenderness, WHC, and color. Factors that affect the ultimate pH (ryanodine gene status, stress that alters muscle glycogen content) also affect these characteristics.

The peak solubility of actin and myosin occurs between pH 5.7 and 6.0 (Scopes 1964). It decreases dramatically as pH drops from 6.0 to 5.6. These proteins are almost completely insoluble below pH 4.9. Sarcoplasmic proteins are soluble between 4.8 and 5.2, regardless of temperature; however, at or above 37 °C, even high pH will not prevent them from precipitating onto myofibrillar proteins. This decreases WHC as well as other quality characteristics of meat. The minimum water-holding capacity of meat occurs around pH 5.0, which corresponds to the isoelectric point of actomyosin. In addition, toughness is negatively correlated with initial pH and rate of pH decline (Zamora et al. 1996). Two-thirds of the WHC losses occurring during rigor are due to loss of ATP, with the remainder due to pH decline. The rate of pH decline is partially genetic, in that pH decreases more rapidly in meat from some breeds, because of the fiber-type distribution in the muscle tissue, than it does in meat from other breeds. Brewer et al. (2002) reported that carcasses from Duroc and Large White pigs experienced postmortem purge losses of 5–6%, while those from Pietrain,

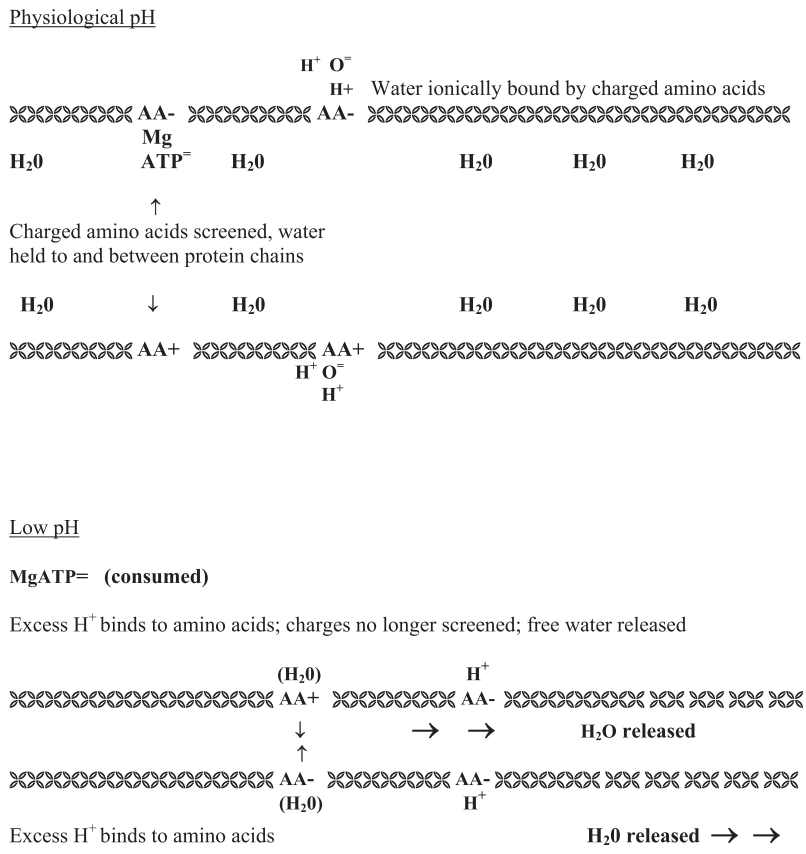

Figure 2.1. Effect of excess hydrogen ion (pH decrease) on water located in muscle tissue.

Duroc/Landrace, and Duroc/Hampshire experienced purge losses of 12–13%. Genetics appears to play a significant role in WHC.

In addition to pH decline, alterations in carcass temperature can have significant effects on meat quality (tenderness and WHC). Loss of circulatory and respiratory competencies at slaughter allows accumulation of metabolic heat. Carcass temperatures can increase to over 42 °C during the first 45–60 minutes postmortem. At this temperature, a rapid pH decline can result in denaturation of myofibrillar proteins such that WHC is ultimately quite low, even if ultimate pH (24 h) is within normal ranges. Rapid postmortem glycolysis is associated with the high drip loss, poor WHC, and pale color of pale, soft exudative (PSE) pork (Bendall and Wismer-Pedersen 1962). Development of the PSE condition may also be due to denaturation and precipitation of sarcoplasmic proteins onto myofibrillar proteins (Joo et al. 1999). The genetic profile of pigs that produce PSE pork is advantageous for production reasons. Brewer et al. (2002) reported that chops from Duroc-sired pigs were more tender than those from Duroc/Landrace- and Pietrain-sired pigs. Brewer et al. (2002) reported similar effects on "texture" of chops from Halothane positive (nn) and negative (NN) Pietrain, RN- Hampshire, rn+ Hampshire, Berkshire, and Duroc lines of pigs.

Hambrecht et al. (2005) reported that high stress conditions (long transport, short lairage) decreased muscle glycolytic potential and increased plasma lactate, cortisol, muscle temperature, rate of pH decline, ultimate pH, and b* values (yellowness) of pork. Other color measures were unaffected by high stress but water-holding properties were impaired. Because supplemental dietary magnesium is related to postmortem glycogen breakdown of lactic acid and concomitant muscle pH decline, it has been shown to help offset damage to color and water-holding capacity that result from the stress involved in transport and handling (Frandson and Spurgeon 1992). Feeding swine magnesium during the finishing phase results in higher initial and/or ultimate muscle pH values and a decrease in the incidence of PSE (D'Souza et al. 1998; Swigert et al. 2004).

Flavor

Meaty Flavor

"Flavor" results from the combination of the basic tastes (sweet, sour, bitter, salt, umami) derived from water-soluble compounds and odors derived from a variety of substances present in the raw meat. Flavor- and odor-active volatiles include alcohols, aldehydes, aromatic compounds, esters, ethers, furans, hydrocarbons, ketones, lactones, pyrazines, pyridines, pyrroles, and sulfides (Shahidi 1994). The relationship between some of the more common volatiles and their respective flavors is shown in Table 2.1.

The lipids present in muscle tissue (subcutaneous fat, intramuscular fat, intermuscular fat, intramyocellular lipid, and structural phospholipids) at slaughter serve as a source of many of these flavor constituents. These lipids are composed of fatty acids that may be saturated, unsaturated and/or methyl-branched (Fig. 2.2). They may be derived directly from the diet, produced as the result of biohydrogenation of dietary lipids, or via endogenous synthesis. Increased marbling, because of the increased amount of fat available for formation of flavor compounds, has traditionally been considered to have a relatively large impact on the ultimate flavor of the meat product.

"Meaty flavor," the generic background flavor of all types of red meat, is associated with the lean portions of meat. Phospholipids (0.5–1% of the lean tissue) contain a high proportion of fatty acids with four or more double bonds (C18:4, C20:4, C20:5, C22:5, C22:6; Table 2.2) that are susceptible to oxidation and likely to make specific flavor contributions to the meat (Elmore et al. 1999). Endogenous antioxidant enzymes, especially catalase and GSH-Px, can potentially delay the onset of oxidative rancidity (Pradhan et al. 2000). Some meat processing operations reduce the activity of these systems (Decker and Mei 1996). Of the 60-plus compounds that contribute specifically to "meaty" aromas, most are sulfur- or carbonyl-containing compounds (Shahidi 1994). Phospholipids are also the source of several sulfides that are generated when they react with cysteine and/or ribose to produce mild, slightly meaty-flavor/odor compounds, such as 2-methyl-3-[methylthio]thiophene (Rowe 2002).

Species-Specific Flavor

Species-specific flavor has traditionally been associated with the lipid portion of meat. It may result from quantitative differences of several compounds (3,5-dimethyl-1,2,4,trithiolane, 2,4,6-trimethylperhydro-1,3,5-dithiazine, mercaptothiophenes, mercaptofurans; Shahidi et al. 1994). A beef-like aroma compound, 12-methyltridecanal, is an important contributor to species flavor (Mottram et al. 1982). It occurs in much smaller amounts in species other than beef. Other species-specific flavor compounds include 2-methyl-3-[methyl]-furan and

Table 2.1. Flavors and aromas associated with volatile compounds in meat

Compound	Flavors and Aromas
Pentanal	Pungent
Hexanal	Green, grassy, fatty
Heptanal	Green, fatty, oily
Nonanal	Soapy
Methional	Cooked potato
12-methyltridecanal	Beefy
Nona-2(E)-enal	Tallowy, fatty
Deca-2(E), 4(E)-dienal	Fatty, fried potato
Butanoic Acid	Rancid
Hexanoic Acid	Sweaty
3-Hydroxy-2-butanone	Buttery
2-propanone	Livery
2,3-Octanedione	Warmed over flavor, lipid oxidation
1-Octen-3-ol	Mushroom
2-Pentyl furan	Metallic, green, earthy, beany
2-methyl-3-[methylthio]furan	Meaty, sweet, sulfurous
4-hydroxy-5-methyl-3(2H)-furanone (HMF)	Meaty
Pyrazines	Nutty, cracker-like, roasted
Amino acids: glycine, alanine, lysine, cysteine, methionine, glutamine, succinic	Sweet
Organic acids: lactic, inosinic, ortho-phosphoric, and pyrrolidone carboxylic	Sweet
Amino acids: aspartic acid, histidine, asparagines	Sour
Organic acids: succinic, lactic, inosinic, ortho-phosphoric, pyrrolidone carboxylic	Sour
Hypoxanthine, anserine, carnosine	Bitter
Amino acids: arginine, leucine, tryptophan	Bitter
Monosodium glutamate (MSG), inosine and guanosine monophosphate (IMP,GMP)	Savory, brothy, beefy.
Bis(2-methyl-3-furyl) disulfide	Roasted meat
2-methyl-3-furanthiol	Roasted meat
4-hydroxy-5-methyl-3(2H)-furanone (HMF)	Meaty
4-hydroxy-2,5-dimethyl-3(2H)-furanone	Meaty
3-hydroxy-4,5-dimethyl-2(5H)-furanone	Meaty

MacLeod and Ames, 1986; Ha and Lindsay, 1991; Spanier et al., 1992; Spanier and Miller, 1993; MacLeod, 1994; Imafidon and Spanier, 1994; Maga, 1998; Mottram, 1998; Shahidi, 1998; Rowe, 2002; Gorraiz et al., 2002.

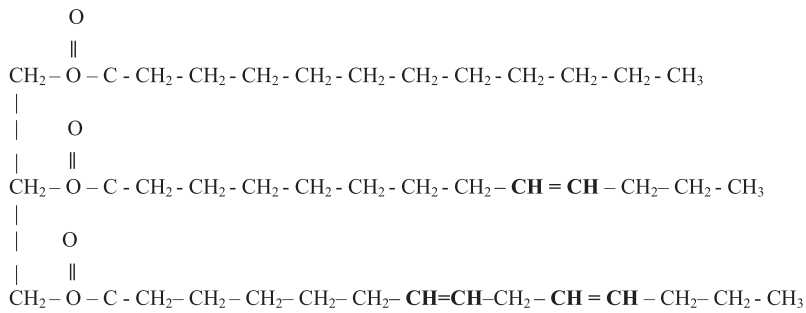

Figure 2.2. Triglyceride with saturated, mono-unsaturated, and poly-unsaturated fatty acid.

Table 2.2. Fatty acid composition of selected types of meat[1]

Total lipid g/100 g	Total sat. fatty acids	12:0	14:0	16:0	18:0	16.1	18.1	20:1	22:1	18:2	18:3	18:4	20:4	20:5 n – 3	22:5 n – 3	22:6 n – 3
Chicken[2]																
Breast 3.57	1.01	0	0.3	0.69	0.25	**0.15**	**1.03**	0.03	0	**0.59**	0.03	0	0.06	0.01	0.01	0.02
Dark 9.73	2.66	0.03	0.07	1.84	0.63	**0.49**	**2.97**	0.05	0	**1.87**	0.09	0	0.14	0.01	0.03	0.05
Turkey																
Breast 3.46	2.10	0	0.01	0.05	1.28	**0.40**	**1.98**	0.01	0.01	**1.45**	0.08	0	0.16	0	0	0
Dark 7.22	2.45	0.02	0.05	1.28	0.72	**0.24**	**1.35**	0.03	0.02	**1.75**	0.07	0	0.26	0	0.04	0.06
Beef[3]																
3.54	1.31	0	0.09	0.78	0.43	**0.11**	**1.31**	0	0	**0.12**	0.01	0	0.02	0	0	0
Pork[3]																
3.53	1.21	0.01	0.45	0.76	0.38	**0.10**	**1.42**	0.02	0.30	**0.30**	0	0	0	0	0	0
Lamb[4]																
9.23	3.30	0.02	0.24	1.79	1.10	—	—	—	—	**0.63**	**0.12**	0.09	—	—	—	—
Ocean Perch																
2.09	0.31	0	0.08	0.18	0.04	**0.10**	**0.27**	0.13	0.29	**0.04**	0.0	0.03	0.01	0.10	0.03	0.30
Atlantic Salmon																
12.35	2.50	—	0.57	1.90	0.32	**0.77**	**2.05**	1.37	—	**0.67**	—	—	1.27	0.69	—	1.46
Tuna																
5.97	0.95	0.009	0.011	0.152	0.051	**0.025**	**0.018**	0.007	0.014	**0.008**	**0.012**	0.005	0.028	0.037	0.013	0.18

[1] Source: USDA National Nutrient Database for Standard Reference, Release 20 (2007) http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/

[2] Chicken, broilers or fryers, separable fat, raw; contains less than 0.5 g 4:0, 6:0, 8:0, and 10:0

[3] Beef, top sirloin, separable lean only, trimmed to 1/8″ fat, select, raw; contains less than 0.5 g 4:0, 6:0, 8:0, and 10:0

[4] Lamb, domestic, rib, separable lean only, trimmed to 1/4″ fat, choice, raw

3-methylcyclopentanone (Imafidon and Spanier 1994). Methyl-branched compounds appear to arise from phosphoglycerides (Werkoff et al. 1993; Mottram 1998). These compounds are affected by diet, breed, and muscle.

Muscles vary in their concentrations of compounds important to meat flavor/odor. Stetzer et al. (2008) reported that beef *Complexus* contained twice the concentration of 2,3-octanedione, nonanal, and butanoic acid, and 30% more hexanoic acid than the *Gludeus medius*, *Rectus femoris*, *Vastus lateralic*, *Vastus medialis*, *Psoas major*, and *Longissimus dorsi*.

Off-Flavors

Muscle tissue also contains compounds that contribute to off-flavors in the finished product as a result of genetics, sex of the animal, heme content of the muscle tissue, and diet. Livery flavor is an objectionable, off-flavor in beef that increases as iron content increases (Campo et al. 1999; Calkins and Cuppett 2006; Yancey et al. 2006). Sulfur-containing compounds (thiols, sulfides, thiazoles, sulfur-substituted furans) can interact with carbonyl compounds to produce a livery flavor (Werkhoff et al. 1993). Muscles often exhibiting liver-like flavor, such as the *Psoas major* (loin) and *Gluteus medius* (round), have higher levels of heme iron and/or myoglobin (Yancey et al. 2006). Compared with beef *Infraspinatus*, *Psoas major*, and *Rectus femoris*, the *Gluteus medius* had the highest liver off-flavor score (Stetzer et al. 2007). Of the *Complexus*, *Serratus ventralis*, *Vastus lateralis*, *Vastus medialis*, and *Longissimus dorsi*, the *Vastus lateralis* had the highest liver off-flavor score and the *Longissimus dorsi* had the lowest (Stetzer et al. 2006). Stetzer et al. (2008) reported that livery off-flavor was positively correlated with pentanal, hexanal, 3-hydroxy-2-butanone, and hexanoic acid.

Sex and carcass maturity also affect off-flavors. Beef from bulls has a more livery, bloody flavor than that from heifers, which appears to be related to higher 2-propanone and ethanol contents (Gorraiz et al. 2002). To the extent that carcass maturity affects iron content, it can increase metallic, rancid, bloody, salty, and bitter flavor notes (Calkins 2006). Volatile compounds impact these flavor notes as well. Higher concentrations of phospholipids, phosphatidylcholine, and phosphatidylethanolamine increase livery and ammonia flavors in beef (Larick et al. 1989). Several muscles (*Triceps brachii*, *Vastus lateralis*, and *Vastus intermedius*) with livery off-flavor have more heptanol, hexanal, hexanol, B-pinene, 1-octene-3-ol, and nonanal.

Because of their effects on desirable and undesirable flavor components, diet, animal sex, age at slaughter, genetics, and muscle must be considered when meat tissues are to be used for specific products (fresh, whole cuts vs. cured, smoked products).

Factors Affecting Tenderness/Texture

In general, consumers rate tenderness as the major factor that determines the eating quality of meat (Brewer and Novakofski 2008). Tenderness embodies all the mouth feel characteristics perceived kinesthetically: those perceived prior to mastication (particle size, oiliness), during mastication (tenderness, juiciness), and after mastication (fibrous residue, mouth coating; Bourne 1992). Tenderness is composed of mechanical (hardness, cohesiveness, elasticity), particulate (grittiness and fibrousness), and chemical components (juiciness and oiliness; Bourne 1992). Minimally, meat tenderness is affected by myofibrillar, connective tissue, and compositional components. The myofibrillar component can be affected by cold shortening and proteolytic degradation; the

connective tissue component can be affected by animal age, degree of activity, mechanical tenderization, and composition (Pearson and Young 1989). Muscle foods have an inherent set of textural characteristics associated with them by the nature of the raw material. These include fibers, fluid/fat exudation, and connective tissue. Textural parameters of interest are those that are affected by these raw materials characteristics as well as those that are affected by exogenously induced alterations (formulation, aging).

Tenderness of the final product depends on the muscle(s) from which the meat was derived. Beef *Psoas major* was more tender than the *Gluteus medius*, *Infraspinatus*, and *Rectus femoris* (Stetzer et al. 2007). Of the *Complexus*, *Serratus ventralis*, *Vastus lateralis*, *Vastus medialis*, and *Longissimus dorsi*, the *Longissimus dorsi* was the most tender and the *Vastus lateralis* was the least (Stetzer et al. 2006). In general, meat that is the most tender is derived from muscles that were least used when the animal was alive, while meat that is the most tough is derived from muscles that are used the most (locomotor, postural). However, both genetics and age affect tenderness. Meat from two-year-old Angus/Wagyu heifers was as tender and juicy as that from yearlings. However, meat from two-year-old pure Angus lines was less tender and juicy than that from yearlings or that from Angus/Wagyu animals (Rentfrow et al. 2004).

Aging

Aging Effects on Tenderness

Sarcomere length, muscle, connective tissue proteins, and proteolytic degradation account for most of the variation in tenderness (Koohmaraie et al. 2002). Tenderness depends, in part, on proteolytic degradation of structural and myofibrillar proteins (Koohmaraie et al. 2002). Large variation in aging-induced improvement occurs among animals and among muscles within an animal; this may relate to initial tenderness (Novakofski and Brewer 2006; Stolowski et al. 2006). A major factor in this variation is high growth rate that requires a high plane of nutrition. During growth, rapid protein turnover increases proteolytic activity, which contributes to the aging process (Zgur et al. 2003). This increased proteolytic activity enhances aging because proteolytic cathepsins degrade some structural proteins, allowing the sarcomere to relax (Kristensen and Purslow 2001). This allows the inflow of water previously expelled during rigor. This inflow may be driven by the difference in protein concentration existing between intra- and extracellular compartments of the muscle cell.

Tenderness improvement with aging varies between animals within a breed, and between muscles within an animal. It depends on several factors that may also be related to initial tenderness (Wicklund et al. 2005; Novakofski and Brewer 2006). Wicklund et al. (2005) reported that changes in tenderness of strip steaks required 14 days of aging. Novakofski and Brewer (2006) reported that the mean improvement in shear with aging over the first week differed depending on the shear value starting point (original shear value); however, no differences occurred between 7 and 14 days. Rentfrow et al. (2004) reported that Warner Bratzler shear values decreased and tenderness increased in beef from one- and two-year-old heifers during aging; however, maximum improvement occurred after only 7 days of aging. Bruce et al. (2005) indicated that aging for up to 14 days increased tenderness.

Aging Effects on Flavor

The effects of aging on flavor are unclear (Mottram 1998). It can alter the makeup of the aroma and flavor precursors, which ultimately affects the characteristics of the cooked product. Aging can increase carbon-

yls derived from lipid oxidation, which may contribute to off-flavors, decrease flavor identity, and increase metallic flavor (Yancey et al. 2005). It can also increase fatty flavor and negative attributes such as painty, cardboard, bitter, and sour (Spanier et al. 1992; Gorraiz et al. 2002; Bruce et al. 2005). Positive flavor compounds, such as 3-hydroxy-2-butanone, 2-pentyl furan, 2,3-octanedione, and 1-octene3-ol, decrease with aging; and negative compounds, such as pentanal, nonanal, and butanoic acid, increase with aging (Stetzer et al. 2008). Aging beef can result in changes in umami taste. Glutamic acid content more than doubles during the first 7 days of aging (Bauer 1983).

The potential benefits of aging for selected muscles for flavor development and tenderization must be weighed against the potential development of off-flavors.

Color

Color and appearance of fresh meat are major factors in consumer purchase decisions because they are presumed to be indicators of meat freshness and quality (Brewer et al. 2002). Meat color is due to the concentration of heme pigments (myoglobin, hemoglobin), their chemical states, and the light-scattering properties of the meat (Lawrie 2002). At high pH, the heme iron is predominantly in the ferrous state (Fe^{2+}); low pH accelerates ferrous iron conversion to the ferric state (Fe^{3+}, Table 2.3). Oxygen can bind to heme iron only if it is in the ferrous state (Fe^{2+}). However, many other ligands (CN, NO, CO, N_3) can bind to either the ferrous (Fe^{2+}) or ferric (Fe^{3+}) form. Water (H_2O) can bind to myoglobin (Mb) only if the iron is in the ferrous form. Under low oxygen tension conditions, Mb exists in the purple-colored, reduced form (Fe^{2+}). Exposed to oxygen for a short period of time, the central iron (Fe^{2+}) reversibly binds oxygen, producing oxymyoglobin (MbO_2), which is bright pink or red. However, when exposed to O_2 for an extended period, the central iron atom can lose an electron (oxidized to Fe^{3+}), producing metmyoglobin (MetMb), which is grey-brown. Immediately post slaughter, the oxidized form can be reduced by endogenous reducing systems in the meat, as long as reducing equivalents (NADH) are available and the globin fraction is in its native state (undenatured). Over time, these reducing equivalents are depleted and the pigment is irreversibly oxidized. Oxidation also occurs rapidly if the globin moiety is denatured by rapidly declining pH while the carcass is "hot" or by excessively low ultimate pH.

In pigs, color variations may have been inadvertently selected for as pigs were bred for high gain/feed ratios and leanness. Brewer et al. (2002) reported that genetic line had significant effects on a* value (redness), which ranged from 9.2 to 11 (on a 15-point scale) among pigs from genetic lines known

Table 2.3. Characteristics of various states of myoglobin

Pigment	Ligand	Conditions	Iron State	Color
Deoxymyoglobin	H_2O	Very low oxygen tension (<5 mm Hg). H_2O is ionically (reversibly) bound to Fe^{++}	Fe^{++}	Purple-red/purple-pink
Oxymyoglobin	$:O_2$	High O_2 tension (70–80 mm Hg). O_2 is covalently bound to Fe^{++}	Fe^{++}	Bright red/bright pink
Metmyoglobin	—	Low O_2 tension (~10 mm Hg). An electron is lost from Fe^{++}	Fe^{+++}	Brown/grey
Carboxymyoglobin	CO:	CO is preferentially bound to Fe^{++} (compared to O_2). Stable during storage and heating	Fe^{++}	Bright red

to suffer from color defects (Halothane+, Pietrain, and RN-Hampshire). Because color is a function of rate of pH decline, genetics can influence both the absolute color (dark pink, pale pink) and the evenness of the color. Visual two-toning was higher in pork loins from Duroc cross lines (Duroc/Landrace and Duroc/Hampshire) than in those from Pietrain, RN- Hampshire, rn+ Hampshire, Berkshire, and Large White carcasses. Pork from Pietrain-sires was lightest and least pink. The color defects observed in PSE pork are also linked to postmortem pH decline and carcass temperature. The abnormal color that occurs in PSE pork has been duplicated in a model system by Friese et al. (2005) by holding chops for 60 to 120 minutes at 42 °C or higher. Chops with a pH <5.8 lightened until L* values (lightness) were similar to those typical of PSE pork.

PSE pork is undesirable to packers because of high drip/purge loss, which represents value lost as "shrink," while the light color makes PSE pork less appealing to consumers (Brewer and McKeith 1998). Pigs do differ in susceptibility to PSE regardless of genotype. Variation in fiber type appears to account for some of this variation because of myosin isoform-specific differences in denaturation characteristics (Depreux et al. 2002).

Animal age and muscle from which the meat is derived also affect color. L* values of beef from the *Psoas major* were significantly higher (lighter) than those from the *Longissimus lumborum* and the *Gluteus medius* (Rentfrow et al. 2004). L* values of the latter were higher in meat from yearling heifers than in that from two-year-old heifers.

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Chapter 3

Meat Decontamination

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Introduction

Food animals, whether infected or asymptomatic carriers, are sources of spoilage and pathogenic microorganisms. More specifically, animals carry microorganisms on their external surfaces, including the gastrointestinal tract, which contaminate the environment as well as food and water, forming a complete cycle. In general, external animal surfaces, as well as their feces and the environment, may serve as sources of contamination for: (i) carcasses during the slaughtering, dressing, chilling, and cutting processes; (ii) meat products during processing, storage and handling; (iii) water and other foods through contaminated manure; or (iv) direct transfer and infection of humans (Sofos 2002). More specifically, sources of microbial contamination for meat include air, water, feces, knives used during exsanguination and cutting, hides, fleece, feathers, the gastrointestinal tract through accidental spillage of its contents during evisceration, and lymph nodes if inspected by incision or otherwise cut. Furthermore, contact with other carcasses, employees, as well as the processing environment (e.g., equipment, water, or air) may also contribute to carcass and meat contamination (Sofos 1994, 2002; Sofos et al. 1999a, b, c; Elder et al. 2000; Childs et al. 2006). Although multiple species of microorganisms may be introduced onto the carcass during hide removal (Bell 1997), the majority of these microorganisms consist of nonpathogenic spoilage bacteria and indicator microorganisms, such as coliforms and *Escherichia coli* (at levels 10 to 10^7 CFU/cm^2 or higher). However, there is also a potential for contamination with pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Campylobacter* spp., and others (Gill and Bryant 1992; Sofos et al. 1999a; Bacon et al. 2000; Reid et al. 2002; Koutsoumanis and Sofos 2004; Koutsoumanis et al. 2006).

Reduction of pathogen contamination levels in meat products could reduce the burden of food-borne diseases, and thus, decrease the estimated $12 billion annual economic losses in medical costs, lost productivity, recalls, legal fees, and loss of businesses in the United States (Buzby et al. 1996; Stopforth and Sofos 2005). Therefore, there is increased interest in improving the microbiological status of meat. The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) has declared *E. coli* O157:H7 an "adulterant" in ground beef and other nonintact beef products and has issued a zero-tolerance policy that requires removal of all visible contamination, including feces, ingesta, and udder contents, from beef carcasses by knife-trimming or steam-vacuuming prior to washing and chilling (Kochevar et al. 1997a; Bacon et al. 2000). Furthermore, FSIS has changed the meat and poultry inspection regulations to require: (i) establishment of sanitation standard operating procedures (SSOP); (ii) operation under the hazard analysis critical control point (HACCP) system; (iii) microbial testing of

carcasses for *E. coli* for verification of the effectiveness of control measures against fecal contamination; and (iii) establishment of microbiological performance standards for *Salmonella* prevalence as a means of tracking pathogen reduction (USDA-FSIS 1996c; Sofos et al. 1999a, b, c, d; Rose et al. 2002). The need for compliance with zero tolerance and microbiological criteria imposed by regulatory authorities or the industry, as well as the fact that knife-trimming may not be adequate for efficient removal of microbial contamination, resulted in evaluation and commercial application of washing and decontamination treatments (Smulders and Greer 1998; Sofos and Smith 1998; Sofos 2005; Stopforth and Sofos 2006) before slaughtering, during slaughtering at the pre- and post-evisceration stage, during chilling, and post-chilling (Fig. 3.1).

Decontamination treatments may be physical or chemical in nature, while the combination of both as multiple interventions is also used (Smulders and Greer 1998; Sofos and Smith 1998; Bacon et al. 2000; Geornaras and Sofos 2005; Kalchayanand et al. 2008). Physical methods aim to mechanically remove soil from the external surfaces of animals (e.g., hides) or carcasses, as well as to reduce microbial populations. They include animal washing/cleaning and/or hair-trimming before slaughtering, dehairing and defeathering, knife-trimming, and washing of carcasses, as well as thermal treatments, such as use of steam/hot water-vacuum generating equipment for spot-cleaning, "steam pasteurization," or spraying with hot water. Chemical treatments involve the application of organic acid or other chemical solutions for chemical dehairing and as rinses for contamination reduction. Thermal treatments (hot water and steam) or organic acid solutions are commonly used alone or in combination (Sofos 2005). Alternative decontamination methods/agents include ionizing radiation, ozonated water, nisin, gluconic acid, lactoferrin, high hydrostatic pressure, sonication and pulsed light, or pulsed electric fields (Cutter and Siragusa 1994a; Reagan et al. 1996; Naidu and Bidlack 1998; Sofos and Smith 1998; Huffman 2002; Castillo et al. 2003; Sofos 2005; Aymerich et al. 2008; Kalchayanand et al. 2008). However, most of these alternatives are still under investigation and have not been applied in practice yet. The main focus of the following paragraphs is to discuss commercially applied decontamination interventions on animals and carcasses.

Animal Washing

Before slaughter, internal tissues of healthy animals are considered sterile (Sofos 1994). Microbial contamination of meat usually starts during conversion of live animals into carcasses and meat by the slaughter/dressing process and more specifically by the removal of the hide, pelt or feathers, and viscera. Contamination is an unavoidable problem, which may occur even in the best-managed slaughter facilities. Nevertheless, highly soiled animals with long wool and visible fecal contamination are expected to introduce in the slaughter plant higher microbial populations than shorn and "clean" animals (Biss and Hathaway 1995; Hadley et al. 1997; Duffy et al. 2005; Childs et al. 2006). Therefore, presentation of clean animals for slaughter is desirable because it reduces the likelihood of pathogen presence and transfer onto carcasses (Biss and Hathaway 1995; Hadley et al. 1997; Bolton et al. 2002; Arthur et al. 2004; Duffy et al. 2005).

A first step in efforts to minimize sources of carcass contamination at slaughter is to wash animals before knife incision (Sofos and Smith 1998). Pre-slaughter washing of sheep is a common intervention in New Zealand (Biss and Hathaway 1995). In addition, Australia has adopted washing of cattle, which is also practiced in certain slaughter plants in the United States (Sofos 2002). The outcome of animal washing is variable and

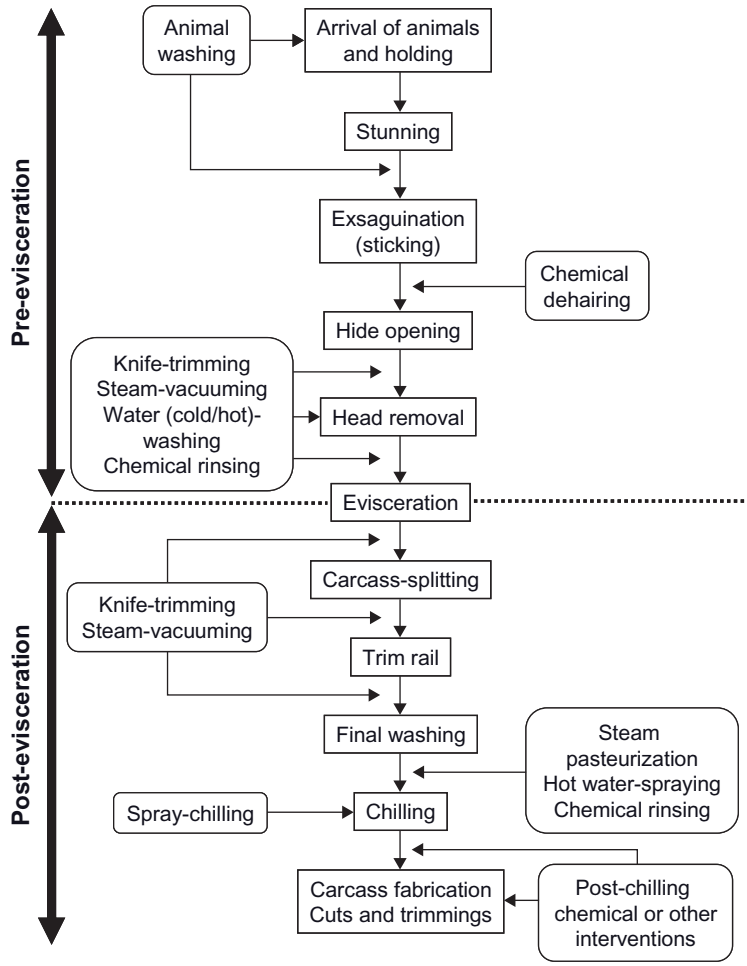

Figure 3.1. Stages of beef-slaughtering dressing process and points where various physical or chemical decontamination interventions may be applied. Based on Bell (1997); Gill and Bryant (1997b); and Edwards and Fung (2006).

depends on climate, type of animal, and availability of facilities (Sofos and Smith 1998; Kain et al. 2001). Indeed, although animal washing may remove almost all visible contamination from animal surfaces, it may have limited effectiveness (<1 $\log_{10}$ CFU/cm^2) in reducing microorganisms (Biss and Hathaway 1995; Kannan et al. 2007). A concern is that it may release microorganisms from feces and redistribute microbial contamination, resulting in washed animals carrying higher microbial loads than unwashed animals (Biss and Hathaway 1995; Mies et al. 2004). For instance, single or double pre-slaughter washing with water or chemicals (e.g., lactic acid and chlorine) increased the percentage of positive *Salmonella* samples on hides of live cattle from 35–60% pre-treatment to 40–72% after treatment (Mies et al. 2004). However, other studies have demonstrated promising results against pathogen contamination. For instance, power-hosing of pigs or cattle for 1–3 minutes, upon arrival at the abattoir, with

potable water (19°C) did not reduce the number of bacteria, but reduced the incidence of *Salmonella* on the neck, belly, and ham areas of live animals, from 27% (before washing) to 10% (Bolton et al. 2002), and caused 3 $\log_{10}$ CFU/cm^2 reductions of artificially inoculated *E. coli* O157:H7 (Byrne et al. 2000).

U.S. regulatory guidelines require cattle to be dry, or at least not dripping, when they are slaughtered (Reed and Kaplan 1996; Sofos and Smith 1998), which can be a constraint when animal washing is considered before slaughter. Nevertheless, when animals are wet or excessively soiled, slaughter speeds should be reduced to minimize accidental transfer of contamination from the exterior of the animals onto the carcass or the plant environment (Sofos 2002). Furthermore, modifications in the steps involved in hide removal, or in equipment used for hide removal, may help in minimizing transfer of contamination onto the carcass surface (Hadley et al. 1997). Considering the above as well as the reported low magnitude of microbial reduction achieved by this intervention, animal washing, is mostly accepted as a means to improve visual appearance, due to removal of visible contamination of animals presented in a "dirty" state, rather than to enhance the microbial quality of meat (Bolton et al. 2002). Van Donkersgoed et al. (1997) found poor correlation between coliform and *E. coli* counts on carcasses with the presentation status of animals before slaughtering (e.g., score and surface wetness) and the slaughtering speed, suggesting that there is significant variability in factors affecting carcass contamination.

Poor sanitation, hygiene and manufacturing practices pre-harvest, as well as during slaughtering, fabrication, and processing may lead to excessively contaminated meat, even when less heavily soiled animals are processed. Especially, pre-harvest practices play a key role on the microbial contamination of the external animal surfaces. Studies have shown that *E. coli* O157:H7 serotypes present on animal hides matched those on the feedlot and transportation trailers (Childs et al. 2006; Woerner et al. 2006). Thus, ensuring hygienic transportation and handling of animals prior to slaughtering, followed by hygienic slaughtering practices, are likely more essential than simply improving the presentation status of animals (Duffy et al. 2000; Kain et al. 2001; Childs et al. 2006; Woerner et al. 2006). An effective practice, for example, could be the combination of animal washing with separation of washed or clean from unwashed animals and application of pre-evisceration decontamination treatment with chemicals. Such practice has been shown to improve the microbial quality of cattle carcasses by reducing aerobic plate count (APC) levels and the prevalence of *E. coli* O157:H7 from 56% to 34% (Bosilevac et al. 2004b). However, physical separation of the processing of highly contaminated from that of clean animals may be impractical in some systems of animal production, marketing, distribution, and slaughtering (Gill 1998).

Other methods for reducing microbial contamination on external animal surfaces, include hide-on multiple interventions with chemicals, such as chlorinated water, or steam of subatmospheric pressure (at 75–80°C), on shackled animals before the dehiding process (i.e., after stunning or exsanguination; McEvoy et al. 2001, 2003; Bosilevac et al. 2004b, 2005). Other examined chemicals include cetylpyridinium chloride (CPC; 1%), sodium hydroxide (SH; 1.6%), trisodium phosphate (TSP; 4%), or phosphoric acid (4%; Bosilevac et al. 2004a, b, 2005). Evaluation of such treatments on a laboratory-scale in model spraying-cabinets has demonstrated reductions of APC and total coliform count (TCC) of up to 4 $\log_{10}$ CFU/cm^2 and of *E. coli* O157:H7 prevalence from 44% to 17% on cattle (McEvoy et al. 2001, 2003; Bosilevac et al. 2004a, b, 2005). Furthermore, in a comparative evaluation of

simulated low-pressure (2.07 bar, 7 s) chemical sprays on whole beef hides inoculated with *E. coli* O157:H7 and *Salmonella*, Carlson et al. (2008a, b) found that 10% of warm (55°C) lactic or acetic acid, or cold (23°C) solutions of sodium metasilicate (SM; 4%), SH (3%), and SH (1.5%) in combination with chlorinated water (200 ppm) reduced the inoculated levels of the above pathogens by >2 $\log_{10}$ CFU/cm^2. The above chemical treatments also caused similar reductions to the populations of total bacteria, coliforms, and *E. coli* (Carlson et al. 2008a).

In conclusion, strict hygienic measures need to be applied during animal transportation to the abattoir and further handling before the dressing process. Animal washing may reduce microbial contamination on the external animal surfaces. However, this intervention has variable results and its effectiveness is uncertain. Nevertheless, application of low-pressure spray rinses with approved chemicals, such as detergents, organic acids, hydrogen peroxide, and chlorine, on animal hides is practiced in the United States and Australia (Midgley and Small 2006; Stopforth and Sofos 2006).

Dehairing

Chemical dehairing of cattle hides is a patented process (Bowling and Clayton 1992) that received FSIS-approval for experimental testing by the industry (Sofos and Smith 1998). The aim of chemical dehairing is to remove hair, mud, manure, other extraneous matter, and associated microbial contaminants from animal hides before their removal from carcasses. Applications (*in vitro*) of chemical dehairing on artificially inoculated bovine hide samples suggested that treatment with 10% sodium sulfide (SS) for 16 seconds could achieve >3 log cfu/cm^2 reductions of inoculated *E. coli* O157:H7, *Salmonella* spp., *L. monocytogenes*, as well as of APC and TCC (Castillo et al. 1998a; Graves Delmore et al. 1998).

In commercial applications, chemical dehairing reduced visible contamination (i.e., hair and carcass defects) and the amounts of waste derived from carcass trimming compared to the conventional process, but it had negligible effect in reducing carcass bacterial loads (Schnell et al. 1995). This was attributed to the fact that dehairing was evaluated during breaks on days when non-dehaired animals were also processed in the same facility, and thus, the plant environment carried microbial contamination (e.g., *via* aerosol, human, and equipment) from conventionally slaughtered animals. Nou et al. (2003) evaluated the APC, *Enterobacteriaceae* counts, and prevalence of *E. coli* O157:H7 on 240 conventionally processed beef carcasses, 240 hides that were chemically dehaired before removal, and on two respective sets of 240 carcasses immediately after hide removal at pre-evisceration in a plant that processed only dehaired animals. It was shown that the APC and *Enterobacteriaceae* populations on carcasses at pre-evisceration that had received chemical dehairing were lower than those on conventionally processed carcasses by 2 and 1.8 $\log_{10}$ CFU/cm^2, respectively. Similarly, the prevalence of *E. coli* O157:H7 on dehaired and nondehaired carcasses was reduced from 67% (pre-treatment) to 1% and from 88% to 50%, respectively (Nou et al. 2003). When simulated chemical dehairing systems were tested *in vitro* on removed whole beef hides inoculated with *E. coli* O157:H7 and *Salmonella*, Carlson et al. (2008b) found that deluging with SS (6.2%, 30°C) or potassium cyanate (PC; 2.4%, 30°C) reduced *E. coli* O157:H7 by 4.8–5.1 $\log_{10}$ CFU/cm^2 and *Salmonella* by 0.7 (PC) and 4.2 (SS) $\log_{10}$ CFU/cm^2.

Physical separation of the dehairing process from the hide removal operations would limit microbial aerosols and the spread of hydrolyzed hair and residual dehairing

chemicals (SS and hydrogen peroxide) released by the dehairing process from coming in contact with dehided carcasses. Concerns with chemical dehairing include the corrosiveness and hazardous potential of the sulfides and their potential effect on the skin and mucus membranes of workers (Edwards and Fung 2006). Moreover, recycling systems are needed to treat and recover the liquid waste generated by dehairing (Edwards and Fung 2006).

In contrast to cattle, pigs and chickens are rarely skinned, as they are scalded (with hot water that may contain wetting agents or alkali at up to 66°C) immediately after bleeding (Gill and Bryant 1992; Bolton et al. 2002). Scalding and singeing are known to cause up to 4.5 $\log_{10}$ CFU/cm^2 reductions of total aerobic bacteria and 6.0 $\log_{10}$ CFU/cm^2 reductions of pathogens, such as *S.* Typhimurium and *C. jejuni,* on poultry (Yang et al. 2001) or swine carcasses (Gill and Bryant 1992; Yu et al. 1999). However, despite these potential reductions in microbial counts, contamination levels may increase on scalded pork carcasses during subsequent polishing and/or mechanical shaving (Gill and Bryant 1992; Gill and Jones 1997a; Yu et al. 1999; Gill et al. 2000), and on poultry carcasses during chilling. Therefore, there is a need for strict hygiene control and additional decontamination strategies in the slaughtering and chilling process, in order to reduce microbial contamination on carcasses and maintain it at low levels.

Spot-Carcass Decontamination Treatments

Knife incision for bleeding (sticking) and for hide removal (skinning) is the first exposure of carcasses and muscle to contamination; the hide also introduces animal contamination on the carcass and in the plant environment, equipment, and workers as it is removed from the carcass. Therefore, spot-treatments of carcasses are the first interventions after hide removal, applied for removal of visible soil from external surfaces of carcasses. Such treatments include knife-trimming and steam-vacuuming. Both spot-carcass interventions are approved by FSIS for use in meat processing in order to achieve zero tolerance of visible contamination on carcasses. However, given that such interventions are based on visual inspection, and thus, invisible carcass contamination may be overlooked, they should be combined with pre- and/or post-evisceration decontamination treatments.

Knife-Trimming

As indicated, knife-trimming is required by FSIS in the United States for removal of visible contamination (i.e., feces, soil, hair, milk fluids, and bruised tissue) from carcasses before any spraying with water or decontamination liquids. In addition to cosmetic reasons, it is assumed that knife-trimming removes microbial contamination through proper removal of soiled tissue (Sofos and Smith 1998; Sofos 2002). The magnitude of reported reductions of naturally occurring total bacteria and coliforms by knife-trimming of beef carcasses ranges from <1 to 2 $\log_{10}$/cm^2 (Gorman et al. 1995b; Reagan et al. 1996; Kochevar et al. 1997b; Phebus et al. 1997; Castillo et al. 1998c). Higher reductions, such as 2.9 to 4.9 $\log_{10}$/cm^2, have been observed on inoculated *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes*, which may be associated with the higher inoculum levels applied on the beef tissue compared with naturally occurring levels of contamination on carcasses (Hardin et al. 1995; Cabedo et al. 1996; Phebus et al. 1997; Castillo et al. 1998c). However, knife-trimming may also increase microbial load of treated spots (Gill et al. 1996) or disperse contamination to adjacent areas (Gorman et al. 1995a). The efficiency of this intervention depends on the skills of personnel perform-

ing the trimming and the proper sanitation of the knives used. To ensure better decontamination of knives after use on a carcass, the industry in the United States has introduced the two- or three-knife system. As one knife is used, the others remain immersed in hot water for decontamination. Furthermore, given that knife-trimming removes visible contamination, it is likely that low-level contamination, as well as contamination which is not associated with visible soil, may be ignored (Edwards and Fung 2006). Thus, alternative decontamination methods, such as steam-vacuuming, but especially whole-carcass treatments in the form of multiple interventions, such as consecutive application of trimming and spraying with hot (66 to 87.8°C) water or chemical solutions, are necessary to improve the microbiological quality of carcasses (Dorsa 1997).

Steam/Hot Water-Vacuuming

In response to the need for interventions alternative to knife-trimming for removal of visible contamination from carcasses, FSIS approved the cleaning of carcass spots of up to 2.5 cm in diameter with handheld equipment, applying steam and vacuum; larger spots must be removed only by knife-trimming (USDA-FSIS 1996a). The aim of the steam-vacuuming process is to clean visible soil as well as to remove or kill associated microorganisms (Kochevar et al. 1997a). A very large portion of the U.S. meat industry applies this economical process throughout the slaughter chain (Gill and Bryant 1997b). Commercial steam-vacuuming units release steam (at 104–110°C, 2.07–3.45 bar) and/or hot water (at 82–94°C, 0.34–1.03 bar) in conjunction with the application of vacuum and are continuously heat-sanitized (Dorsa et al. 1996a, b, 1997b; Kochevar et al. 1997a; Castillo et al. 1999a). The principle of the method is described as follows: initial application of steam or hot water loosens soil and causes bacterial inactivation, while subsequent vacuum removes the contaminants. Kochevar et al. (1997a) tested two commercial steam-vacuuming systems according to the operation protocol applied in operating plants. They found that steam-vacuuming caused 1.7–2.0 and 1.7–2.1 $\log_{10}/cm^2$ reductions of APC and TCC, respectively, which were slightly higher than reductions achieved by knife-trimming. Decrease in microbial populations due to steam-vacuuming may also exceed those achieved with water spray-washing (Castillo et al. 1999a). In general, reported reductions of APC, TCC, and *E. coli* by steam-vacuuming of naturally contaminated carcasses have ranged from 0.5 to 2 $\log_{10}$ CFU/cm^2 (Gill and Bryant 1997b; Kochevar et al. 1997a), whereas those on tissues artificially inoculated with bovine feces are commonly higher, such as 3–4 $\log_{10}$ CFU/cm^2 (Dorsa et al. 1996a; Castillo et al. 1999a). Considerable effectiveness of steam-vacuuming has also been shown against pathogens artificially inoculated on beef carcass short plates or cutaneous trunci muscles. Specifically, average reported reductions of *L. innocua*, *L. monocytogenes*, *S.* Typhimurium, and vegetative cells of *Clostridium sporogenes* ranged from 2 to 3.4 $\log_{10}$ CFU/cm^2 (Dorsa et al. 1997b; Phebus et al. 1997), whereas a reduction of 5.5 $\log_{10}$ CFU/cm^2 has been reported for inoculated *E. coli* O157:H7 (Dorsa et al. 1996b).

The duration of vacuum application, the processing stage, and the type or state of beef tissue seem to play an important role on the efficiency of steam-vacuuming. Bacon et al. (2002a) evaluated post-chilling steam-vacuuming (130°C, 1.72 bar) on *Salmonella*-inoculated cold beef adipose tissue surfaces and found reduction of less than 1 $\log_{10}$ CFU/cm^2. The limited efficacy of the applied treatment was attributed to the short contact time of the steam-vacuuming unit with the adipose surface, in combination with the protective effect of the hardened cold surface on microcolonies imbedded or attached to the tissue.

However, steam-vacuuming seems to be a useful decontamination approach on hot carcasses during dressing, as it may deliver significant reductions of APC and TCC. Implementation of water/steam-vacuuming reduces the amount of trimming needed on carcass-processing lines and hence, limits the effect of human subjectivity, as well as the risks of contamination by insufficient sanitation of knives and improper personnel hygiene. In addition to visible contaminated spots, steam-vacuuming operators in U.S. slaughter plants also apply the intervention on certain carcass sites (e.g., hide cutting pattern lines) known to carry microbial contamination even in the absence of visible soil. It should be noted that the effectiveness of this intervention depends on appropriate application and proper state of equipment operation.

Pre-Evisceration Decontamination

Pre-evisceration interventions include washing with cold or hot water and/or rinsing with chemical solutions, such as organic acids (Fig. 3.1). In addition, water-washing of beef carcasses is performed as a final step before decontamination and chilling (Fig. 3.1). Washing with cold water may be considered equally or less effective than knife-trimming in removing bacterial contamination, whereas the combination of both treatments may not be more effective than each treatment alone (Gorman et al. 1995a, b; Gill et al. 1996; Graves Delmore et al. 1997; Reagan et al. 1996). However, this comparison may be unjustified, since knife-trimming applies to defined carcass segments, while washing applies to whole carcasses. Compared with cold water, which physically removes bacteria, hot water-washing has an additional lethal effect on microbial contamination (see relevant section of this book chapter).

Apart from water-washing, rinsing with chemical solutions may also be effective in reducing microbial contamination after hide removal but before evisceration (Fig. 3.1). Organic acids, such as lactic and acetic, are the chemicals of choice in pre-evisceration treatments. However, due to the strong flavor of acetic acid, lactic acid is the preferred compound. Details on the effect of chemical solutions on the reduction of carcass microbial contamination are presented in the "chemical decontamination" section of this chapter.

Given that bacterial attachment increases with time elapsed between exposure of the carcass to contamination and application of the decontamination treatment, the sooner the application of spray-washing after hide removal the higher the expected result. It is believed that pre-evisceration washing is effective, as it may limit the adherence of bacteria to carcass by reducing the surface tension (i.e., the surface free energy and contact angle) of the latter (Dickson 1995). Consequently, spray-washing before evisceration may result in significant reduction of initial contamination because of the short time for bacterial and soil attachment after hide removal, and because the wet surface limits subsequent bacterial attachment during evisceration and before final carcass-washing (Sofos and Smith 1998).

Final Carcass-Washing

As indicated, water-washing is also applied at the end of the dressing process (post-evisceration), following zero-tolerance inspection, before carcass-chilling. Typically, carcass-washing may take place manually with hand-held hoses in small operations or by automated spraying systems in cabinets (Anderson et al. 1981), in which the nozzle type and configuration, and the spraying pressure determine the droplet size and temperature of water as it hits the carcass surface (Bacon 2005). The main objective of final carcass-washing is to improve the appearance of the carcass by removing blood and sawdust generated during carcass-splitting.

Common temperatures of water-washing range from 10 to 40°C (and rarely up to 56°C) and pressures from 343 to 4134 kPa, whereas the duration varies from 5 seconds up to 2 or 10 minutes for manual spraying (Sheridan 2004; Bacon 2005). In contrast to automated spraying systems, hand-washing lacks consistency because of human error, such as lack of operator attention and fatigue (Anderson et al. 1981; Sheridan 2004). Studies evaluating the effectiveness of spray-washing on whole carcasses or excised carcass tissues have used either commercial or model spray-washing cabinets. Specifically, pre-evisceration washing of beef, veal, sheep, or lamp carcasses with cold (2 to 35°C) or slightly warm water (e.g., 40 or 56°C) for 5–90 seconds, reduced counts of total bacteria and indicator organisms, such as *E. coli* and coliforms by <1 to 2.3 $\log_{10}$ CFU/cm^2, either inoculated through fecal paste (Gorman et al. 1995a, b; Reagan et al. 1996; Dorsa et al. 1996b, 1997a, 1998a, b, c; Kochevar et al. 1997b; Graves Delmore et al. 1997, 1998; Castillo et al. 1998b, c; Penney et al. 2007) or naturally present on carcasses (Gill et al. 1996; Kain et al. 2001). The reported reductions for pathogens such as *E. coli* O157:H7 and *Salmonella* inoculated on carcass tissues are in the range 1.9 to 3.5 $\log_{10}$ CFU/cm^2 (Castillo et al. 1998b, c; Hardin et al. 1995; Cutter and Rivera-Betancourt 2000).

In addition to washing time, temperature and pressure, the effectiveness of washing also depends on bacterial population densities, the condition (temperature of carcass based on postmortem and/or pre-chilling time) of the tissue, and the extent of bacterial attachment (Bacon 2005; Hardin et al. 1995). Bacterial adhesion, which depends on factors such as formation of exopolymeric substances, wool, moisture of tissue, and microbial species, as indicated, also increases with time of carcass tissue exposure to fecal contamination (Bacon 2005). Furthermore, it has been suggested that washing appears relatively more effective when the initial contamination level is high (Gill and Landers 2003b). This may be explained on the basis that large numbers of bacteria are likely associated with solid particles, which are easily washed off, whereas lower bacterial populations may be associated more firmly with surface tissue (Gill and Landers 2003b). Another reason could be that the higher the initial contamination the more bacteria exist to be removed.

The efficacy of cold water (2 to 40°C) in reducing microbial contamination of carcasses may be enhanced as spraying pressure increases from 27 to 41 bar (1 bar = 100 kPa), and/or as contact time increases from 5 to 90 seconds (Kelly et al. 1981; Gorman et al. 1995a, b; Gill et al. 1996; Bell 1997; Castillo et al. 1998c; Kain et al. 2001; Yang et al. 2001; Edwards and Fung 2006). For example, an approximately tenfold increase in spraying pressure (i.e., from 2–13 to 20–27 bar) of water (16–35°C) increased the reduction of APC from <1 $\log_{10}$ to 1.24–1.35 CFU/cm^2 on beef (Gorman et al. 1995a, b) and lamb adipose tissue (Kochevar et al., 1997b). Certainly, the increase in pressure also improves the presentation of carcasses (Kochevar et al. 1997b). However, careful selection of washing pressure needs to be made, because application of high pressure water-washing may lead to potential penetration of bacterial contamination to inner layers of tissue, while low pressures may simply result in translocation of the microbial cells on the carcass surface (De Zuniga et al. 1991; Anderson et al. 1992; Ellerboek et al. 1993; Gorman et al. 1995a). Castillo et al. (1998c) inoculated beef carcass tissue with feces mixed with *S.* Typhimurium and *E. coli* O157:H7 and found that spray-washing with water caused spreading of 0.7 and 1.8 $\log_{10}$ CFU/cm^2 of the above microorganisms, respectively, to sites of carcasses adjacent to the inoculated areas. This was attributed to washing run-off. Likewise, Bell (1997) found that pre-chilling washing (517.5 kPa)

of beef carcasses spread the microbial contamination in a posterior to anterior direction, following the flow of wash water, down the carcass (i.e., from hind leg to forequarter). Similarly, power-hosing of pork carcasses after singeing increased bacterial numbers on ham, belly, and neck by almost 3 $\log_{10}$ CFU/cm^2 (Bolton et al. 2002). Polishing of pig carcasses following singeing and/or scalding may increase APC, further necessitating a pre-chilling washing/decontamination step (Gill et al. 1995, 2000; Yu et al. 1999). However, due to the aforementioned limitations, pre-chilling washing may be insufficient to reduce the microbial contamination of carcasses entering the dressing process, especially if the dressing process is not implemented under controlled hygienic conditions (Gill et al. 2000). In a comparative evaluation of the hygienic performance of eight pork plants, it was found that APC, TCC, and *E. coli* after final washing were equal to or higher than after polishing in the majority of plants, suggesting that microbial contamination was either deposited on carcasses or not removed from carcasses during the dressing process (Gill et al. 2000).

In conclusion, spray-washing with tap/potable water may have a moderate effect on microbial reduction but it is very effective in improving the visual appearance of carcasses. Nonetheless, if not done properly, spray-washing may lead to: (i) increased surface tissue moisture; (ii) entrapment, embedding, and subsequently potential proliferation of bacteria into tissues; entrapment and embedding may also act as a physical barrier against subsequent decontamination interventions; (iii) reduction of endogenous spoilage microflora and thus of their competitive effect on pathogens; and, (iv) redistribution or translocation of microbial contamination from heavily contaminated to cleaner parts of carcasses (Cabedo et al. 1996; Bacon 2005). Therefore, it is suggested that water spray-washing should be done properly, with well-functioning equipment. Simple water-washing alone may not be sufficient for major improvements in carcass hygiene, unless it is followed by other physical or chemical decontamination methods, which are discussed in the next sections.

Thermal Decontamination

Research on meat decontamination since the 1980s has shown that the effectiveness of physical decontamination methods may be significantly enhanced when the carcass surface temperature is raised above 70°C (Kelly et al. 1981; Davey and Smith 1989; Smith 1992). Currently, commercially applied thermal treatments worldwide include hot water (70–85°C) or saturated steam to reduce microbial contamination on carcasses. Both interventions are commercially applied post-evisceration, after final carcass-washing (Fig. 3.1), whereas hot water has also been evaluated as potential decontamination treatment of carcass trimmings. While hot water treatments may be applied in spraying cabinets equipped with proper nozzles, steam pasteurization requires the installation of more expensive steam cabinets. The commercial application of steam pasteurization costs more than hot water. According to a recent review by Midgley and Small (2006), hot water requires approximately $400,000–$500,000 for the installation of proper equipment and results in an average cost of $0.60–$0.70 per carcass, whereas the respective costs estimated for steam pasteurization are $650,000 and up to over $1 million and $0.75–$0.80 per carcass, depending on the number of carcasses processed per hour.

Hot-Water Decontamination

Hot water (74–97°C) exerts decontamination effects through physical removal and thermal inactivation of bacteria present on the surface of meat. It is an intervention of high perfor-

mance that may deliver microbial reductions additional to those caused by previous water-washing or knife-trimming (Gorman et al. 1995b; Reagan et al. 1996; Graves Delmore et al. 1997; Castillo et al. 1998b, c). The effectiveness of hot-water treatments mainly depends on the temperature, duration, and pressure of application (Dorsa et al. 1996b; Graves Delmore et al. 1997; Kochevar et al. 1997b; Gill et al. 1996, 1999, 2001; Bacon 2005). Spraying of hot water is carried out with properly designed nozzles to meet the requirements of flow rate, droplet size, pressure of water, as well as the spray angle (Bacon 2005). The temperature achieved on the carcass surface is commonly 6–10°C lower than that of sprayed water, depending on duration of treatment and other factors (Dorsa et al. 1996b; Castillo et al. 1998b; Eggenberger-Solorzano et al. 2002). Furthermore, the distance of nozzles from the carcass and the header temperature affect the turbulence of water flow and hence, the heat transfer from water to the targeted anatomical location of the carcass by the time of contact (Bacon 2005). As with all water-washing interventions, the earlier the hot water is applied the more effective it is in killing bacteria (Barkate et al. 1993).

Studies evaluating the efficacy of hot (>70°C) or warm (50–60°C) water-washing on chicken, pig, and beef carcasses have involved immersion, spraying or deluging with sheets of water. Immersion of whole broiler carcasses into water (95°C) for 3 minutes was capable of reducing APC from approximately 3–4 and 2–3 $\log_{10}$ CFU/cm^2 before treatment on skin and deboned subcutaneous meat, respectively, to <10 CFU/cm^2 (Avens et al. 2002). On scalded and/or polished uneviscerated pork carcasses, the maximum reported reduction of natural microbial contamination (e.g., mesophilic aerobic bacteria and *E. coli*) by commercial application of hot water ranged from 2 to 3 $\log_{10}$/cm^2 (Gill et al. 1995, 1997; Eggenberger-Solorzano et al. 2002). Such reductions on pork carcasses are achieved by water of 65 or 80°C for 5 seconds (Eggenberger-Solorzano et al. 2002) or 85°C for 15 or 20 seconds (Gill et al. 1995, 1997). The highest reported reductions (4.0 to >4.8 $\log_{10}$/cm^2) of *E. coli* O157:H7, *S.* Typhimurium, APC, TCC, and *Enterobacteriaceae* were achieved by spraying beef carcass surfaces artificially contaminated with inoculated bovine feces with water at 95°C for 5 seconds (Castillo et al. 1998b, c). Lower hot-water temperatures, such as 72°C (for 12 to 15 s), 74°C (for 12, 18 or 26 s), or 77°C (for 8 s), were also effective in carcass decontamination (Gorman et al. 1995b, 1997; Reagan et al. 1996; Dorsa et al. 1997b, 1998a, b, c; Graves Delmore et al. 1997). Average reductions of APC, TCC, *E. coli*, and inoculated *E. coli* O157:H7 and *S.* Typhimurium achieved at 72°C were 3.7, 2.7, 2.7, 1.8, and 2.7 $\log_{10}$ CFU/cm^2, respectively (Dorsa et al. 1998b, c; Cutter and Rivera-Betancourt 2000). Increasing the water temperature by 2°C (i.e., 74°C) enhanced microbial inactivation, compared to 72°C, and markedly decreased the prevalence of pathogens, such as *E. coli* O157:H7 (from 27% to 5%), on bovine heads (Bosilevac et al. 2006; Kalchayanand et al. 2008), as well as the levels of APC on lamb adipose tissue (Kochevar et al. 1997b). In contrast, lower temperatures, such as those of warm water (56°C), did not contribute to meat decontamination more than washing with cold (<30°C) water (Cutter et al. 1997).

One concern with hot water treatments is their potential undesirable effect on the appearance of carcass tissue. Hot water treatments at 82°C for 10 seconds may cause temporary bleaching of the tissue, as the meat regains its redness after chilling (Barkate et al. 1993). Other studies indicate that water temperatures of 85°C for 10–20 seconds may confer both substantial microbial reductions and acceptable appearance of carcasses (Gill et al. 1995, 1997; 1999; Gill and Badoni 1997). Long carcass hot water-washing dura-

tions, such as >20 seconds at temperatures 80–85°C, do not improve the decontamination efficiency of hot water, while they may cause irreversible discoloration of meat (Gill et al. 1995, 1999, 2001; Edwards and Fung 2006). Deleterious effects on the color of meat are also caused by very high water temperatures (e.g., 90°C), irrespective of exposure time (Barkate et al. 1993; Gill et al. 1995; Gill and Badoni 1997).

Apart from the immediate impact on microbial reduction, hot-water sprays have no residual antimicrobial effects during product storage, while in some cases hot-water application may actually enhance microbial growth; however, it may facilitate reduction or inhibition of growth of injured cells by subsequent organic acid treatments (Ikeda et al. 2003; Koutsoumanis et al. 2004). Specifically, although application of hot (72 or 74°C) or cold (33°C) water for 12 seconds on beef tissue caused 2.1–2.6 $\log_{10}$ CFU/cm^2 reductions in APC, TCC, *L. innocua*, *E. coli* O157:H7, and *C. sporogenes,* microbial growth occurred at least 1000-fold within 21 days of storage at 4–5°C in vacuum packages, with no pronounced differences in bacterial counts compared to untreated samples (Dorsa et al. 1997a, 1998a, b, c). Furthermore, hot water (75°C, 30 s) enhanced growth of *L. monocytogenes* during subsequent storage of treated meat in vacuum packages at temperatures of 4, 10, and 25°C, compared to colder (55°C) water or untreated meat (Ikeda et al. 2003; Koutsoumanis et al. 2004). This has been attributed to the potential of hot water to increase available nutrients on the surface of meat possibly *via* denaturation of components or extraction from inner tissue, reduce natural psychrotrophic competitors of the pathogen, and increase free water available for microbial growth (Ikeda et al. 2003). Significant delay in growth of natural spoilage flora has been reported on adipose tissue treated with hot water. Gorman et al. (1997) observed that APC were reduced by 0.8–2.8 $\log_{10}$ CFU/cm^2 following treatment of beef adipose tissue with hot water (74°C), whereas subsequent storage at 4°C allowed APC to reach only 4.3 $\log_{10}$ CFU/cm^2 compared with untreated samples in which APC exceeded the level of 6 $\log_{10}$ CFU/cm^2. This is potentially due to cell injury caused by hot-water treatments.

In conclusion, hot water is more effective than cold water in reducing microbial spoilage and pathogenic contamination of carcasses. However, proper selection of pressures, temperature, and duration of the application is necessary in order to ensure microbial reductions and avoid permanent discoloration of the carcass tissue. Furthermore, hot water is a physical intervention that involves no chemical risk and requires lower capital investment. For these reasons, it has received commercial application in meat-processing industries worldwide.

Steam Pasteurization

Use of steam, instead of hot water, is an alternative thermal decontamination treatment. A patented (Steam pasteurization™) process has been approved by FSIS for application in the United States (USDA-FSIS, 1996a) and is used commercially at the post-evisceration stage, after final washing and before chilling (Gill and Bryant 1997b; Nutsch et al. 1997) (Fig. 3.1). Steam pasteurization™ consists of the following steps (Bacon 2005): (i) removal of water by vacuum from the meat surface in order to allow better penetration of steam; (ii) application of "saturated" steam (commonly for 6.5–10 s) raising surface temperature to 80–90°C; and, (iii) cooling of treated tissues in order to prevent visual defects or microbial growth. Moist heat treatment using a commercial steam cabinet was more effective in reducing bacterial counts than cold (15 °C) or warm (54°C) water, but equally effective for hot (82°C) water, on lamb carcasses at pre-evisceration after water-washing

(15–82°C for 10 s; Dorsa et al. 1996b). Nonetheless, combination of steam pasteurization with knife-trimming or steam-vacuuming may increase microbial reductions (Phebus et al. 1997).

Post-evisceration steam pasteurization of carcasses in a beef slaughtering plant caused decreases in the percentage of positive carcass samples for *E. coli*, coliforms, and *Enterobacteriaceae*, as well as *Salmonella* from 16.4, 37.9, 46.4, and 0.7%, to 0, 1.4, 2.9, and 0%, respectively (Nutsch et al. 1997). However, concerns have been expressed as to whether steam-pasteurized carcasses become more susceptible to bacterial attachment in case of recontamination (Warriner et al. 2001). Reported reductions for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes,* achieved by post-evisceration steam pasteurization (105°C, 6–15 s), range from 3.4 to 3.7 $\log_{10}$ CFU/cm^2 (Phebus et al. 1997). The respective reductions for APC, TCC, and *E. coli* are 2 to 3 $\log_{10}$ CFU/cm^2 (Gill and Bryant 1997b; Nutsch et al. 1997). Furthermore, ultra-high temperature steam (140°C) for 5 seconds yielded 4 $\log_{10}$ CFU/cm^2 reductions of *L. innocua* on the surface of poultry carcasses (Morgan et al. 1996). In addition to steam-flushed chambers, Retzlaff et al. (2004) proposed the use of a static steam system, applying a constant flow of steam at temperatures of 82.2–98.9°C for 6–15 seconds. Steam of 98.9°C for 9 seconds afforded the highest reductions of *E. coli* O157:H7 (4.1 $\log_{10}$ CFU/cm^2), without negatively affecting color and texture of beef (Retzlaff et al. 2004).

Overall, steam pasteurization requires a major capital investment, but it can be an additional intervention that can cause further reductions to those achieved by knife-trimming, washing, and/or chemical rinses before chilling (Dorsa et al. 1996b; Gill and Bryant 1997b; Phebus et al. 1997; Gill and Landers 2003b). Therefore, in the United States, steam pasteurization has been successfully used in beef slaughter.

Chemical Decontamination

In addition to the aforementioned physical decontamination methods, interventions involving chemical agents have also been recommended and used on carcasses (Fig. 3.1) (Smulders and Greer 1998). A significant amount of research has been undertaken into the chemical decontamination of beef carcasses over the last 30 years, in order to reduce total bacterial numbers and presence of pathogens. Nowadays, chemical interventions are applied as components of multiple sequential interventions, including knife-trimming, steam-vacuuming, and thermal (hot-water or steam) treatments (Dickson and Anderson 1991; Hardin et al. 1995; Gorman et al. 1997; Castillo et al. 1998c, 1999a, b, 2001a, b, 2003; Stopforth et al. 2004, 2005, 2007; Geornaras and Sofos 2005; Table 3.1). Chemicals used or proposed for use in carcass decontamination include organic acids, acidic calcium sulfate, acidified sodium chlorite, a peroxyacetic acid-based solution, potassium hydroxide, and other commercially available mixtures of approved chemicals (USDA-FSIS 2008c). Chemical rinses may be applied pre-evisceration, or post-evisceration before chilling, as well as during spray-chilling and after chilling as carcasses are removed to be fabricated (Fig. 3.1).

Organic Acids

Lactic and acetic acid have been widely used at concentrations of up to 5% and have resulted in 2.2–5, 1.4–4.9, 0.40–1.6, 3.6–4.7, 1.6–4.9 and 1.5–>3.6 $\log_{10}$ CFU/cm^2 reductions of *Salmonella*, *E. coli* O157:H7, *L. monocytogenes, Yersinia enterocolitica*, *Enterobacteriaceae*, and APC, respectively, on beef, pig, lamb, or poultry carcass tissues (Anderson et al. 1988; Gorman et al. 1995b, 1997; Hardin et al. 1995; Goddard et al. 1996; Podolac et al. 1996; Kochevar et al. 1997b; Castillo et al. 1998c, 2001a, b; van Netten et al. 1997; Cutter 1999;

Table 3.1. Populations (log_{10} CFU/cm^2) of indicator organisms and pathogens before and after application of decontamination interventions on hides, beef carcasses and beef or pork trimmings.

Surface	Interventions in sequence				Microorganism(s)	Log_{10} CFU/cm^2 or g		Reference
	1st	2nd	3rd	4th		Before	After	
Hides	Water (35°C)	Water (35°C)	NA[a]	NA	Coliforms	5.9	4.3	Bosilevac et al. (2005)
	Water	Water	Vacuum	NA		5.9	2.3	
	Water	ASC[b] (200 ppm)	NA	NA		5.7	2.8	
	Water	ASC (200 ppm)	Vacuum	NA		5.7	2.8	
	Chloroform (4%)	Water	NA	NA		6.2	2.6	
	Chloroform (4%)	Water	Vacuum	NA		6.2	2.6	
	Sodium hydroxide (1.6%)	Water	NA	NA		5.7	4.2	
	Sodium hydroxide (1.6%)	Water	Vacuum	NA		5.7	1.8	
	Sodium hydroxide (1.6%)	ASC (500 ppm)	NA	NA		5.9	2.2	
	Sodium hydroxide (1.6%)	ASC (500 ppm)	Vacuum	NA		5.9	2.0	
	Phosphoric acid (4%)	Water	NA	NA		5.8	3.3	
	Phosphoric acid (4%)	Water	Vacuum	NA		5.8	2.3	
	Phosphoric acid (4%)	ASC (200 ppm)	NA	NA		5.9	2.3	
	Phosphoric acid (4%)	ASC (200 ppm)	Vacuum	NA		5.9	1.6	
	Phosphoric acid (4%)	ASC (500 ppm)	NA	NA		5.9	1.5	
	Phosphoric acid (4%)	ASC (500 ppm)	Vacuum	NA		5.9	0.2	
	Trisodium phosphate (4%)	Water	NA	NA		5.9	4.4	
	Trisodium phosphate (4%)	Water	Vacuum	NA		5.9	3.4	
Beef carcass tissue	Water (20°C for 5 s)	NA	NA	NA	*E. coli* O157:H7	6.1	4.6	Penney et al. (2007)
	PAA[c] (180 ppm; 20°C for 5 s)	NA	NA	NA		6.1	2.3	
	Water	PAA (180 ppm)	NA	NA		6.1	2.7	
	Water (82.2°C, 10 s)	NA	NA	NA	APC[d]	6.6	3.3	Dorsa et al. (1996b)
	Steam-vacuum (30 s)	NA	NA	NA		6.2	3.2	
	Water (82.2°C, 10 s)	Steam-vacuuming (30 s)	NA	NA		6.7	2.7	
	Water (72°C, 12 s)	NA	NA	NA		5.7	3.7	
	Water (32°C, 12 s)	NA	NA	NA		5.8	3.8	
	Water (72°C, 12 s)	Water (32°C, 12 s)	NA	NA		5.7	3.2	
	Steam-vacuuming (30 s)	Water (72°C, 12 s)	Water (32°C, 12 s)	NA		6.1	3.0	

	Water (72°C, 12 s)	Water (32°C, 12 s)	NA	NA	Coliforms	5.2	1.9	
	Steam-vacuuming (30 s)	Water (72°C, 12 s)	Water (32°C, 12 s)	NA		5.0	0.8	
				NA	*E. coli*	4.9	0.6	
	Steam-vacuuming (6 s)	NA	NA	NA	APC	6.7	2.7	Castillo et al. (1999a)
					Coliforms	5.3	2.7	
					E. coli	5.2	2.8	
	Steam-vacuuming (6 s)	Hot water (95°C, 5 s)	NA	NA	APC	6.7	4.4	
					Coliforms	5.3	5.1	
					E. coli	5.2	5.2	
	Steam-vacuuming (6 s)	Lactic acid (2%, 55°C, 11 s)	NA	NA	APC	6.7	3.5	
					Coliforms	5.3	4.4	
					E. coli	5.2	4.4	
	Steam-vacuuming (6 s)	Hot water (95°C, 5 s)	Lactic acid (2%, 55°C, 11 s)	NA	APC	6.7	4.4	
					Coliforms	5.3	4.4	
					E. coli	5.2	4.5	
	Steam-vacuuming (6 s)	Lactic acid (2%, 55°C, 11 s)	Hot water (95°C, 5 s)	NA	APC	6.7	4.3	
					Coliforms	5.3	4.3	
					E. coli	5.2	4.4	
	Water (82°C, 30 s)	Lactic acid (5%, 55°C, 30 s) after 48 h of chilling	NA	NA	*E. coli O157:H7*	6.0	2.4	Stopforth et al. (2005)
					Salmonella	6.0	2.3	
	Sodium metasilicate (1%, 82°C, 30 s)	Water (82°C, 30 s)	Lactic acid (5%, 55°C, 30 s pre-chilling)	NA	*E. coli O157:H7*	6.0	2.7	
					Salmonella	6.0	2.9	
Beef trimmings	Ozonated water (1%, 15 min, 7.2°C)	Acetic acid (5%, 15 min, 7.2°C)	NA	NA	*E. coli*	6.8	5.4	Pohlman et al. (2002a)
					Coliforms	6.0	4.2	
					APC	7.1	5.8	
					Salmonella Typhimurium	5.8	4.2	

(continued)

Table 3.1. Populations (log_{10} CFU/cm^2) of indicator organisms and pathogens before and after application of decontamination interventions on hides, beef carcasses and beef or pork trimmings. *(cont.)*

Surface	Interventions in sequence				Microorganism(s)	Log_{10} CFU/cm^2 or g		Reference
	1st	2nd	3rd	4th		Before	After	
	Ozonated water (1%, 15 min, 7.2°C)	CPC[e] (0.5%, 15 min, 7.2°C)	NA	NA	*E. coli*	6.8	5.1	
					Coliforms	6.0	4.1	
					APC	7.1	5.6	
					Salmonella Typhimurium	5.8	4.0	
	ClO_2 (200 ppm, 15 min, 7.2°C)	Trisodium phosphate (10%, 15 min, 7.2°C)	NA	NA	*E. coli*	6.8	6.2	
					Coliforms	6.0	5.7	
					APC	7.1	6.8	
					Salmonella Typhimurium	5.8	5.5	
	Acetic acid (5%, 3 min)	CPC (0.5%, 3 min)	NA	NA	APC	7.1	5.3	Pohlman et al. (2002b)
					Salmonella Typhimurium	5.8	3.8	
	ClO_2 (200 ppm, 3 min)	CPC (0.5%, 3 min)	NA	NA	APC	7.1	5.9	
					Salmonella Typhimurium	5.8	4.4	
	CPC (0.5%, 3 min)	Trisodium phosphate (10%, 3 min)	NA	NA	APC	7.1	6.2	
					Salmonella Typhimurium	5.8	4.6	

	Water (5 passes)	Hot water (82°C, 3 passes)	Hot air (510°C for 6 passes)	Lactic acid (2%, room temperature, 3 passes)	APC	2.5	0.1–0.5	Kang et al. (2001a)[f]
					Coliforms	2.0	0	
					Psychrotrophs	3.5–4.0	0–1	
					LAB[g]	2	0–0.5	
Lean or fat beef trimmings	Water (5 passes)	NA	NA	NA	APC	5	4	Kang et al. (2001b)[f]
					Coliforms	3.5	2.5	
					E. coli	3.5	2.2–2.4	
					Psychrotrophs	5.0	4.0	
					LAB	4.5	3.5	
	Lactic acid (2%, 3 passes)	NA	NA	NA	APC	5	3.8	
					Coliforms	3.5	2.2–2.4	
					E. coli	3.5	2.0	
					Psychrotrophs	5.0	3.8	
					LAB	4.5	3.6	
	Water	Hot water (65°C, 1 pass)	Hot air (510°C, 5 passes)	Lactic acid	APC	5	3.2	
					Coliforms	3.5	1.9	
					E. coli	3.5	1.8	
					Psychrotrophs	5.0	3.5	
					LAB	4.5	3.0	
	Water	Hot water (82°C, 1 pass)	Hot air (510°C, 5 passes)	Lactic acid	APC	5	3.2	
					Coliforms	3.5	1.9	
					E. coli	3.5	1.8	
					Psychrotrophs	5.0	3.5	
					LAB	4.5	3.0	
	Water	Hot water (82°C, 3 passes)	Hot air (510°C, 6 passes)	Lactic acid	APC	5	3.0–3.1	
					Coliforms	3.5	1.5	
					E. coli	3.5	1.1	
					Psychrotrophs	5.0	3.0–3.2	
					LAB	4.5	2.9–3.0	

(continued)

Table 3.1. Populations (log_{10} CFU/cm^2) of indicator organisms and pathogens before and after application of decontamination interventions on hides, beef carcasses and beef or pork trimmings. *(cont.)*

Surface	Interventions in sequence				Microorganism(s)	Log_{10} CFU/cm^2 or g		Reference
	1st	2nd	3rd	4th		Before	After	
Fat pork trimmings	Water (15°C, 120 s)	NA	NA	Lactic acid	APC	4.9–5.0	2.5	Castelo et al. (2001a)[f]
					Coliforms	4.7–4.8	1.1	
					E. coli	4.5	1.1	
					Psychrotrophs	3.8–3.9	0.5	
					LAB	7.0	3.0	
	Water	Lactic acid (2%, 15°C, 75 s)	NA		APC	4.9–5.0	1.0	
					Coliforms	4.7–4.8	0.1–0.2	
					E. coli	4.5	0.0	
					Psychrotrophs	3.8–3.9	0.1–0.2	
					LAB	7.0	1.5	
	Water	Hot water (65.5°C, 15 s)	Hot air (510°C, 60 s)	Lactic acid	APC	4.9–5.0	0.9	
					Coliforms	4.7–4.8	0.1–0.2	
					E. coli	4.5	0.1–0.2	
					Psychrotrophs	3.8–3.9	0.0	
					LAB	7.0	1.5	
	Water	Hot water (82.2°C, 15 s)	Hot air (510°C, 75 s)	Lactic acid	APC	4.9–5.0	0.9–1.0	
					Coliforms	4.7–4.8	0.0	
					E. coli	4.5	0.0	
					Psychrotrophs	3.8–3.9	0.0	
					LAB	7.0	1.1–1.2	
	Water	Hot water (82.2°C, 45 s)	Hot air (510°C, 90 s)	Lactic acid	APC	4.9–5.0	0.0	
					Coliforms	4.7–4.8	0.0	
					E. coli	4.5	0.0	
					Psychrotrophs	3.8–3.9	0.0	
					LAB	7.0	<1.0	

Lean beef trimmings	Water (15°C, 120 s)	NA	NA	NA	APC	5.0	4.0	Castelo et al. (2001b)[f]
					Coliforms	3.5	2.8–2.9	
					E. coli	3.5	2.8–2.9	
					LAB	6.8	6.0	
	Water	Lactic acid (2%, 15°C, 75 s)	NA	NA	APC	5.0	2.9	
					Coliforms	3.5	1.8–1.9	
					E. coli	3.5	1.8–1.9	
					LAB	6.8	5.5	
	Water	Lactic acid (2%, 15°C, 75 s)	Hot air (510°C, 90 s)	NA	APC	5.0	2.5	
					Coliforms	3.5	1.5	
					E. coli	3.5	1.5	
					LAB	6.8	5.5	
	Hot air	Water	Hot air (510°C, 90 s)	NA	APC	5.0	4.1	
					Coliforms	3.5	3.0	
					E. coli	3.5	3.0	
					LAB	6.8	5.8–5.9	

[a] not available.
[b] Acidified sodium chlorite.
[c] Peroxyacetic acid.
[d] Aerobic plate counts.
[e] Cetylpyridinium chloride.
[f] Approximate reductions based on data from figures.
[g] Lactic acid bacteria.

Eggenberger-Solorzano et al. 2002; Bosilevac et al. 2006). Furthermore, acetic (1.5–3%) and lactic (2%) acid spraying have also reportedly decreased populations of spoilage bacteria of lamb or beef, such as psychrotrophic Gram-negative flora (e.g., pseudomonads), lactic acid bacteria, yeasts, and *Brochothrix thermosphacta* (Anderson et al. 1988; Dorsa et al. 1997a, 1998a, b, c; Koutsoumanis et al. 2004). The observed reductions were higher than those achieved by washing with cold (32°C) or hot (72°C) water (Dorsa et al. 1998 a, b, c). Even though fumaric acid (1% and 1.5% at 55°C for 5 s) alone or as a mixture with acetic acid has also been found effective in reducing pathogens on beef carcass tissue (Bell et al. 1986; Podolak et al. 1996), it is not commercially applied. Fumaric acid has a negative impact on the appearance and flavor of meat, even at low concentrations (0.046%), while it has not been included in the list of safe and suitable ingredients for use in the production of meat and poultry products (USDA-FSIS 2008c).

The efficiency of organic acid rinses in reducing contamination is affected by the type of acid, its concentration, the temperature and duration of application, the type of microorganism and level of contamination, the coverage of the carcass surface with the solution, the carcass surface region, the type of tissue (e.g., lean or adipose, frozen or fresh tissues), and the stage of application (Anderson et al. 1988; Anderson and Marshall 1989; Cutter and Siragusa 1994b; Hardin et al. 1995; Cutter et al. 1997; van Netten et al. 1997; Castillo et al. 2001b; Gill and Landers 2003b). Lactic acid (2%, 55°C) was found more effective than acetic acid in reducing inoculated *E. coli* O157:H7 on beef carcass surfaces (Hardin et al. 1995). This may be associated with the higher reduction of surface pH caused by lactic acid (pKa = 4.73) than acetic acid (pKa = 3.86), when applied at the same concentrations. Perhaps the higher effectiveness of lactic compared to acetic acid also explains why spraying temperature (30–70°C) did not influence the effectiveness of 2% acetic acid for 15 seconds (Cutter et al. 1997), while an increase in temperature from 55 to 65°C had a significant influence on the effectiveness of 2% or 4% lactic acid for 15 or 30 seconds (Castillo et al. 2001b). With respect to the concentration alone, the effectiveness of lactic, acetic, and citric acid spraying rinses (24 or 32°C) against total bacterial counts, *L. innocua*, *E. coli* O157:H7, and *C. sporogenes* on beef carcass tissue was found to increase with concentration from 1.5% to 5% (Castillo et al. 2001b; Cutter and Siragusa 1994b; Dorsa et al. 1997a). Furthermore, an increase in the concentration, temperature, and duration of post-chilling lactic acid treatment from 2% to 4%, 55 to 65°C, and 15 to 30 seconds, respectively, enhanced reduction of *E. coli* (Castillo et al. 2001b). Nonetheless, concentrations exceeding 2% may cause discoloration or compromise flavor, and thus, levels of 1.2% to 2% at 55°C were recommended (Woolthuis and Smulders 1985; Bell et al. 1986; Dickson and Anderson 1991; Goddard et al. 1996); however, levels of up to 5% are also used even post-chilling.

Organic acid treatments are more effective against low- compared with high-contamination levels (Greer and Dilts 1992; Cutter et al. 1997). The target organisms and meat tissue also affect microbial reductions caused by organic acids. For instance, *P. fluorescens* was less resistant to lactic, acetic, and citric spray rinses than *E. coli* (Cutter and Siragusa 1994b) and so was *S.* Typhimurium compared with *E. coli* O157:H7 when exposed to spray rinses with lactic or acetic acid (Hardin et al. 1995). Higher microbial reductions were obtained on adipose than on lean tissues (Cutter and Siragusa 1994b; Dickson 1988). However, the inside round of carcass, in which fecal material and associated microorganisms may be imbedded in muscle bundles as well as between fat and lean, was the most difficult carcass surface region to decontaminate even with organic

acid rinses (Hardin et al. 1995). *E. coli* O157:H7 survived less on post-rigor frozen beef tissue, treated with 2% acetic acid (56°C, 15 s), compared with pre-rigor fresh tissue, presumably due to higher bacterial attachment on fresh than on frozen surfaces (Cutter et al. 1997). In general, however, the efficacy of organic acid treatments is reduced on chilled compared with hot (fresh) carcasses, because bacterial attachment increases with time from slaughtering to chilling, and the temperature of the rinsing solution is reduced by the chilled surface (Bacon et al. 2002b).

Application of organic acid early after slaughtering, such as after dehiding or after evisceration, is highly effective (Prasai et al. 1991). For instance, pre-chilling treatment of inoculated beef samples with lactic acid and water resulted in 5.2 $\log_{10}$ CFU/cm^2 reductions of inoculated *S.* Typhimurium and *E. coli* O157:H7, while post-chilling lactic acid spraying reduced these organisms by only 1.6 and 2.4 $\log_{10}$ CFU/cm^2, respectively (Castillo et al. 2001b). Spraying of carcass tissue with 5% Tween 20 (a nonionic surfactant) 15 minutes before acid treatment was found to loosen or prevent bacterial attachment, thereby facilitating the subsequent decontamination by lactic acid spraying (Calicioglu et al. 2002). Such methods would probably be more effective on fat-covered surfaces, such as briskets and ribs or poultry skin.

In addition to initial microbial reductions, the residual levels of acids, and thus, the reduction of pH on treated meat surfaces, may suppress pathogen proliferation or even exert bactericidal effects during storage (Koutsoumanis et al. 2004). Specifically, spray-washing with acetic, lactic, or citric acid at concentrations 1–5% reduced the pH on the surface of lean tissues from 5.60 to 3.79–4.96 (with an average of 4.3–4.6) immediately after treatment, whereas after 1 day, the pH rose to 5.29–5.39 (Cutter and Siragusa 1994b). The respective pH changes on adipose tissue were 3.24–4.39 after treatment and 3.74–4.84 after 1 day (Cutter and Siragusa 1994b). Following prolonged storage, such as 21 days at 4–5°C, the pH on the surface of meat may increase up to the initial values of 5.5–5.6 (Dorsa et al. 1997a, 1998a, b, c; van Netten et al. 1997). For pathogens that cannot grow at refrigeration temperatures, such as *E. coli* O157:H7, *S. aureus*, *C. jejuni,* and *S.* Typhimurium, beef decontamination with 1.5–5% lactic, or 1.5–3% acetic acid (25–65°C, 15 or 30 s) contributed to reductions of 2–3 $\log_{10}$ CFU/cm^2 and/or eliminated populations during storage in air or vacuum at 4–5°C (Podolak et al. 1996; Dorsa et al. 1997a, 1998a, b, c; van Netten et al. 1998; Berry and Cutter 2000; Cutter and Rivera-Betancourt 2000; Castillo et al. 2001b; Calicioglu et al. 2002; Gozález-Fandos and Dominguez 2006). Other studies (van Netten et al. 1997, 1998; Dorsa et al. 1998a, b, c; Ikeda et al. 2003; Koutsoumanis et al. 2004) have found that organic acids at 2% and 5% suppressed growth of the same pathogens at 12°C, and of *L. monocytogenes* and *Y. enterocolitica* during aerobic storage or storage in vacuum sealed packages at 4 to 10°C. However, Uyttendaele et al. (2001) found that treatment of beef tissues with 1% or 2% lactic acid/sodium lactate buffer solution did not affect survival of *E. coli* O157:H7 on beef stored (4°C) aerobically. Organic acid applications may also extend the shelf-life of treated meat, by delaying growth of meat spoilage bacteria. Lactic acid caused a shift in the dominant flora of aerobically stored meat from pseudomonads to yeasts and lactic acid bacteria (van Netten et al. 1997; Koutsoumanis et al. 2004). Moreover, treatments (25 or 55°C) of lamb or beef with 2–5% acetic or lactic acid delayed growth of the Gram-negative aerobic flora and to a lesser extend of lactic acid bacteria at −1 to 25°C (Anderson et al. 1988; Kotula and Thelappurate 1994; Goddard et al. 1996; Dorsa et al. 1997a, 1998a, b, c; van Netten et al. 1997, 1998; Koutsoumanis et al. 2004).

In conclusion, lactic acid spray rinses up to 5% to constitute an effective decontamination intervention for both immediate reduction of microbial contamination on carcass surfaces and meat as well as inhibition of growth in packaged meat. Application of lactic acid after water-washing is recommended in order to ensure long-term antimicrobial effects. Nonetheless, excessive use of organic acids may result in corrosion of meat processing equipment (Theron and Lues 2007). Currently, lactic acid decontamination is widely used in U.S. beef slaughtering plants.

Other Chemical Solutions

Chlorine is a well-known sanitizing agent, which is commonly used in the food industry for sanitation of equipment, utensils, and water supplies (Sofos and Smith 1998). It can also be added in the water used for final washing or chilling of carcasses. Chlorine solutions (20–50 ppm) applied at 4–23°C reduced presence of *Salmonella* and *Campylobacter* on chicken carcasses by 56% and 12%, respectively (Kelly et al. 1981; Tsai et al. 1995; Sofos and Smith 1998; Whyte et al. 2001; Park et al. 2002; Fabrizio et al. 2002; Stopforth et al. 2007; Bauermeister et al. 2008). On beef, the bacterial reductions achieved by 50–900 ppm chlorine (pure or electrolytically generated), or 200–500 ppm of acidified chlorine are of similar magnitude to those caused by water-washing (Emswiler et al. 1976; Kochevar et al. 1997b; Bosilevac et al. 2005). Effectiveness of chlorine treatments may be enhanced at high (74°C) compared to low (16°C) washing temperatures (Kochevar et al. 1997b). However, chlorine is considered a less effective decontamination agent than organic acids (Acuff 2005; Edwards and Fung 2006), whereas concerns have been expressed for corrosion of equipment, formation of harmful by-products when reacting with organic matter, and oxidation of meat color and lipids. Indeed, "available" chlorine is decreased by organic material (due to oxidizing-reducing reactions), while effectiveness is reduced at low temperatures, and it is highly affected by the pH of the solution, with maximum activity in the 6.0–7.0 range (Oomori et al. 2000; Edwards and Fung 2006; Kalchayanand et al. 2008).

Chlorine dioxide (ClO_2) is an alternative to chlorine, which is effective in controlling the natural flora occurring in poultry chill water, with no interference of pH (Tsai et al. 1995). However, it is not more effective in reducing microbial contamination on carcasses than chlorine or spraying with water alone (Cutter and Dorsa 1995; Stopforth et al. 2007). Furthermore, given the lack of information related to potential health risks of ClO_2 (Tsai et al. 1995), it has not been commercially applied as a decontaminant on beef, but, as indicated, it finds use in poultry chill tank water as well as vegetables.

Other chemical agents containing various forms of chlorine, such as 500–1200 ppm acidified sodium chlorite (ASC) and 100 mM sodium chlorate, have been shown to reduce bacterial pathogens, APC, and TCC in chilled meat or poultry carcasses and meat cuts (Kemp et al. 2000; Anderson et al. 2001; Gill and Badoni 2004; Lim and Mustapha 2004; Stopforth et al. 2007). Preliminary *in vitro* studies showed that 20 mM of a commercial sodium chlorite-based oxyhalogen disinfectant (Salmide®) caused 3–5 $\log_{10}$ CFU/cm^2 reductions of *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* in aqueous solutions, whereas when combined with ethylenediaminetetracetic acid (EDTA) the observed reductions were >6 $\log_{10}$ CFU/cm^2 (Mullerat et al. 1995). Spraying of beef carcass samples with ASC (1200 ppm for 10 s at 69 kPa at 22–24°C) activated with phosphoric or citric acid (final pH 2.5–2.9) decreased inoculated *E. coli* O157:H7 and *S.* Typhimurium counts by 4 to 5 $\log_{10}$ CFU/cm^2 and *L. monocytogenes* and *S. aureus* counts by 1–1.9 $\log_{10}$ CFU/cm^2 (Castillo et al. 1999b; Lim and

Mustapha 2004). A mixture of ASC with 0.5% CPC or 0.1% potassium sorbate yielded 1 $\log_{10}$ CFU/cm^2 additional reductions and suppressed growth of pathogens during storage of treated samples at 4°C for 14 days (Lim and Mustapha 2004). A commercially available compound (Sanova®) is comprised of 1200 ppm sodium chlorite and 2% citric acid. In general, chlorine compounds are recommended for use in poultry processing in order to keep contamination low in chilling water.

TSP is a bactericidal agent approved for the treatment of beef and poultry carcasses in the United States. The antimicrobial activity of TSP applied at 8–12% is attributed to its high pH (11–12) and its ability to reduce attachment of pathogens to carcass surfaces (Lillard 1994; Mendonca et al. 1994; Cabedo et al. 1996; Dorsa et al. 1998a, b, c). The latter effect may be further enhanced by combining 5% Tween 80 with 1% TSP for treatment of poultry skins (Hwang and Beuchat 1995). A patented solution of TSP (AvGARD™, Rhone-Poulenc), commonly applied at 8–12% and temperatures from 10–60°C (15–36 s), has been shown to reduce *Salmonella*, *E. coli* O157:H7, *L. monocytogenes, C. sporogenes*, and APC by 1 to >3 $\log_{10}$ CFU/cm^2, on chicken, beef, and pork tissues (Dickson et al. 1994; Lillard 1994; Gorman et al. 1995b; Dorsa et al. 1997a; Kochevar et al. 1997b; Xiong et al. 1998; Dorsa et al. 1998a, b, c; Cutter and Rivera-Betancourt 2000; Whyte et al. 2001). Wang et al. (1997) also demonstrated that increasing the water temperature from 10 to 60°C and the spraying pressure from 206.8 to 1034.2 kPa may improve the effectiveness of 10% TSP (30 s) in reducing *S.* Typhimurium on chicken skin. In conclusion, TSP appears to be effective in inhibiting bacterial attachment, thereby facilitating removal of bacteria by washing. It is not used for treatment of beef, but it may find some application in poultry processing (Sofos and Smith 1998; Whyte et al. 2001).

Aqueous ozone solutions have also been evaluated as decontamination interventions for meat. Ozone is a GRAS substance for use in foods in the United States (Sofos and Smith 1998). In general, water containing 0.5% to 0.95% ozone applied for 16–36 seconds at 24–36°C was not more effective in reducing APC, *E. coli* O157:H7, and *Salmonella* than knife-trimming or washing with pure water alone (Gorman et al. 1995b; Reagan et al. 1996; Castillo et al. 2003; Kalchayanand et al. 2008). Similarly, hydrogen peroxide at levels of 5% has been found only slightly more effective than water-washing and TSP (Gorman et al. 1995b; Cabedo et al. 1996; Reagan et al. 1996; Kochevar et al. 1997b). The antimicrobial effect of hydrogen peroxide is mainly attributed to the formation of radicals that damage fundamental cellular components, such as nucleic acids, lipids, and proteins. There have been contrasting reports on the effectiveness of peroxyacetic acid (PAA) as a decontaminant, although a commercial PAA-based solution (Inspexx 200™, Ecolab Company, St. Paul, MN) has been approved for application on carcasses in the United States. This product has demonstrated approximately 1 $\log_{10}$ CFU/cm^2 reductions when applied at 200 ppm at the post-evisceration stage (Gill and Landers 2003a, b). However, Penney et al. (2007) observed 3–4 $\log_{10}$ CFU/cm^2 reductions of *E. coli* O157:H7 after treatment with 180 ppm PAA. Conversely, under the conditions tested by Gill and Badoni (2004) and Ransom et al. (2003), 0.02–0.04% PAA had little effect on APC, TCC, and *E. coli*. In contrast to treatments with hydrogen peroxide and PAA, application of 1% CPC for 15 seconds at 35°C not only reduced APC and inoculated *E. coli* O157:H7 and *S.* Typhimurium by 6 $\log_{10}$ CFU/cm^2, but also totally inhibited their growth during storage of vacuum-packaged beef at 4°C (Cutter et al. 2000). Furthermore, two consecutive washings of cattle hides (for 3 and 1 min) with 1% CPC decreased prevalence of *E. coli* O157:H7 on

pre-evisceration carcasses from 23% to 3%, whereas APC and *Enterobacteriaceae* counts on treated surfaces decreased by 1 $\log_{10}$ CFU/cm^2 (Bosilevac et al. 2004a). In poultry decontamination, studies have shown that 0.1% or 0.5% CPC for 30 seconds to 3 minutes at 15–60°C reduced *S.* Typhimurium by 1.7 to 2.5 $\log_{10}$ CFU/cm^2, with reductions increasing with temperature, duration, and concentration of spraying solution (Kim and Slavik 1995; Wang et al. 1997; Xiong et al. 1998). Moreover, reductions of 5 $\log_{10}$ CFU/cm^2 in *S.* Typhimurium were obtained either by spraying 0.4% CPC for 3 minutes or 0.2% CPC for 10 minutes (Breen et al. 1997).

A variety of other antimicrobial substances have been proposed and evaluated with varying effectiveness for the decontamination of meat and poultry. Cutter and Siragusa (1994a) illustrated 1.79 to 3.54 $\log_{10}$ CFU/cm^2 reductions of beef spoilage bacteria and *L. innocua* by spraying with nisin solution of 5000 AU/ml. Bovine lactoferrin (LF) is an iron-binding protein, commonly found in milk, saliva, tears, seminal fluids, and secondary granules of neutrophils. "Activated lactoferrin" is an LF derivative through a patented process that has received a GRAS status in the United States for use on fresh beef (Naidu 2001, 2002). It is thought to interfere with bacterial adhesion on surfaces, causing detachment of microbial contamination (Naidu et al. 2003). Lactoferricin B is a peptide, deriving from hydrolysis of LF that binds to the outer membrane of Gram-negative bacteria, reported to inhibit *E. coli* O157:H7 at the minimum level of 8 μg/ml (Shin et al. 1998). USDA-FSIS has approved the use of LF either as 2% water-based antimicrobial spray, for decontamination of beef carcasses and parts, or as a spray that would deliver 1 g of LF per dressed carcass, followed by washing with temperate water and lactic acid rinse (USDA-FSIS 2008c).

Other potential decontaminants tested, with limited effectiveness, include gluconic acid at 1.5% and 3% (Garcia Zepeda et al. 1994); Carnatol™ (composed of copper sulfate petahydrate) and Timsen™ (composed of alkyldimethylbenzylammonium chloride) (Cutter et al. 1996); solutions (10% or 1.5% of commercial preparation) of sodium tripolyphosphate, monosodium phosphate, sodium acid pyrophosphate, or sodium hexametaphosphate; and 0.05–1.6% NaOH (Rathgeber and Waldroup 1995; Hwang and Beuchat 1995; Bosilevac et al. 2005), 85 ppm of peracetic acid mixture (comprised of peracetic acid and hydrogen peroxide; Bauermeister et al. 2008), 0.1% ammonium hydroxide, 1–4% SM and 0.005% acidic, or basic oxidized water (Hsu et al. 2004; Stopforth et al. 2005)

Multiple Decontamination Interventions

Application of combinations of hurdles of low intensity, or sublethal, may replace the use of single hurdles of higher or lethal intensity, thereby achieving the desired antimicrobial effect without compromising sensory quality or other properties. Multiple hurdles or interventions of sublethal intensity may be applied sequentially or simultaneously (Sofos and Smith 1998; Sofos 2005). Sequential decontamination treatments, combining physical and chemical methods, have been proven more effective compared to single treatments. Such a concept may improve the effectiveness of some physical methods, such as washing with cold water or knife-trimming, while using lower concentrations of chemicals in subsequent stages (Graves Delmore et al. 1998). For instance, Reagan et al. (1996) found that the combination of knife-trimming and washing of beef carcasses caused almost two-fold and six-fold higher reductions of the incidence of *Listeria* and *Salmonella*, respectively, compared with washing or trimming alone. This study formed the research hypothesis on sequential decontamination interventions. Then, Graves Delmore et al. (1998) validated the concept

by testing the efficacy of multiple spray-washing/rinsing treatments in the laboratory utilizing warm/hot water and/or acetic acid solution separately and in sequence to reduce microbial contamination on beef tissue inoculated with *E. coli*. Treatments applied achieved variable reductions of APC, *E. coli*, and TCC in the range of <1 to 1.7 or 4.3 $\log_{10}$ CFU/cm^2 (Graves Delmore et al. 1998). Furthermore, the combination of steam-vacuuming with hot (72 or 95°C at 24 bar for 5 s) and then cold (30°C) water, and/or 2% lactic acid (55°C, 11 s) increased log-reductions of aerobic bacteria, indicator organisms, and pathogens up to 4.3–5.3 $\log_{10}$ CFU/cm^2, as compared with 1–3 $\log_{10}$ CFU/cm^2 reductions obtained by application of single treatments (Dorsa et al. 1996b; Castillo et al. 1999a; Phebus et al. 1997). Likewise, application of chemical agents, such as 1.8% acetic acid, increased the decontamination efficacy of hot water (80°C) by 2 $\log_{10}$ CFU/cm^2 and also reduced the number of positive *E. coli* samples (Eggenberger-Solorzano et al. 2002). The impact of consecutive water-washings (i.e., first with hot and then with cold water) is weaker compared with the combination of hot water with chemical agents (Dorsa et al. 1996b; Stopforth et al. 2005). Moreover, the order of application of hot water and lactic acid is important for the microbial reduction and residual antimicrobial effects during storage (Castillo et al. 1998c, 1999a; Koutsoumanis et al. 2004). Koutsoumanis et al. (2004) found that application of hot water (75°C) first, followed by lactic acid (2%, 55°C; HW-LA), was more effective than the reverse sequence (LA-HW) in delaying growth of *L. monocytogenes* during storage (4, 10, and 25°C) of vacuum-packaged beef tissues. Possible reasons for HW-LA being more effective than LA-HW include cellular damage by heat and susceptibility of injured cells to subsequent acid exposure, while application of hot water after acid treatment may reduce residual acid levels on the surface of treated meat (Koutsoumanis et al. 2004). Likewise, Castillo et al. (1998c, 1999a) found fewer beef carcasses with *Enterobacteriaceae* above 1 $\log_{10}$ CFU/cm^2, or positive for *Salmonella* and *E. coli* O157:H7, when hot water was applied before lactic acid. However, delaying the application of lactic acid after water-washing further enhances the effectiveness of acid treatment by minimizing the dilution of acid concentration by the residual surface moisture (Gill and Landers 2003b).

Stopforth et al. (2005) performed a study simulating application of multiple decontamination treatments (each one for 30 s), including lactic acid, ammonium hydroxide, SM, and acidified or basic electrolyzed oxidized water, in order to reduce initial *E. coli* O157:H7 and *S.* Typhimurium contamination levels of 5.6–5.7 $\log_{10}$ CFU/cm^2 on beef tissue. Sequential applications involving use of SM or lactic acid application were the most effective, achieving 1.1–2.0 $\log_{10}$ CFU/cm^2 reductions (Stopforth et al. 2005). Furthermore, in a multiple-hurdle decontamination system of carcasses pre- and post-evisceration, as well as pre- and post-chilling, the combinations of either two lactic acid applications, or at least one hot and one lactic acid application, were more effective than multiple hot-water or single lactic acid sprays (Stopforth et al. 2005). More detailed examples of the performance of multiple decontamination interventions are provided in Table 3.1.

The above suggests that all the physical and chemical decontamination treatments described may be used as multiple interventions from the stage of animals arriving at the abattoir, during the dressing process, until chilling and fabrication, in order to reduce microbial contamination on hides, carcasses, and cuts. Currently, the U.S. beef industry uses sequential and simultaneous interventions, such as animal washing, knife-trimming, steam-vacuuming, pre-evisceration washing/rinsing, washing with hot water

or steam pasteurization, post-evisceration washing, chemical rinses, and chilling, that reduce contamination of *E. coli* O157:H7 from above 50% on animal hides to less than 0.5% on carcasses.

Chilling

At the end of the dressing process, carcass-chilling takes place in cold (−5 to 4°C) air chambers with or without intermittent spraying (or misting) carcasses with cold water for variable durations within 12–24 hours, while the total chilling process lasts 24–48 hours (Dickson and Anderson 1991; Hippe et al. 1991; Strydom and Buys 1995; Gill and Bryant 1997a; Gill and Jones 1997b; Greer and Jones 1997; Stopforth and Sofos 2005; Simpson et al. 2006). Spray-chilling has been adopted in various beef plants because it facilitates temperature reductions and reduces losses of carcass surface moisture and weight. Such a process may cause bacterial injury and death either due to localized freezing of surface moisture when cooling below 0°C occurs or due to the evaporative water losses on the carcass surface (Sheridan 2004; Simpson et al. 2006). However, chilling is a step for control of microbial growth rather than a decontamination intervention, since it relies mainly on cold temperatures, unless pre-chilling decontamination interventions have been applied, or antimicrobials are added into the spray-chilling water, which, combined with cold temperatures, may lead to the death of injured cells. Indeed, chilling of pig, lamb, and beef carcasses with cold water may increase numbers of psychrotrophs and inhibit growth of *E. coli* and coliforms (Gill and Bryant 1997a; Gill and Jones 1997b; Jericho et al. 1998). In a study monitoring the microbiological changes on the surface of carcasses after 22–36 hours of chilling with cold water to average temperatures of −1.4 to 2.5°C in four beef packing plants, the total aerobic counts on carcass surfaces increased by 1–2 $\log_{10}$ CFU/cm^2, whereas *E. coli* and coliforms counts remained stable (Gill and Landers 2003b). However, pre-chilling decontamination interventions, such as pre- and post-evisceration spraying with 2% lactic acid and steam or hot water pasteurization, markedly reduced the populations of *E. coli* and coliforms during chilling (Gill and Landers 2003b). The latter may be associated with a pre-chilling injury of cells due to the decontamination treatments and the subsequent death or inability of cells to repair their damage during chilling.

In addition to lactic acid, other chemical agents, such as 0.1 or 0.5% CPC, 0.05–0.1% ammonium hydroxide, 0.12% ASC, 0.02% PAA, 0.01% NaOH, or 0.005% sodium hypochlorite within 48 hours of chilling of beef carcass at 3°C to 1°C have been reported to reduce inoculated *E. coli* O157:H7 by 1 to 3 $\log_{10}$ CFU/cm^2, that is 0.5 to 2 $\log_{10}$ CFU/cm^2 higher than spray-chilling with water alone (Dickson 1991; Stopforth et al. 2004). Stopforth et al. (2004) evaluated the above chemicals in simulated spray-chilling of beef adipose tissue and found that CPC, followed by lactic acid, were the most effective chemical agents, followed by PAA and ASC. However, acid-habituated *E. coli* O157:H7 cells (in acidic washings of pH 4.12) remained at higher levels than non-acid-habituated cultures after 10 hours of spray-chilling with CPC and after spray-chilling with lactic acid and 48 hours of storage of chilled tissues at 1°C (Stopforth et al. 2004). Commercial evaluation of spray-chilling in three poultry processing plants showed that chlorine (20–50 ppm) and ClO_2 (500–1200 ppm ASC) spray-chilling caused up to 1.2 $\log_{10}$ CFU/cm^2 reductions of APC and TCC on poultry carcasses (Stopforth et al. 2007). A mixture of peracetic acid and hydrogen peroxide (PAHP), approved for use in poultry chillers in the United States, reduced *Salmonella* and *Campylobacter* positive poultry carcass samples by 92% and 43% exiting the chiller, respectively, when applied at 85 ppm in a

commercial facility (Bauermeister et al. 2008); PAHP mixture was more effective than 30 ppm chlorinated water.

Nonetheless, chillers may also be sites of cross-contamination. Studies have shown that chilling may increase the incidence of *Salmonella* on poultry carcasses (Stopforth et al. 2007) as well as *E. coli* counts on beef carcasses (Gill and Landers 2003b). Possible routes of contamination include the chiller exit contact surfaces, as well as contact between carcasses and conveyors (Stopforth et al. 2007; Gill and Landers 2003b). Therefore, post-chilling interventions may be useful for reduction of contamination before and after fabrication.

Post-Chilling Decontamination Treatments

Despite the reduction of microbial populations and pathogen prevalence by decontamination interventions during slaughtering, dressing, and chilling, surviving microorganisms or additional contamination may be present on carcasses, primal and sub-primal cuts, and trimmings (Gill and Bryant 1992; Bacon et al. 2002b). Thus, post-chilling decontamination treatments of whole carcass sides and cuts may be useful in further reducing microbiological contamination of meat and preventing pathogen proliferation during storage of packaged meat. For example, post-chilling spraying of beef carcass samples with 4% lactic acid (55°C, 30 s) reduced inoculated *Salmonella* and *E. coli* O157:H7 by 1.9–2.4 $\log_{10}$ CFU/cm^2 and decreased their populations during storage (4°C) of ground meat prepared from treated samples (Castillo et al. 2001b). Beef processors in the United States apply decontamination interventions of lactic acid and Inspexx 200™ or Sanova® on carcass sides as they exit the cooler and enter into fabrication.

Multiple sequential treatments, including hot water and air, as well as chemical solutions, have been suggested for post-chilling decontamination of carcasses and trimmings. Specifically, post-chilling sequential exposure of beef and pork trimmings to temperate (25°C, 75–180 s) or hot (65 or 82°C, 15–45 s) water, lactic acid (2% for 15–120 s or 5% for 30 s) and hot air (510°C, 75 s) reduced total bacteria and preserved ground products derived from these trimmings during refrigerated storage (Castelo et al. 2001a, b; Kang et al. 2001a, b; Stopforth et al. 2005). Pohlman et al. (2002a, b, c) found that single or double sequential treatments of beef trimmings with solutions of chemical antimicrobials, such as 5% acetic acid, 1% ozonated water, 0.5% CPC, 200 ppm ClO_2, and 10% TSP, may significantly reduce *Salmonella* and *E. coli,* TCC, and APC, while maintaining redness and fresh odor of ground beef produced from the treated trimmings during storage under retail display conditions at 4°C. Other antimicrobials, including low-molecular weight polylactic acid (2%) and nisin (2%) (Ariyapitipun et al. 2000), applied alone or in combination on beef cubes, have reportedly suppressed growth of *L. monocytogenes* during vacuum storage at 10°C.

Post-chilling interventions, applied following pre-chilling treatments, may increase the total reductions achieved by pre-chilling interventions. Specifically, Castillo et al. (2001b) found that pre-chilling water and lactic acid (2%) treatments reduced *Salmonella* and *E. coli* O157:H7 by 3 $\log_{10}$ CFU/cm^2, while subsequent post-chilling application of lactic acid (4%) by spraying increased total reductions to >5 $\log_{10}$ CFU/cm^2. Likewise, Stopforth et al. (2005) found that post-chilling application of 5% LA (55°C, 30 s) on beef carcass samples conferred additional reductions to those achieved by pre-chilling sequential treatments with warm (55°C), hot (82°C) water, and 1% SM (82°C), simulating pre- and post-evisceration of beef carcasses and pre-chilling decontamination interventions, respectively.

In general, lower water and acid spray temperatures, as well as shorter exposure durations compared with pre-chilling interventions, are recommended in order to avoid any negative impact on color and odor of cuts or trimmings (Castelo et al. 2001a; Kang et al. 2001a; Kotula and Thelappurate 1994; Table 3.1). Some discoloration may occur on the surface (to a 2–3 mm depth) of trimmings subjected to multiple decontamination treatments, especially in those including heat and acid. The discoloration of meat cuts by hot-water treatment depends on the quality of meat (normal, dark-firm-dry, or pale soft exudative), the type of tissue (i.e., muscle vs. fat, or pork vs. beef), and the rigor (i.e. pre- vs. post-rigor) state (Gill and Badoni 1997). However, the grinding process "dilutes" the surface color, and the resulting product has color similar to that of products from untreated trimmings (Kang et al. 2001b). The decontamination treatments are more effective on fat than on lean beef trimmings because the fat tissue allows higher microbial reductions (Castelo et al. 2001a, b), whereas its color is more stable than that of the lean tissue (Kang et al., 2001a). The decision as to the intensity of post-chilling hot water treatments is also dependent on the commercial use of the product. For example, in case of frozen patties destined for restaurants, discoloration by hot water is of limited commercial importance. Conversely, discoloration may be important for the acceptability of the product by consumers. In this respect, treatment of beef cuts (manufacturing beef) with hot water (85°C, 45 s) reduced APC by 2 $\log_{10}$ CFU/cm^2 without any detectable effect on the color and flavor of frozen beef patties produced from the treated meat (Gill et al. 2001).

Overall, application of decontamination treatments on trimmings may contribute to immediate microbial reductions and residual antimicrobial effects during aerobic storage or storage of trimming in vacuum sealed packages (Castelo et al. 2001a, b; Castillo et al. 2001b; Kang et al. 2001b). However, post-chilling interventions that may change the properties of the product require change in the labeling requirements and the product may have to be presented with a name more complex than "meat," unless the treatments do not cause substantial changes in meat properties and thus can be designated as a "processing aid."

Overview of Practical Improvements Achieved by Decontamination

The Pathogen Reduction final rule (USDA-FSIS 1996c) established microbial testing for meat plants to assess the effectiveness of the control measures undertaken to prevent and reduce contamination of carcasses with fecal material, hair, ingesta, and associated bacteria. The established criteria for *E. coli* and *Salmonella* were based on national baseline studies (1992–1993) that had collected data from various meat plants (USDA-FSIS 1994, 1996b). The adoption of HACCP principles (USDA-FSIS 1996c) and associated critical control points applied as decontamination interventions constitute important measures for control of carcass contamination. Following implementation of decontamination strategies in the U.S. beef plants, microbial testing of carcasses demonstrated significant improvements in the hygiene of the slaughtering process. Specifically, FSIS performed a national baseline data collection program (1997–1998) for cattle and swine carcasses from approximately 1,400 and 1,250 establishments, respectively (USDA-FSIS 1998a, b). Prevalence of *E. coli* and *Salmonella* in 1,881 cattle samples was 16.6% and 1.2%, respectively, and 44.1% and 6.9% in 2,127 swine samples. Of the samples that were positive for *E. coli*, 98.9% of cattle and 91.5% of swine samples had *E. coli* between 0–10 CFU/cm^2. Furthermore, a three-year survey (1998–2000) of 98,204 samples of various meat and poultry prod-

ucts, including broilers, cows, bulls, steers, and heifers, as well as ground beef, ground chicken, and ground turkey collected from large to very small federally inspected U.S. establishments, showed that the prevalence of *Salmonella* was significantly lower after than before the implementation of HACCP (Rose et al. 2002). The above results also showed significant compliance with performance criteria for *E. coli* and standards for prevalence of *Salmonella* (USDA-FSIS 1996c; 1998a, b; Rose et al. 2002). The prerequisites/HACCP verification testing program for *Salmonella* in raw meat (cows, bulls, steers, heifers, hogs) and poultry products in the period 1998–2007 showed that the average percentage of samples from different product categories, such as carcasses, cuts, or ground products, meeting the performance standards for prevalence of *Salmonella* exceeded 90% (USDA-FSIS 2008b). However, the presence of pathogenic bacteria on the surface of carcasses, even though of low prevalence, emphasizes the need for proper refrigeration, handling, and cooking of meat products before consumption. Testing of raw ground beef for *E. coli* O157:H7 from 1994 to 2007 showed an average percentage of less than 0.5% for positive samples from federal establishments and retail stores, and a decreasing trend of *E. coli* O157:H7 prevalence below 0.20% after 2003, in samples taken for the U.S. ground beef testing program (USDA-FSIS 2008a). In order to keep the percentage of positive raw ground beef samples low, FSIS considers it extremely critical to keep the percent of positive ratings for beef trimmings low. Therefore, routine verification sampling of beef manufacturing trimmings intended for use in raw ground beef or beef patty products has also been initiated at the slaughter establishments that produce such trimmings. The results of a one-year (December 2005–January 2007) study for approximately 1,700–1,900 samples of beef trimmings showed that the prevalence of *E. coli* O157:H7 and *Salmonella* was 0.68% and 1.28%, respectively, suggesting implementation of effective decontamination strategies (USDA-FSIS 2007). In all positive samples, pathogens were below 3 MPN/cm^2.

Microbial surveys have also been carried out by individual research groups in slaughtering plants (for steers, heifers, lambs, cows, and bulls) to evaluate whether and how much the applied control measures increased the probabilities of carcasses passing the imposed performance criteria (Sofos et al. 1999b, c, d; Bacon et al. 2000, 2002a, b; Elder et al. 2000; Duffy et al. 2001; Arthur et al. 2004). Some of these studies (Sofos et al. 1999b, c, d) were carried out in 1995–1996, while the industries were preparing to operate under HACCP, and thus, could be used to compare and evaluate the effectiveness of the control strategies to be applied. The establishments involved in these surveys used either single or sequential decontamination interventions. Pre-evisceration interventions (in the following order of application) included: (i) steam-vacuuming; (ii) carcass-washing with water (21–38°C) or organic acids (acetic or lactic acid 1.6 to 2.6%, 43–60°C); and, (iii) hot water (71–77°C) or steam pasteurization. Post-evisceration interventions included: (i) organic acid solution rinsing; (ii) pre-chilling carcass-washing (final washing); and, (iii) 24-hour chilling. Reductions in the magnitude of 3–4 $\log_{10}$ CFU/cm^2 were obtained for total bacterial counts, TCC and *E. coli* starting immediately after hide removal and before any decontamination until the end of chilling (Bacon et al. 2000). Regarding the animal type, *Salmonella* incidence after chilling was higher on cow-bull (0.5–4.4%) than on steer-heifer (0–2.2%) carcasses (Sofos et al. 1999c). The percentage of samples passing the performance criteria for *E. coli* counts ($m < 5$ CFU/cm^2) increased from 68.3% at pre-evisceration to 96.2% after final washing (Sofos et al. 1999b, d). It was more difficult to meet the performance criteria during the wet (November through January) than the

dry (May through June) season (Sofos et al. 1999b, c, d). Likewise, total bacteria on lamb carcasses were lower in the spring than in the winter (Duffy et al. 2001). Elder et al. (2000) and Arthur et al. (2004) in their survey in four and two meat processing plants, respectively, indicated that sanitary procedures and post-evisceration antimicrobial interventions could reduce the prevalence of *E. coli* O157:H7 on carcasses from 20.1–43.4% pre-evisceration to 0–1.8% post-processing. Similarly, Barkocy-Gallagher et al. (2003) found that the percentage of positive *E. coli* O157:H7 and *Salmonella* carcass samples was reduced from 26.7% and 12.7% pre-evisceration to 1.2% and 0.1%, on dressed carcasses, respectively; that is after interventions with chemicals and chilling. Finally, a hot-water (80°C) decontamination system, consisting of a stainless steel spraying cabinet and a recirculation water system, was effective in reducing microbial contamination on carcasses and gained approval by FSIS for commercial use in beef slaughter (Sofos and Smith 1998). Of the aforementioned in-plant decontamination interventions, water/steam pasteurization and spraying with lactic acid are considerably more effective in reducing TCC and *E. coli* than water-washing (Gill and Landers 2003a). Furthermore, combination of lactic acid rinses of carcasses, primal cuts, and contact surfaces for carcass fabrication may reduce the numbers of total bacteria, coliforms, and *E. coli* on carcasses and the processing environment, thereby improving the sanitary conditions of the plant (Bacon et al. 2002a).

The reduction of *E. coli* O157:H7 during the dressing process has been demonstrated in establishments using the best intervention strategies and processing techniques. Pre-harvest strategies that may reduce the prevalence in feces, as well as additional measures to prevent pre-evisceration contamination, would also markedly improve the microbial safety of the final product (Elder et al. 2000). Arthur et al. (2004) observed high correlation ($r>0.99$) between hide and pre-evisceration carcass contamination. Therefore, the establishments should apply prerequisite programs, including good manufacturing practices, sanitation standard operating procedures (SSOPs), HACCP programs, and carcass-decontamination interventions, so that carcasses enter the chillers with reduced contamination (USDA-FSIS 2002). However, even the best decontamination technologies require the foundation of a good plant design and hygienic process control (Sofos et al. 1999a).

Potential Concerns and Risks Associated with Decontamination

An important concern of organic acid decontamination is the potential for selection of strains that may be able to adapt and develop acid resistance. Subsequently, such strains may colonize equipment surfaces, recontaminate carcasses, and resist subsequent decontamination treatments (Samelis and Sofos 2003, 2005). Berry and Cutter (2000) showed that acid-adapted *E. coli* O157:H7 was more resistant against acetic acid sprays than non-adapted cells. Similarly, acid-habituated *E. coli* O157:H7 was more resistant to spray-chilling of beef with chemical solutions than nonhabituated cells (Stopforth et al. 2004). Furthermore, there is a potential risk for extended survival of *E. coli* O157:H7 in environmental niches, where acidic decontamination runoff fluids are mixed with water, forming sublethal pH environments (pH 4.5–5.0). These environments may allow development and maintenance of acid resistance by *E. coli* O157:H7 with increased potential of growth initiation compared to cells that have not been exposed to such adverse conditions (Samelis et al. 2002, 2004; Stopforth et al. 2007; Skandamis et al. 2007, 2009). Such situations may also harden bacterial biofilms on equipment surfaces and render them less sensitive to sanitation agents (Stopforth et al.

2003). Moreover, even though cells exposed to acidic environments formed by diluted organic acid run-off fluids may be injured by sanitizing agents and be undetectable with common plating methods, they may recover and restore their acid resistance during subsequent exposure to fresh meat decontamination run-off fluids (Skandamis et al. 2009). However, the role of acid adaptation on microbial resistance to decontamination treatments possibly depends on the microorganism and product storage conditions, since acid adaptation of *L. monocytogenes* did not seem to affect the survival and proliferation of the organism during storage at 10°C of beef treated with hot water and/or lactic acid (Ikeda et al. 2003). An additional concern is that organic acid treatments may alter the natural flora of the beef carcass, thereby reducing the competitive effect of the background flora against enteric pathogens, or allowing proliferation of acid tolerant organisms, such as lactic acid bacteria or yeasts and molds, and thus, altering the spoilage association in fresh meat (Ikeda et al. 2003; Samelis et al. 2002).

Concerns were expressed at the European level about the development of antimicrobial resistance to chemical agents that may be used for carcass decontamination. Thus, the European Food Safety Authority (EFSA) issued an opinion paper on the potential for pathogens acquiring "reduced susceptibility" to ClO_2, ASC, TSP, and PAA applied for removal of meat surface contamination, or the potential to develop "resistance to therapeutic agents" as a result of exposure to the above biocides (EFSA 2008). According to this opinion, there was no existing evidence that proper use (in terms of concentration) of the aforementioned biocides for carcass decontamination would result in reduced susceptibility in resistance to therapeutic agents. Similarly, it has been reported that disinfecting poultry chiller water with 20 ppm ClO_2 is of negligible risk to human health, due to its reduction of chlorite and chlorate (Tsai et al. 1995). However, excessive doses of the above antimicrobials may allow for antimicrobial resistance (EFSA 2008). Furthermore, *in vitro* studies suggest that exposure of pathogens to sublethal levels of certain meat decontamination chemical agents may induce tolerance to lethal levels of the same biocides, as well as to other (heterologous) stresses (cross-resistance or protection). Specifically, Sampathkumar et al. (2004) found that pre-treatment of *S. enterica* serovar Enteritidis with sublethal levels of TSP (i.e., 1.5%) increased tolerance to higher TSP concentrations, and conferred cross-tolerance to heat (55°C) and alkaline pH (11.0). However, evidence for such cross-tolerance *in situ* is still lacking.

Given that several *Salmonella* strains implicated in foodborne outbreaks have been identified as resistant to multiple antibiotics, the hypothesis that such strains are also resistant to antimicrobial interventions in beef processing has been tested. Bacon et al. (2002c) performed a survey for antibiotic resistant *Salmonella* strains in eight beef-packing plants and found that even though the prevalence of *Salmonella* was reduced from 15.4% on hides to 1.3% on carcasses, approximately 60% of the isolates were resistant to at least one antibiotic. Moreover, the isolation of two antibiotic resistant *Salmonella* strains from carcasses after final washing suggested potential resistance to decontamination interventions (Bacon et al. 2002c). However, studies have found no correlation between susceptibility to antimicrobial agents and resistance to heat (55–61°C) or low pH (2.3 or 3.0) stress (Bacon et al. 2003a, b). Likewise, Arthur et al. (2008) found that the susceptibility of *Salmonella* to various meat decontamination agents, including 2% acetic or lactic acid, electrolyzed-oxidizing, and ozonated (6 ppm) water as well as commercial acid (pH 1.6) products, was not influenced by their multidrug resistance status. Similarly, the ability of certain *E. coli* O157:H7 strains to cause human disease was

not associated with resistance to the above antimicrobial interventions (Arthur et al. 2008).

In conclusion, even though existing laboratory data suggest that acid decontamination interventions may increase the potential of pathogens to develop acid resistance, there is no clear evidence that chemical decontamination poses additional risks due to faster pathogen growth or higher acid resistance during storage of products, compared with physical decontamination. Furthermore, multidrug resistant pathogens are not more resistant to decontamination treatments than susceptible strains. Therefore, the proper use of chemical rinses may lead to significant reduction of pathogens on meat without raising concerns associated with stress-adapted pathogens, provided that hygienic and sanitary practices are applied throughout the processing chain, as zero tolerance inspection assures in the United States.

Legislative Aspects of Decontamination

Two approaches are applied worldwide relative to microbial control during meat production (Bolton et al. 2001; Midgley and Small 2006): (i) the "intervention HACCP"; and, (ii) the "nonintervention HACCP." The intervention HACCP uses decontamination interventions along the production line to reduce microbial contamination and is adopted by the United States and many meat-processing plants in Canada and Australia. More specifically, the USDA-FSIS has recognized and approved that one or more physical or chemical decontamination steps should be included in the slaughter/dressing process as critical control points under HACCP. In Canada, the use of lactic and acetic acid sprays is approved as part of the Good Manufacturing Practices (GMP) during the carcass-dressing process, provided that proper facilities, equipment, and quality control are available (Theron and Lues 2007; http://www.inspection.gc.ca/english/fssa/meavia/man/mane.shtml). In Australia, beef plants exporting meat to the United States have used lactic acid decontamination technologies (Smulders and Greer 1998). However, no organic acid-treated meat is exported to Europe. Finally, even though chemical hide washing is approved in Australia, chemical dehairing is not allowed (Midgley and Small 2006), while in the United States chemical dehairing needs to be approved on a case-by-case basis; no application exists at this time.

The nonintervention HACCP relies on monitoring, and application of hygiene measures (strict adherence to GMP) that prevent occurrence of contamination (Bolton et al. 2001). It is currently adopted by the European Union (EU) meat industry, even though the new EU regulations have approved or provide the basis for approval of decontamination interventions. More specifically, the existing Regulation 852/2004 on food hygiene (Commission of the European Communities 2004a) and the need for implementation of HACCP principles in the entire food chain have forced establishments to improve their hygiene and processing procedures, as well as to verify and validate their systems. Furthermore, according to Regulation 2160/2003, proper and effective measures should be taken for detection and control of zoonotic agents throughout the food chain (Commission of the European Communities 2003). In principle, such methods include hygienic practices during feed production, at the farm level and during transportation of the animals, good animal husbandry practices, record-keeping, and traceability (Commission of the European Communities 2003). Application of strict hygiene measures and good slaughter practices may be considered sufficient to avoid problems associated with carcass contamination during slaughter under conditions of slow slaughter speeds. In addition to these practices, thermal decontamination interventions, such as hot water and steam pasteurization are also per-

mitted and potentially used as critical control points in HACCP systems applied in slaughtering operations within the EU. Moreover, carcass-chilling involving application of cold air and reduction of surface water activity at the end of the dressing processes is a mandatory practice worldwide (Bolton et al. 2002). Chemical decontamination treatments have not yet received official approval in the EU, even though Regulation 853/2004 (Commission of the European Communities 2004b) permits the use of substances other than potable and clean water for decontamination of surfaces of foods of animal origin intended for human consumption. Moreover, in agreement with U.S. authorities, EFSA suggests the use of chemical decontamination as a supplementary (not primary) measure to reduce and control microbial contamination of carcasses, as part of an integrated control program (EFSA 2008). Permission for use of chemical decontaminants under EU legislation is provided when preceded by a thorough scientific evaluation in collaboration with EFSA for the impact of suggested chemicals on public health (EFSA 2008). Lack of such information has precluded the approval of chemical decontamination in the EU so far. In this respect, EFSA encourages further research on the scientific evaluation of the efficacy of antimicrobial treatments, their toxic effect on humans, as well as the potential for development of resistant clones.

Future Trends

In view of demands by consumers for high-quality, natural, nutritious, fresh in appearance, and convenient meat products that maintain their freshness for extended periods, alternative mild methods for improving safety of meat have been developed and evaluated. The potential for adaptation of pathogens and the development of resistance to current decontamination technologies further necessitate the development of new methods. An overview of such methods can be found in the book chapter by Guan and Hoover (2005) and the review article by Aymerich et al. (2008). More specifically, these technologies include high hydrostatic pressure (HHP; 300 to 600 MPa, 2–10 min), irradiation with 1.5–5.5 kGy, pulsed electric fields, shock waves, high-intensity light, carbon dioxide treatment (supercritical CO_2), ultrasonics, gas plasma (ionized gas) treatment, and oscillating magnetic fields (2–100 tesla; Tinney et al. 1997; Arthur et al. 2005; Aymerich et al. 2008; Guan and Hoover 2005). Most of these treatments are still in the experimental stage and may require a long time and work before they find commercial application as meat decontamination technologies. However, HHP at 600 MPa for 2 to 10 minutes has been commercially used in meat products, such as ham and pre-cooked meals, as well as chicken and pork cuts, to control *L. monocytogenes* during storage at 4°C (Hugas et al. 2002; Garriga et al. 2004; Guan and Hoover 2005). In addition to the lethal effect on bacteria, HHP does not compromise the nutritional characteristics of the product (Aymerich et al. 2008). Nevertheless, this method is not applicable on carcasses or big pieces of meat.

Although studies have demonstrated the effectiveness of irradiation in reducing pathogens on fresh meat and it is approved in the United States, the process has been commercially applied only to a limited extent, due to consumer concerns for potential adverse health effects. Presently, a petition is pending in the United States for use of irradiation to decontaminate carcasses after dressing. On the other hand, active packaging systems, including antimicrobial coating or incorporation of agents in the packaging film, may control microbes during product storage (Aymerich et al. 2008; Coma 2008). Antimicrobials to be potentially used in such packaging systems include compounds of plant, microbial, or animal origin, such as

essential oils, nisin, and lactoferrin, which also meet the demands of consumers for more natural products. Such emerging antimicrobial treatments, in combination with good hygiene practices and efficient control measures during slaughtering (such as decontamination interventions), may further enhance meat safety. However, further research is needed in order to evaluate the effect of such interventions on the sensory properties of foods and the feasibility of their application on an industrial scale. Furthermore, prior to application of novel decontamination strategies, the potential of such interventions to induce microbial resistance through adaptation should also be considered.

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Chapter 4

Aging/Tenderization Mechanisms

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Introduction

Humans (both men and women alike) continue to look for ways to slow down, control, or virtually eliminate the aging process, often looking for the infamous "fountain of youth." Scientists (Strehler 1986) from the Molecular Biology Division at the University of California, Los Angeles, have demonstrated that gene loss is the primary cause of the human aging process. On the contrary, the "aging" of meat is viewed positively, and the practice of storing meat for extended periods of time after the death of an animal, in order to improve its texture, has been a practice followed by many for a very long time.

Most consumers do not understand the basic notion that all meat—red, white, or pink—is muscle. Its origin is muscle, which converts over time into meat, and this conversion is by no means instantaneous. The widely held view that postmortem storage of meat at refrigerated temperatures (referred to as aging) results in a significant improvement in meat tenderness has been adhered to for a very long time, and aging still remains an important method used for producing tender meat. The first scientific reports looking at postmortem tenderization of meat were those of Bouley (1874) and Lehman (1907), who reported that there was an increase in meat tenderness during extended postmortem storage. Hoagland et al. (1917) provided indication of protein breakdown by showing that there was an increase in noncoagulable nitrogen during the storage of meat. Postmortem "aging" has been called "conditioning" or "ripening" and is a natural process when meat is subjected to controlled refrigerated storage conditions. While meat from any species could be aged, postmortem aging is generally limited to beef, due to the relative youth of pork, lamb, and veal at the time of slaughter. Muscle from poultry carcasses also undergoes aging, but the time course for aging in poultry muscle is hours and minutes as compared with days for beef.

Tenderness development in postmortem skeletal muscle is a very complex phenomenon. Immediately post-exsanguination, muscle is soft and tender until the onset of rigor mortis causes irreversible linkages between the actin and myosin proteins, which cause the meat to toughen. With aging, however, protein degradation and ultrastructural changes occur that cause tenderization of the meat. During this tenderization phase, degradation of myofibrillar and cytoskeletal proteins such as troponin, desmin, vinculin, dystrophin, nebulin, and titin occurs within the muscle (Taylor et al. 1995; Robson et al. 1997). As these proteins degrade, muscle ultrastructure changes as: (1) breaks at the junction of the I-band and Z-disk occur, (2) Z- to Z-line attachments are disrupted by the degradation of intermediate filaments, and (3) Z- and M-line attachments to the sarcolemma are disrupted. Ultimately, the ultrastructural changes that occur within muscle during aging are thought to be the result of enzymatic degradation of myofibrillar and

cytoskeletal proteins. Thus, the purpose of this chapter is to provide an overview of the established theory of enzymatic aging tenderization and to review recent developments that contribute to a more complete understanding of the underlying mechanisms that influence postmortem proteolysis and the aging tenderization of meat.

Enzyme Systems

Skeletal muscle contains numerous enzymes that drive various metabolic pathways in the living tissue. Many of these enzyme systems are thought to remain active in postmortem muscle and influence meat-quality development. In particular, the cathepsin, calpain, and proteasome enzyme systems have been extensively studied to determine their roles in the proteolysis associated with the aging tenderization of meat.

Cathepsins

Cathepsins are acid proteases usually located in the lysosomes (DeDuve et al. 1955) and in phagocyte cells but have also been found in the sarcoplasmic reticulum of muscle cells (Allen and Goll 2003). Ultrastructural studies indicate lysosomes are prevalent in fetal muscle tissue but occur much less frequently in adult skeletal muscle. Cathepsins are distinguished by their active sites (aspartic, cysteine, and serine proteases) and substrate specificity. Over 15 lysosomal cathepsins have been identified, but only eight (B, L, H, S, F, K, D, E) have been found in skeletal muscle fibers (Barnier 1995; Hopkins and Thompson 2002; Sentandreu et al. 2002).

Cathepsins often are not considered as important in meat tenderization because their membrane-bound location is thought to limit substrate accessibility. Lysosomes are incapable of engulfing the myofibril structure and no myofibrillar fragments have been identified within the lysosome. On the other hand, lysosomes have fragile membranes that may rupture with the decreasing temperature and pH in the muscle during postmortem storage. Only cathepsins B, D, H, and L have been found to degrade the same proteins as observed during postmortem aging (Etherington 1984; Etherington et al. 1987, 1990; Prates 2002; Sentandreu et al. 2002; Li et al. 2008). Inhibition of cathepsins B and L, however, does not prevent tenderization (Hopkins and Thompson 2001). Cathepsin B degrades myosin, and actin to a lesser extent, while cathepsin D degrades both actin and myosin into small peptide fragments. Cathepsin L acts on myosin, actin, α-actinin, troponin-T, and troponin-I. Both an endo- and exopeptidase, cathepsin H is reported to act on myosin (Allen and Goll 2003). Although cathepsins probably do not account for the bulk of postmortem proteolysis during aging, their contributions cannot be completely discounted.

Calpains

Calpains are Ca^{2+}-dependent cysteine proteases with optimal activity at neutral pH and are found in all kinds of living organisms, including animals, plants, fungi, and bacteria. Discovered about a decade after cathepsins (Guroff 1964), calpains have been extensively researched and the subject of many reviews on muscle proteolysis (Goll et al. 1992, 1998, 2003; Geesink et al. 2000; Ilian et al. 2001; Friedrich and Bozóky 2005; Geesink et al. 2005; Koohmaraie and Geesink 2006; Geesink and Veiseth 2009). Currently, at least 15 different calpains have been identified in mammals (Suzuki et al. 2004). Six different calpains are expressed as mRNA in the mammalian skeletal muscle, but only μ- and m-calpains and p94/calpain 3 isoforms can be detected at the protein level (Sorimachi et al. 1990; Spencer et al. 1995; Sorimachi and Suzuki 2001; Huang and Wang 2001). The Ca^{2+} requirements, optimum pH and

temperature of activity, autolysis, and inhibitors of calpains have been extensively reviewed (Allen and Goll 2003; Geesink and Veiseth 2009). Calpain 1, or μ-calpain, requires micromolar (10–50 μM) calcium concentration for full activity. Calpain 2, m-calpain, is activated at 0.3–1.0 mM Ca^{2+}, while the Ca^{2+} requirement of p94/calpain 3 is reported to be at submicromolar levels (Branca et al. 1999; Ono et al. 2004). Chicken muscle expresses a distinct μ/m-calpain, intermediate to μ- and m-calpains in Ca^{2+} requirements for activation (Sorimachi et al. 1990; Sorimachi and Suzuki 2001; Lee et al. 2008). In postmortem chicken muscle, μ/m-calpain activity remains steady during aging, in contrast to μ-calpain activity, which disappears by 6 hours postmortem (Lee et al. 2008). From this finding it was postulated that chicken muscle tenderizes more rapidly than beef due to greater activation of the calpain system (Lee et al. 2008).

Ubiquitous μ- and m-calpains are heterodimers that dissociate in the presence of Ca^{2+} into a regulatory 28 kDa subunit that is identical in both isoforms (Carafoli and Molinari 1998) and into a large catalytic subunit (~80 KDa). It is the catalytic subunit (Dutt et al. 2002) that dictates the Ca^{2+} level for activation. The μ- and m-calpains are located in the sarcoplasm and are concentrated around the Z-disk region bound to their inhibitor, calpastatin (Allen and Goll 2003). The equilibrium binding of calpains to calpastatin also is Ca^{2+}- and pH-dependent, with binding decreasing as pH decreases (Dransfield 1993). Moreover, the Ca^{+2} required for calpains to bind to calpastatin is significantly lower than that for activating μ-calpain proteolysis (Cong et al. 2000, 2002).

The p94/calpain 3 isoform, unique to skeletal muscle, is not fully inhibited by and can degrade calpastatin (Ono et al. 2004); however, its role in postmortem aging is not fully understood (Parr et al. 1999; Ilian et al. 2000, 2001a, b, 2004; Stevenson et al. 2002; Geesink et al. 2005). Although the mRNA levels for p94 are 10 times greater than μ- and m-calpain mRNA (Kinbara et al. 1998), purification from muscle is difficult because it is highly unstable. The p94 has both a cysteine protease domain and a calmodulin-like Ca^{+2} binding domain in the same polypeptide chain that binds to the N2A and M-line regions of titin (Ojima et al. 2007). The endogenous N-terminal (but not C-terminal) domain of p94 is localized in the Z-bands and also directly binds to sarcomeric α-actinin, suggesting incorporation of proteolytic fragments into the Z-bands. It has been suggested that p94 protects titin from degradation by μ- and m-calpains (Ojima et al. 2007; Beckmann and Spencer 2008; Hayashi et al. 2008). Because p94 is active even in the absence of Ca^{2+} (Sorimachi and Suzuki 2001), it is thought to have a role in the regulation of μ- and m-calpain activity or be a negative regulator of apoptosis (Goll et al. 2003).

Less than 10% of total calpain is normally activated in the skeletal muscle (Goll et al. 2003). Research has demonstrated that the optimal condition for calpain activity is pH 7.5 at 25°C (Zeece et al. 1986), but activity is still detected at pH 5. Meat tenderization begins at about pH 6.3 (approximately 6 h postmortem in beef), as μ-calpain is activated at low Ca^{+2} concentrations. M-calpain, or calpain 2, is optimally active at pH range of 6.5 to 8.0 and 1–2 mM Ca^{2+} but shows minimal activity at pH 5.5 and 5°C, conditions achieved in 24 to 48 hours postmortem in the beef carcass. It is estimated that approximately 30% of m-calpain remains inactive and can be detected up to 56 days postmortem (Geesink and Koohmaraie 1999). With this limited postmortem activity range and reported discrepancies between *in vitro* and intracellular Ca^{2+} concentrations, some researchers doubt that calpains alone are responsible for aging of meat. Furthermore, purification of calpains and calpastatin is difficult to accomplish, and techniques for mea-

suring calpain under different pH/temperature and ionic strength combinations may not provide an accurate estimation of activity because of precipitation of calpain or the alteration of the interaction with substrates. These issues have been the topic of much debate in the literature (Prates 2002; Goll et al. 2003; Geesink and Veiseth 2009), but the prevailing belief is that μ-calpain is the essential and predominant enzyme responsible for postmortem proteolysis and that combined m- and μ-calpain activity may be responsible for up to 85% of postmortem meat tenderization (Geesink et al. 2000; Geesink et al. 2006)

Proteasomes

Evidence has been accumulating that calpains are necessary to initiate the degradation of myofibrillar proteins by releasing them from the surface of the myofibril and making them available for subsequent degradation. Given that calpains cleave proteins at a limited number of sites and produce large polypeptide fragments rather than small peptides or amino acids, it is clear that other proteases may be involved in the bulk degradation of sarcomeric structures (Goll et al. 2003). For the subsequent breakdown of myofibrillar proteins, once calpains have released them from the sarcomere, the main candidate is the proteasome (Attaix et al. 1998, 2001; Delbarre-Ladrat et al. 2006; Yamamoto et al. 2009; Geesink and Veiseth 2009).

The proteasome, or multicatalytic proteinase complex (MCP), is a multisubunit protease complex with an apparent sedimentation coefficient of 20S. Two types of regulatory complexes bind to both ends of the cylindrical 20S. One complex, the 26S proteasome, is a eukaryotic ATP-dependent protease (Tanaka 1998) and hydrolyzes ubiquitin-conjugated proteins (Tanaka 1998; Delbarre-Ladrat et al. 2006). The proteasome, first isolated in 1980 (Wilk and Orlowski 1980), is a barrel-shaped polypeptide structure with active sites in its interior core (Mykles and Harie 1995; Attaix et al. 1998, 2001; Glickman and Cienchanover 2002). A 20S latent form is part of the 26S proteasome (Attaix et al. 1998, 2001) and can be activated by mild denaturing treatments (Yamamoto et al. 2009) such as heat, chemicals, or high pressure. At least five multipeptidase activities (Mykles and Harie 1995) have been described: trypsin-like, chymotrypsin-like, branched-chain amino acid-preferring, small neutral amino acid-preferring, and peptidylglutamyl peptide hydrolase (PGPH). The MCP proteasome enzymes have optimal pH activity at pH 7.0–8.0, and the proteasome is found in the sarcoplasm (Foucrier et al. 2001) of skeletal muscle. Control of indiscriminate proteolysis appears to be regulated by two methods. The MCP preferentially degrades polypeptides that have been ubiquitinated and secondly by physical size limitation; that is, only polypeptides that can pass through the narrow 10–13Å opening to the central core of the barrel are easily degraded into 6–12 amino acid fragments in a single pass (Attaix et al. 1998, 2001; Glickman and Cienchanover 2002). It is likely that another protease, calpains for example, acts in concert or synergy to release proteins from the myofibrillar assembly in order to make large proteins available for degradation into amino acids by the MCP (Hasselgren 1999; Allen and Goll 2003). The MCP plays a major role in degrading sarcoplasmic proteins and myofibrillar fragments; however, there is insufficient evidence that MCP breaks down the same proteins in postmortem muscle as in in vitro tests (Huang et al. 2007). Proteasomes remained relatively stable throughout 7 days of aging in beef and rabbit muscle (Yamamoto et al. 2009), supporting their potential role in meat tenderization.

Current Enzymatic Model of Postmortem Proteolysis and Aging Tenderization

In order to identify enzymes responsible for the postmortem aging of meat, researchers have used the criteria that candidate enzyme systems must: (1) be endogenous to skeletal muscle and have access to substrates, and (2) have the ability to degrade the same proteins that are degraded during the postmortem storage of muscle (Goll et al. 1983, 2003, 2008; Koohmaraie 1996). Of the three major enzyme systems investigated, only calpains meet both criteria. Calpains have access to substrates and have been shown to have limited proteolytic capabilities (Goll et al. 2003), cleaving myofibrillar proteins at a specific number of sites to produce large polypeptide fragments similar to those observed after the postmortem storage of muscle. In contrast, lysosomal cathepsins and proteasomes are capable of exhaustively degrading proteins into small peptides or short amino acid segments but cannot disassemble the myofibril and do not generate the same degradation patterns of myofibrillar proteins observed during meat aging. Furthermore, the location of cathepsins in lysosomes is thought to restrict their access to substrates. Thus, the prevailing theory is that the calpain/calpastatin system is the predominant driver of postmortem proteolysis and aging tenderization.

While it is widely accepted that proteolysis of key myofibrillar proteins by the calpain enzyme system is primarily responsible for increased tenderness during postmortem storage (Koohmaraie et al. 1991; Uytterhaegen et al. 1994; Goll et al. 2003; Koohmaraie and Geesink 2006), it can be argued that calpains alone are not sufficient to fully explain postmortem proteolysis and meat tenderization. Questions regarding the role of calpains in postmortem tenderization initially centered on: (1) the observation that calpain activity substantially diminishes early postmortem, and (2) the fact that many of the conclusions regarding the calpain role in tenderization are based on indirect evidence. Within the pH range of 7.4 to 5.8, both μ-calpain and m-calpain retain enzymatic activity, but as muscle pH drops more autolysis of μ-calpain occurs and proteolytic activity diminishes (Koohmaraie 1992). Over the first 24 hours postmortem, bovine μ-calpain retains <20% of its activity when assayed at pH 7.0 and 5°C (Koohmaraie 1992). Even though Koohmaraie (1996) demonstrated that μ-calpain in muscle retains 5% to 10% of its original activity even after 14 days storage, the question still remains whether this level of activity is sufficient to explain the protein degradation observed in muscle beyond 24 to 48 hours postmortem. Direct evidence that μ-calpain is involved in the postmortem proteolysis associated with tenderization was strongly provided by two studies in which postmortem proteolysis of myofibrillar proteins was severely diminished in μ-calpain knockout mice (Geesink et al. 2006) and in mice over-expressing calpastatin (Kent et al. 2004). Despite such strong evidence for the role of μ-calpain, some postmortem proteolysis was still detected in both these studies, suggesting that μ-calpain does not account for postmortem proteolysis in its entirety.

Many recent studies on meat tenderness have confirmed the importance of the calpain system but have further indicated that aging tenderization is a highly complex process that stretches beyond the explanation provided by the current calpain theory of postmortem tenderization. A growing body of evidence suggests that multiple enzymes and interdependent muscle factors may be necessary to fully explain postmortem proteolysis and its link to tenderization. The remainder of this chapter will focus on recent novel findings that contribute to a more complete understanding of the underlying mechanisms

that control postmortem proteolysis and aging tenderization of meat.

Apoptosis Theory of Aging Tenderization

Recent data has indirectly shown that the process of apoptosis may play a role in postmortem proteolysis and meat tenderization (Herrera-Mendez et al. 2006; Ouali et al. 2006). In living organisms, apoptosis, or programmed cell death, is a complex mechanism by which cells can be eliminated without damaging surrounding cells (Kerr et al. 1972; Fidzianska et al. 1991). Apoptosis is initiated and regulated by either the target cell or the central nervous system, and is mediated by the caspase enzyme system. As a result of the slaughter process, the muscle tissue will be deprived of oxygen and nutrients due to the loss of the blood supply. The hypothesis is that under these anoxic conditions, the muscle cells will have no alternative but to initiate apoptosis, which through the caspase system would induce a series of biochemical and structural changes important in the tenderization process. Thus, the traditional model of the conversion of muscle to meat would include a phase corresponding to the initiation of cell death in addition to the phases of rigor mortis development and aging tenderization (Ouali et al. 2006). The apoptotic process would then occur until muscle conditions (pH, ionic strength, energy availability) would be unfavorable for enzyme activity (Ouali et al. 2006). Direct evidence to support this emerging hypothesis is still lacking, however.

Caspases

Apoptosis within the cell occurs through the action of the caspase enzyme system. Detailed information on the structure, activity, activation, and inhibition of caspases can be found in the review of Fuentes-Prior and Salvesen (2004). In brief, caspases are a group of neutral cysteine proteinases that upon activation, which involves cleavage of the pro-domain and dimerization, cleave proteins at specific aspartic acid residues (Sentandreu et al. 2002; Fuentes-Prior and Salvesen 2004; Herrera-Mendez et al. 2006). To date there are 14 caspases that are divided into three classes based on biological function: cytokine activators that function in inflammation, apoptosis initiator caspases, and effector caspases (Fuentes-Prior and Salvesen 2004; Herrera-Mendez et al. 2006). During the apoptosis process, the initiator caspases (caspases 8, 9, 10, and 12) activate the downstream effector caspases (caspases 3, 6, and 7), which cleave specific target proteins (Earnshaw et al. 1999). Since the primary *in vivo* function of caspases is to enzymatically degrade cellular structures (Creagh and Martin 2001), in regards to meat tenderization it has been postulated that caspases would probably initially degrade proteins involved in the spatial organization of myofibrils and that further degradation of cellular components would proceed with the contribution of additional proteolytic systems such as the calpains, cathepsins, and proteasomes (Ouali et al. 2006). Similar to calpains, caspases have been shown to degrade a large number of muscle proteins (Earnshaw et al. 1999; Nicholson 1999; Fischer et al. 2003). In particular, caspase 3 has been shown to cleave myofibrillar proteins in muscle during catabolic conditions (Du et al. 2004).

Only a few studies, however, have investigated caspases in skeletal muscle in regards to their potential contribution to postmortem proteolysis and meat tenderization. Using porcine *trapezius*, *psoas*, *longissimus dorsi,* and *semitendinosus* muscle, it was demonstrated that caspases and the caspase inhibitor apoptosis repressor with caspase recruitment domain (ARC) can be detected in different muscle types at varying levels of expression (Kemp et al. 2006a). Incubation of recombinant caspase 3 with porcine myofibrils

resulted in the degradation of desmin, troponin I, actin, troponin T, and myosin light chains under *in vitro* conditions similar to those found in muscle during postmortem aging (Kemp and Parr 2008). One study investigated the *in vivo* behavior of the caspase system by measuring caspase 3/7 and caspase 9 activities and the degradation of caspase substrates, alpha II spectrin, and poly (ADP-ribose) polymerase (PARP) in porcine *longissimus* muscle between 0 and 8 days postmortem (Kemp et al. 2006b). In this study, caspases were found to be most active early postmortem (<4 hr), and caspase activity diminished with postmortem time. It was also observed that caspase activity (caspase 3/7 and caspase 9) and the abundance of alpha II spectrin degradation products were negatively correlated to Warner-Bratzler shear force measurements. This led to the conclusion that the changes in caspase activity and caspase-mediated cleavage of muscle proteins observed during postmortem aging may be associated with meat tenderization.

Other research, however, contends that caspases are not likely to play a major role in the postmortem proteolysis associated with meat tenderization. In beef muscle, it was observed that caspase 3 activity is present immediately after slaughter but that it decreases with time postmortem (Underwood et al. 2008). In this study, pro-caspase 3 was not activated during postmortem storage and caspase 3 activity was not correlated with Warner-Bratzler shear force in beef *longissimus*. The data from one study using muscle from callipyge and normal lambs indicated that caspase 3/7 and caspase 9 activities decreased between 1 and 21 days postmortem but did not directly support or reject the involvement of the caspase system in meat tenderization (Kemp et al. 2009).

There is some speculation that caspases may influence postmortem proteolysis through their interaction with the calpain/calpastatin enzyme system. Numerous studies have observed cross-talk between the calpain and caspase systems during apoptosis, in which calpain activity is indirectly up-regulated by caspase enzymes cleaving calpastatin (Wang et al. 1998; Porn-Ares et al. 1998; Neumar et al. 2003). Calpains have also been shown to impact caspase activity during apoptosis (Nakagawa and Yuan 2000; Chua et al. 2000; Neumar et al. 2003). There is relatively little data, however, on the interaction between caspases and the calpain enzyme system in skeletal muscle as it relates to postmortem proteolysis. A negative relationship between peak caspase 3/7 activity at 8 hours postmortem and calpastatin activity at 0 and 2 days postmortem has been observed in the muscles of normal lambs but not in callipyge lambs (Kemp et al. 2009). Thus, while there is no direct evidence that caspases contribute significantly to postmortem tenderization, data suggest that they may play an indirect role by degrading calpastatin. More data is needed, however, to determine definitively the direct and indirect contribution of the caspase system to postmortem proteolysis and meat tenderization.

Heat Shock Proteins

Due to their anti-apoptotic functions in living tissue, small heat shock proteins (HSP) are increasingly being investigated as potential factors influencing the conversion of muscle to meat and meat quality. In living muscle tissue, HSP such as alpha β-crystallin, HSP20, and HSP27 have a homeostatic function in which they stabilize unfolded proteins, help refold denatured proteins, and prevent protein aggregation (Liu and Steinacker 2001). Due to their abilities to protect cellular proteins from denaturation and loss of function, HSP expression is up-regulated in living tissues in response to stress. It is speculated that HSP expression could be stimulated after slaughter in response to the muscle cell stress and death during the conversion of muscle to meat and

that they may influence postmortem proteolysis (Ouali et al. 2006). Several recent studies have demonstrated that HSP are up-regulated in postmortem muscle (van Laack et al. 1993; Bouley et al. 2004; Hwang et al. 2005; Jia et al. 2006a, b; Sayd et al. 2006; Jin et al. 2006). In a study comparing microarrays between high and low quality meat groups from beef *longissimus* muscles, the down-regulation of alpha β-crystallin and HSP27 in muscle samples taken at 10 minutes postmortem was associated with improved tenderness, juiciness, and flavor (Bernard et al. 2007). In this study, the expression of DNAJA1, which encodes for a member of the large 40 kDa heat shock protein family, was negatively correlated to tenderness measurements after 14 days of aging and alone explained 63% of the variability in sensory assessed tenderness (Bernard et al. 2007). From this it was suggested that the anti-apoptotic activity of this gene could slow cellular death during the conversion of muscle to meat and lower meat tenderization. HSP27 content in fresh beef muscle and levels of HSP27 fragments in 14-day aged beef were found to explain up to 91% of the variation in sensory tenderness scores (Morzel et al. 2008). In pre-rigor beef, HSP20 and alpha β-crystallin levels peak at 0.5 and 3 hours postmortem and then decline until 22 hours postmortem (Pulford et al. 2008). HSP content postmortem is also influenced by postmortem muscle pH (Pulford et al. 2008). Similarly, high levels of alpha β-crystallin at 22 hours postmortem are associated with diminished protein degradation in beef muscle with low ultimate pH, suggesting that HSP may shield the muscle structure from proteolytic degradation during aging (Pulford et al. 2009). Further data are needed, however, to determine if HSP levels in postmortem muscle are merely indicators of postmortem proteolysis and meat tenderization or if they play a mechanistic role in the aging process.

Instrinsic Muscle Factors Influencing Postmortem Proteolysis

In order to fully understand how calpains contribute to postmortem proteolysis and aging tenderization, researchers have also looked at postmortem characteristics of muscle that could influence either calpain activation or the efficacy of calpain-mediated proteolysis. Differences in muscle factors such as protein oxidation levels and sarcomere length have been investigated for their potential to account for some of the variability observed in the rate and extent of postmortem myofibrillar protein degradation.

Effect of Oxidation on Calpain-Mediated Proteolysis

There is increasing evidence that postmortem proteolysis and aging tenderization are influenced by dynamic changes that occur in the microenvironment of the muscle cells during the conversion of muscle to meat. In addition to a decline in pH and an increase in ionic strength, there is a rise in the formation of reactive oxygen species and an increase in protein oxidation within postmortem muscle (Martinaud et al. 1997; Harris et al. 2001; Rowe et al. 2004a, b). Using vitamin E supplementation and irradiation to generate a range of oxidation levels in beef *longissimus* muscle, researchers have found that increased oxidation of muscle proteins early postmortem (<24 h) negatively impacts meat tenderness (Rowe et al. 2004a, b). Similar to the reversible inactivation of calpain that occurs *in vivo* (Guttmann and Johnson 1998), oxidative conditions in postmortem muscle can diminish calpain activity and reduce myofibrillar proteolysis (Rowe et al. 2004a, b; Maddock et al. 2006) and limit tenderization (Rowe et al. 2004a, b). Since both μ- and m-calpain have a cysteine residue at their active sites and require reducing conditions

for activity, it is not surprising that oxidizing conditions inhibit proteolysis by μ-calpain (Guttmann et al. 1997). It has also been demonstrated that oxidation alters calpain activity and the inhibition of calpains by calpastatin differently, depending on the pH and ionic strength conditions of the muscle (Maddock et al. 2006). Overall, these studies indicate that oxidative conditions in postmortem muscle may influence the postmortem proteolysis associated with the aging tenderization of meat.

Effect of Muscle Shortening on Calpain-Mediated Proteolysis

The direct impact of muscle shortening and sarcomere length on meat tenderness has been well-established and it is thought that sarcomere shortening dictates tenderness early postmortem (<24 h) while variations in proteolysis control differences in tenderness during aging (Wheeler and Koohmaraie 1994). Numerous studies have demonstrated that muscles with longer sarcomeres have lower resistance to shearing than those with shorter sarcomeres (Herring et al. 1965; Herring et al. 1967; Hostetler et al. 1972; Marsh and Carse 1974; Smulders et al. 1990; Wang et al. 1994). Past data has reported that sarcomere length does not impact postmortem proteolysis and that the negative impact of short sarcomeres on tenderness is solely due to the increased overlap of the thick and thin filaments (Young et al. 1980; Locker and Wild 1982; Jaime et al. 1992; Wheeler and Koohmaraie 1999). More recently, however, several studies have demonstrated that there may be some interaction between sarcomere length and the degradation of myofibrillar proteins (Weaver et al. 2008, 2009). Using a muscle stretching model to generate muscle samples with a wider range in sarcomere lengths than have been used in previous investigations, one study found that sarcomere length contributed to differences in the extent of troponin-T proteolysis in excised beef *semitendinosus* muscle from 2 to 10 days postmortem (Weaver et al. 2008). Using an *in vitro* model, in which myofibrils with varying sarcomere lengths were digested with exogenous μ-calpain, it was observed that sarcomere length influenced the rate and extent of troponin-T degradation (Weaver et al. 2009). In both of these studies, less proteolysis was observed in samples with shorter sarcomeres. The limited protein degradation with shorter sarcomeres was hypothesized to be a function of limited substrate availability (Weaver et al. 2008, 2009), but more data is required to elucidate this mechanism. It is also unclear from these studies if the range in sarcomere lengths that resulted in proteolytic differences is indicative of the natural variation in sarcomere length that exists in muscles from commercially processed beef. Nevertheless, these studies indicate that besides having a direct effect on tenderness, large differences in sarcomere length may also impact the postmortem proteolysis responsible for the aging tenderization of meat.

Nonenzymatic Mechanisms of Aging Tenderization

Since there is nearly universal agreement that aging reduces meat toughness in all but sarcomere-shortened muscle, and an overwhelming number of studies have shown that a decrease in toughness of meat is accompanied by a corresponding increase in protein degradation and protein solubility as meat ages, most research efforts have focused solely on the endogenous proteolytic enzymes as the primary mechanism regulating tenderization. The accumulated evidence on proteolytic systems, however, has numerous contradictions and more importantly, does not fully explain the large variation in meat tenderness or great differences in tenderization rates among species. It has been demon-

strated that a minimal amount of proteolysis occurs during the first 3 days of aging, yet the largest changes (~65%) in postmortem tenderization occur during the first 3 days postmortem (Wheeler and Koohmaraie 1994; Taylor et al. 1995). Although much less investigated, some mechanisms not related to proteolysis appear to contribute to postmortem tenderization.

Calcium Theory of Tenderization

The rise in free sarcoplasmic Ca^{2+} from 10^{-4} mM in living skeletal muscle to 0.2 mM in postmortem muscle has been hypothesized to be responsible for postmortem tenderization, regardless of proteolysis (Takahashi 1992, 1996, 1999). The calcium theory of meat tenderization is based on evidence that all structural weakening of myofibrils and rigor linkages, which contain molecular constituents with an affinity for binding with Ca^{2+}, are fully induced when the concentration of free Ca^{2+} increases to more than 0.1 mM (Takahashi 1992, 1996, 1999). This concept, however, has not received widespread acceptance. Based on reports over the last four decades, the mechanism underlying the weakening of myofibrils (Takahashi et al. 1967; Hattori and Takahashi 1979) has been related to the liberation of phospholipids from Z-disks (Ahn et al. 2003), and the fragmentation of cytoskeletal structure proteins titin (Tatsumi et al. 1999), nebulin (Tatsumi and Takahashi 1992, 2003), and desmin (Takahashi 1996) through direct binding reactions with free Ca^{2+}. The second key element, weakening of rigor linkages, was attributed to the translocation of paratropomyosin from the A-I junction region onto thin filament actin (Hattori and Takahashi 1988; Takahashi et al. 1995; Fei et al. 1999). All these ultrastructural changes were demonstrated specifically by 0.1 mM Ca^{2+} ion treatments in vitro, in muscles from beef, pork, chicken, and rabbits. With regard to the different speeds of meat tenderization among animal species, evidence was presented that the rate of free calcium increase paralleled the respective tenderizing rates for different species in the order of chicken (fastest), rabbit, pork, and beef (slowest) muscles (Yamanoue et al. 1994; Ji and Takahashi 2006). A prevalent argument that proteolysis could not be a factor is that meat during aging is under nonphysiological conditions, so the activity levels of the highly pH and temperature dependent proteolytic systems are too low or inactive (Kanawa et al. 2002) in a postmortem cellular environment (ultimate pH 5.5–5.8; 2°–5°C temperature) to elicit the postmortem changes observed. One concern regarding all these studies is the absence of objective measurements of tenderness to further support the calcium theory of tenderization. One report (Geesink et al. 2001) observed a rise in sarcoplasmic Ca^{2+} in postmortem muscle and correlated it to the myofibrillar fragmentation index (MFI) and shear force, which seems to support the calcium theory, but provided alternative interpretations of these results that contradicted the calcium theory of tenderization.

Osmotic Pressure

One of the most extensively investigated factors during the development of rigor mortis is the postmortem fall in pH. The intracellular osmotic pressure (i.e., ionic strength) increases nearly twofold and has a close relationship with pH ($r = 0.97$) during the time course of *rigor mortis* (Ouali 1990), yet it has received comparatively little attention in meat research studies. It was suggested that the pH drop was likely the major cause for the large increase in osmotic pressure through alteration of proteins to which ions (mainly Na^+, K^+, Ca^{2+}, and Mg^{2+}) are normally bound (Ouali et al. 1991). In general, salt concentrations above physiological values (~0.15 M) raise myofibrillar protein solubility; consequently, it was postulated the ionic strength attained at the com-

pletion of rigor (0.24–0.30 M) could be high enough to induce partial dissociation of the myofibrillar structure and increase proteolytic susceptibility of myofibrillar proteins. The high ionic strength in postmortem muscle (0.3 M) was shown to be responsible for the solubilization of structural proteins (C-protein, M line protein, troponoin T, actin, tropomyosin, and α-actinin; Wu and Smith 1985, 1987) and changes in myofibrillar ATPase activity with aging (Ouali 1992). This was further supported by the fact that the highest osmotic pressure values coincided with the contraction speed of muscles (i.e., fast-twitch white muscles tenderize faster than slow-twitch red muscles; Geesink et. al. 1992; Ouali et al. 1991, 1992). From these studies it was concluded that elevated osmotic pressure, in addition to proteolytic enzymes, has a physico-chemical impact on myofibrillar proteins that could be associated with improvements in tenderness.

Results so far do not support a synergistic role of elevated ionic strength with proteolysis. The pH/ionic strength conditions in postmortem muscle induce conformational changes in the substrate proteins, consequently altering their susceptibility by rendering specific cleavage sites inaccessible to proteolytic attack. Secondly, an increase in ionic strength was also shown to inhibit the activity of μ- and m-calpain (Huff-Lonergan et al. 1995; Geesink and Koohmaraie 2000; Li et al. 2004; Maddock et al. 2005). Increased ionic strength, similar to the fall in pH, is an important variable to examine in determining the relative contribution of proteolytic enzymes to postmortem tenderization.

Emerging Use of Proteomic Approaches to Study Aging Tenderization

While the basic understanding of the mechanisms that control postmortem proteolysis and aging tenderization have not changed substantially over the last decade, technological advances during that time frame have helped uncover new pieces to the puzzle. Traditionally, most of the research on postmortem proteolysis and meat aging was done using classic SDS-PAGE and western blotting techniques to document protein degradation in either tissue sampled from aged intact muscle cuts or from protein extracts following the in vitro digestion of isolated myofibrils and muscle proteins. Researchers are increasingly taking a proteomics approach to understanding protein changes related to meat quality by utilizing two-dimensional electrophoresis (2DE) combined with protein identification by mass spectrometry (MS). Rather than just investigating a few proteins at a time, this powerful tool allows researchers to simultaneously and efficiently separate a wide range of proteins expressed in muscle tissue and to identify numerous protein changes that occur in postmortem muscle.

Lametsch and Bendixen (2001) first demonstrated in porcine muscle the use of proteome analysis to determine postmortem protein changes. Using 2DE to separate proteins from 5–200 kDa with pIs ranging from pH 4–9, 15 significant changes were observed in the proteome patterns of porcine *longissimus* muscle between slaughter and 48 hours postmortem. A subsequent study using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to identify proteins demonstrated that peptide fragments from three structural proteins (actin, myosin heavy chain, and troponin-T) and six metabolic proteins (glycogen phosphorylase, creatine kinase, phosphopyruvate hydratase, myokinase, pyruvate kinase, and dihydrolipoamide succinyltransferease) accumulated in porcine *longissimus* muscle between slaughter and 48 hours postmortem (Lametsch et al. 2002).

Several studies have investigated protein changes in porcine *longissimus* muscle between 0 and 72 hours postmortem (Lametsch et al. 2003; Morzel et al. 2004).

From these studies, myofibrillar proteins or fragments of myofibrillar proteins found to change with postmortem storage include actin, myosin heavy chain, titin, myosin light chain I, myosin light chain II, CapZ, cofilin, troponin-T, cypher proteins, and myozenin. Sarcoplasmic proteins enolase, phosphoglycerate kinase, pyruvate dehydrogenase, glycogen phosphorylase, triosephosphate isomerase, myokinase, eukaryotic translation initiation factor 5A, α-crystallin, creatine kinase, and pyruvate kinase were also found to change with postmortem storage in these studies (Lametsch et al. 2003; Morzel et al. 2004). Using a model in which isolated myofibrils were incubated with μ-calpain under simulated postmortem conditions and protein degradation was measured by combining MALDI-TOF MS with SDS-PAGE and 2DE, one study observed that desmin, actin, myosin heavy chain, myosin light chain I, troponin-T, tropomyosin, thioredoxin, and CapZ were degraded *in vitro* by μ-calpain (Lametsch et al. 2004). In both bovine *longissimus* and *semitendinosus* muscles, levels of cofilin, lactoylglutathione lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27, and HSP20 were found to be different between samples removed at 0 and 24 hours postmortem (Jia et al. 2006). This study also found 15 additional proteins that changed during postmortem storage in either the *longissimus* or *semitendinosus* muscles (Jia et al. 2006). In a similar study, thirty-nine proteins were identified from bovine *longissimus* muscle that significantly change during the first 24 hours postmortem (Jia et al. 2007). Proteins undergoing changes included metabolic enzymes, cellular-defense and stress-response proteins, structural proteins, and proteolytic enzymes.

Some of the proteomic changes observed during postmortem aging have been correlated with tenderness measurements. The abundance of actin fragments, myosin heavy chain fragments, myosin light chain II, and triose phosphate isomerase at 72 hours postmortem correlate to shear force measurements at 1 and 4 days postmortem in porcine *longissimus* muscle (Lametsch et al. 2003). Similarly, spots corresponding to actin, myokinase, F-actin capping protein, HSP27, myosin light chain I, peroxiredoxin 2, triosephosphate isomerase, and troponin T were correlated to shear force changes between 1 and 7 days postmortem in porcine *longissimus* muscle (Hwang et al. 2005). In *longissimus* muscle from Korean native cattle, researchers identified seven proteins that are differentially expressed in samples from carcasses segregated into high- and low-quality beef grades (based on marbling, lean color, fat color, maturity, and tenderness) (Kim et al. 2008). Both HSP27 and inositol 1,4,5-triphosphate (IP3R1), which is involved in the intracellular pathways that mediate Ca^{2+} release from intracellular stores (Berridge and Lipp 2000), were higher in low-quality beef, and HSP27 was positively correlated to 2-day postmortem shear force measurements.

Proteomic studies on postmortem muscle have led to new insights into the mechanisms of aging tenderization in meat. Based on studies using one-dimensional SDS-PAGE, it has been accepted for years that actin is not degraded postmortem (Bandman and Zdanis 1988; Huff-Lonergan et al. 1995; Koohmaraie 1994). Several studies using 2DE separation, however, have demonstrated that fragments of actin accumulate with postmortem storage (Lametsch et al. 2002; Lametsch et al. 2003; Morzel et al. 2004; Hwang et al. 2005) and that the abundance of actin fragments correlates to tenderness (Lametsch et al. 2003; Hwang et al. 2005). Similarly, these studies have also observed that fragments of myosin accumulate with postmortem storage (Lametsch et al. 2002, 2003; Morzel et al. 2004). While aging tenderization is usually thought to be a manifestation of changes to

the myofibrillar and cytoskeletal components of muscle, the findings of proteomic studies on postmortem muscle suggest that metabolic enzymes and other water-soluble proteins within muscle may be potentially useful as markers for meat tenderness. With the limited number of proteomic-based and high-resolution studies on aged muscle, however, it is still unclear if the protein changes observed during postmortem storage play a mechanistic role in aging tenderization or if they are simply indicators of proteolysis.

Conclusions

In summary, we can state that skeletal muscle is a product of exceedingly structured and oriented proteins containing numerous enzymes that drive various metabolic pathways in the living tissue and is destined to undergo a variety of changes after death. Dramatic changes take place both during chilling and aging but most markedly during the immediate postmortem period as muscle is transformed to meat. The subject of utmost importance in the minds of many is where do we go from here in research on quality and palatability of meat? Postmortem aging is a process that occurs naturally in all muscle tissues, whether vacuum packaged or in the form of carcasses or wholesale cuts. In the conversion of muscle to meat, postmortem aging of carcasses and cuts is a natural process that usually improves tenderness under refrigerated conditions. Endogenous enzymes found in muscle cells fragment key proteins in such a way that the fundamental integrity of the muscle ultrastructure is altered, resulting in improved meat tenderness. Although postmortem aging has a profound effect on meat tenderness, it does not ensure total and uniformly tender meat. Aging tenderization of meat is a complex phenomenon with numerous factors (both antemortem and postmortem) that influence the underlying enzymatic mechanisms.

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Chapter 5

Freezing/Thawing

Christian James and Stephen J. James

Introduction

Modern commercial meat freezing has a surprisingly long history. It is believed that the first modern meat freezing works were established at Darling Harbour in Sydney, Australia, in 1861 (Critchell and Raymond 1912). In the next decade, there were a number of attempts to transport frozen meat. The first entirely successful frozen meat shipment was that of the S.S. *Paraguay* from Buenos Aries to Havre in 1877. Due to a collision, the ship took seven months to complete her journey; however, the 5,500 mutton carcasses were still reported to be in "in tip-top condition" when the ship arrived at Havre. A second voyage was planned but never happened, and to quote a Dr. Bergés (Critchell and Raymond 1912), "As has often happened in the history of industries, it has been the French who have made the discoveries, and the English have turned them to account to their profit." Thus it was that the arrival of the S.S. *Strathleven* in London on December 8, 1880 with its cargo of 40 tons of frozen Australian beef and mutton may be said to have truly started the international frozen meat trade. This meat was sold for up to three times its value in Australia and, as stated in the *Daily Telegraph* at the time, "It has been tested by the ordinary method of cooking, and found to be in such good condition that neither by its appearance in the butchers' shops, nor by any peculiarity of flavour when cooked for the table, could it be distinguished from freshly killed English meat" (Critchell and Raymond 1912).

Despite this glowing report, soon frozen meat began to suffer from a perception that its eating quality was not as good as that of "fresh" chilled meat. In the middle 1950s, Swift and Company tried to introduce frozen red meat to the consumer but found that "the consumer indicated she was not interested in purchasing frozen red meats" (Bernholdt 1974). In Australia in 1986, 24% of respondents in a survey of retail and consumer handling of beef considered that freezing "definitely affected quality" and a further 13% felt it would "under certain circumstances" (Walker and Mitchell 1986). Today some retailers and media still pedal this perception. For example, one online store in the United States quotes that "When you are buying steaks online, you want to get a good value, and you want to get great quality, right? We do too! That means we want unfrozen steaks" (Anonymous 2008). However, contrary to this, consumers appear happy to purchase chilled meat and freeze it at home. A U.S. survey found that approximately 80% of a major retailer's customers did this (Payne et al. 2002). A similar New Zealand survey reported that while the majority of red meat (84.6%) purchased by consumers surveyed was fresh (rather than frozen), approximately 64% of the fresh meat they purchased was subsequently frozen in the home (Gilbert et al. 2007).

Currently, meat for industrial processing is usually frozen in the form of carcasses,

quarters, or boned-out primals in 25-kg cartons. It is not unusual for meat to be frozen twice before it reaches the consumer. During industrial processing, frozen raw material is often thawed or tempered before being turned into meat-based products (i.e., pies, convenience meals, burgers, etc., or consumer portions, fillets, steaks, and so on). These consumer-sized portions are often refrozen before storage, distribution, and sale.

The Effect of Freezing and Thawing on Meat Quality

There is a general view that fast freezing offers some quality advantage, with "quick frozen" appearing on many meat products with the expectation that consumers will pay more for a quick-frozen product. Studies have shown that freezing rate influences ice crystal size, location (intra- or extra-cellular), and morphology (Grujic et al. 1993). However, there are little data in the literature to suggest that, in general, the method of freezing or the rate of freezing has any substantial influence on the quality characteristics or final eating quality of meat. Slightly superior chemical and sensory attributes have been found in meat cryogenically frozen in a few trials (Dobrzycki et al. 1977; Sebranek et al. 1978; Sebranek 1980), but other trials have not shown any appreciable advantage (Lampitt and Moran 1933), especially during short-term storage (Hill and Glew 1973). As an example, in a study comparing frozen beef burgers, no significant difference could be seen in cooking losses or eating quality between samples frozen using either spiral, impingement, or cryogenic methods, even after 2 months storage (Sundsten et al. 2001). In terms of increased throughput, however, the study did reveal some slight commercial advantages of fast freezing.

High-pressure freezing and in particular "pressure shift" freezing is attracting considerable scientific interest (LeBail et al. 2002). The meat is cooled under high pressure to sub-zero temperatures but does not undergo a phase change and freeze until the pressure is released. Rapid nucleation results in small even ice crystals. However, studies on pork and beef (Fernandez-Martin et al. 2000) and on pork (Zhu et al. 2004) failed to show any real commercial quality advantages, and an increase in toughness was found in the later study.

There are few published data relating thawing processes to the palatability of meat, and eating quality is generally independent of thawing method. However, two reports indicated that cooking directly from the frozen state produced less juicy lamb rib-loins (Woodhams and Smith 1965) and less tender beef rolled rib joints (James and Rhodes 1978) when compared with meat that had been thawed before cooking.

Tenderness and Texture

To quote an Australia CSIRO report (1988), "Toughness (in meat) is caused by three major factors—advancing age of the animal, 'cold shortening' (the muscle fiber contraction that can occur during chilling), and unfavorable meat acidity (pH)." There is general agreement on the importance of these factors, with many experts adding cooking as a fourth equally important influence.

The texture of frozen meat will have been generally fixed by what happened to the meat during the primary chilling of the carcass. Chilling can have serious effects on the texture of meat if it is carried out too rapidly when the meat is still in the pre-rigor condition, that is, before the meat pH has fallen below about 6.2 (Bendall 1972). In this state, the muscles contain sufficient amounts of the contractile fuel adenosine triphosphate (ATP) for forcible shortening to set in as the temperature falls below 11°C, the most severe effect occurring at about 3°C. This is the so-called "cold shortening" phenomenon, first observed by Locker and Hagyard (1963) and its mechanism described by Jeacocke (1986).

The meat "sets" in the shortened state as rigor comes on, and this causes it to become extremely tough when it is subsequently cooked. If no cooling is applied and the temperature of the meat is above 25°C at completion of rigor, then another form of shortening rigor or "heat shortening" will occur (Dransfield 1994), also on cooking.

The severity of cold shortening is highly pH-dependent. It is much greater if muscle temperatures below 10°C are achieved while the pH is 6.8 (i.e., exceptionally rapid chilling) than at pH 6.2 (i.e., at an easily attainable commercial rate of chilling). To allow a safety margin, and taking into account the fact that some carcasses will show high initial pH values in the eye muscle, it is recommended that any part of a beef or lamb carcass should not be chilled below 10°C until at least 10 hours after slaughter. In pork, cold shortening occurs if temperatures between 3 and 5°C are reached before the onset of rigor (normally 3 to 8 hours); this will only occur in rapid pork chilling systems and is not as common. Avoiding cold shortening in beef through the use of slow chilling rates can lead to problems of "bone-taint" (James and James 2002). Electrical stimulation of the carcass after slaughter can allow rapid chilling to be carried out without much of the toughening effect of cold shortening. However, electrical stimulation followed by moderate cooling may affect tenderness in an unpredictable way and could result in tougher meat (Buts et al. 1986). Electrical stimulation will hasten rigor and cause tenderization to start earlier at the prevailing higher temperature. In beef, meat from carcasses given high- or low-voltage stimulation and slow cooling can obtain adequate aging in about half the time of non-stimulated meat.

If freezing is applied immediately after slaughter, cold shortening may be prevented. However, a more severe shortening, thaw shortening, will occur on thawing (Bendall 1974). An entire lamb carcass can be frozen in 6 hours, thus freezing all the meat in a pre-rigor state. However, pork and beef carcasses, with their greater insulation of fat, cool more slowly; thus thaw shortening is rarely encountered in these meats. Thaw shortening may be prevented in lamb carcasses by applying electrical stimulation prior to freezing, or by tempering the meat at temperatures between −2 and −5°C for up to 6 days during thawing (Dransfield 1974).

When meat is stored at above freezing temperatures it becomes progressively more tender. This process, known as "aging" (or, alternatively, as conditioning or maturation), is traditionally carried out by hanging meat carcasses for periods of 14 days or longer (in the case of beef) in a controlled environment at between −1 and 5°C (so called "dry aging"). Alternately, the carcass may be divided into sub-primals and aged in vacuum packs (usually referred to as "wet aging"). The rate of aging differs significantly between animal species (Dransfield 1986) and necessitates different times for tenderization. Beef, veal, and rabbit age at about the same rate and take about 10 days at 1°C to achieve 80% of aging. Lamb ages slightly faster than beef but more slowly than pork. The ultimate tenderness will depend on the initial "background" tenderness of the meat and the tenderization that has occurred during chilling. The age of the animal is also important.

Frozen meat that has been aged prior to freezing is more tender than that frozen within 1 or 2 days, and the difference has been shown to be maintained throughout frozen storage for 9 months (Jakobsson and Bengtsson 1973). However, there is evidence that aging shortens the frozen storage life. Chilled storage of lamb for one day at 0°C prior to freezing can reduce the subsequent storage life by as much as 25% when compared to lamb that has undergone accelerated conditioning and only 2 hours storage at 0°C (Winger 1984). It has been shown that pork that has been held for 7 days prior to freezing deteriorates at a faster rate during subsequent

frozen storage than carcasses chilled for 1 to 3 days prior to freezing (Harrison et al. 1956). Aging for periods greater than 7 days was found by Zeigler et al. (1950) to produce meat with high peroxide and free fatty acid values when stored at −18°C or −29°C. Although shorter aging times appear to have a beneficial effect on storage life, there is obviously a necessity for it to be coupled with accelerated conditioning to prevent any toughening effects.

There is some evidence that freezing rate affects the rate of tenderizing after thawing but not the ultimate tenderness (Dransfield 1986). Freezing at −10°C more than doubles the rate, while freezing in liquid nitrogen almost trebles the rate. Freezing is known to cause structural damage by ice crystal formation. It seems likely that ice crystals, particularly small intracellular ice crystals formed by very fast freezing rates, enhance the rate of aging by release of enzymes (Dransfield 1986). Repeated freeze-thaw cycles using relatively low freezing rates do not seem to cause any enhanced tenderising (Locker and Daines 1973). There is little evidence of any relationship between chilling rates and subsequent frozen storage life.

Whether aged or unaged, chilled or frozen, it is in the cooked final product that the consumer will assess tenderness and texture. Thus, the way the meat is cooked must always be considered. The consumers' environment or setting can also influence their appreciation of tenderness. In one study, consumers were found to be more critical of the tenderness of beef steaks cooked in the home than those cooked in restaurants (Miller et al. 1995). The Warner-Bratzler force transition level for acceptable steak tenderness was between 4.6 and 5.0 kg in the home and between 4.3 and 5.2 kg in restaurants.

Drip Production

When meat is frozen, its water-hold capacity is reduced. This in turn gives rise to increased "drip." Drip can be referred to by a number of different terms including "purge loss," "press loss," and "thaw loss," depending on the method of measurement and when it is measured. The protein concentration of drip is about 140 mg ml^{-1} (i.e., about 70% of that of meat itself). The proteins in drip are the intracellular, soluble proteins of the muscle cells. The red color is due to the protein myoglobin, the main pigment of meat. Drip loss occurs throughout the cold chain and represents a considerable economic loss to the red meat industry. The potential for drip loss is inherent in fresh meat and related to the development of rigor mortis in the muscle after slaughter and its effect on pH. It is influenced by many factors. Some of these, including breed, diet, and physiological history, are inherent in the live animal. Others, such as the rate of chilling, storage temperatures, freezing, and thawing, occur during processing.

When meat is frozen quickly, the water, both that released by the fibrils as the meat has gone into rigor and that which is still held, is frozen simultaneously. Consequently, there is no change in the water's relative positions or amounts. At slower freezing rates, however, the water balance is altered, the extracellular water freezing first. As freezing, continues, the existing ice crystals grow at the expense of water from the intrafibrillar space. When meat is thawed, the reverse of freezing process occurs. Water that has been frozen is released and has to reestablish equilibrium with the muscle proteins and salts. Obviously, if the muscle proteins have been denatured, they will reabsorb less water. Since the fibers have been squeezed and distorted by ice formation, this nonreabsorbed water will lie in wider channels within the meat structure, thus increasing the potential drip. If cell walls have also been damaged by freezing, even less water will be reabsorbed and will exude as drip.

Drip potential clearly appears to be related to species. In general, beef tends to lose pro-

portionately more drip than pork and lamb. The potential for drip loss is inherent in fresh meat and related to the development of rigor mortis in the muscle after slaughter and its effect on pH. In pigs, especially, there are large differences in drip loss from meat from different breeds. Taylor (1972) showed that there was a substantial difference, up to 2.5-fold, in drip loss between four different breeds of pig.

There can be large differences in drip loss between different muscles. Taylor (1972) showed that there was a 1.7- to 2.8-fold difference in drip between muscle types in pigs. Since most of the exudate comes from the cut ends of muscle fibers, small pieces of meat also drip more than large intact carcasses, and the way that different muscles are cut will also have an influence on drip.

A number of scientific investigations, which can be compared to commercial practice, have defined the effect of freezing rate on drip production. Petrovic et al. (1993) stated that the optimal conditions for freezing portioned meat are those that achieve freezing rates between 2 and 5 $cm\,h^{-1}$ to −7°C. Grujic et al. (1993) suggest even tighter limits: 3.33 to 3.95 $cm\,h^{-1}$. They found that "slow freezing" up to 0.39 $cm\,h^{-1}$ resulted in decreased solubility of myofibrillar proteins; increase in weight loss during freezing, thawing, and cooking; lower water-binding capacity; and tougher cooked meat. "Very quickly frozen" meat (>4.9 $cm\,h^{-1}$) had a somewhat lower solubility of myofibrillar proteins, lower water-binding capacity, and somewhat tougher and drier meat. The samples were thawed after storage times of 2 to 3 days at −20°C so the relationship between freezing rates and storage life was not investigated. Sacks et al. (1993) found that after 2.5 months, drip loss from mutton samples frozen using cryogenics was >2% less than in those using air freezing.

These results are scientifically very interesting; however, in industrial practice most meat is air frozen in the form of large individual pieces or cartons of smaller portions. In commercial situations, freezing rates of 0.5 $cm\,h^{-1}$ in the deeper sections would be considered "fast," and there would be considerable variation in freezing time within the meat. The samples frozen by Sacks et al. (1993) were much smaller (77.6 g in weight) than most commercial products. Even with such small samples, there was no significant difference in drip after 48 hours between cryogenic freezing at −90°C and a walk-in freezer operating at −21°C.

Even partial freezing will increase drip. Hence tempering of meat to aid cutting, dicing, slicing, etc. will increase drip loss, though not to the same extent as full freezing. Irie and Swatland (1993) found that drip loss from 3 mm thick slices of pork that had been "lightly frozen" before slicing average 8.0 ± 4.2% over a 4-day period. Drip losses from samples that had been kept in a freezer at −10°C for 6 days had a higher drip loss of 14.0 ± 4.3%.

Excessive drip may have a small effect on the eating quality of meat. Perceived juiciness is one of the important sensory attributes of meat. Dryness is associated with a decrease in the other palatability attributes, especially with lack of flavor and increased toughness (Pearson 1994). However, moisture losses during cooking are typically an order of magnitude higher than most drip losses during refrigeration. Consequently, small differences in drip loss will have little effect on eating quality.

Odor and Flavor

There is no evidence that freezing and thawing has any effect on meat flavor. However, meat flavor can alter during frozen storage. This is principally caused by lipid (fat) oxidation, also referred to as oxidative rancidity, which results in unacceptable "off" or "rancid" flavors. The importance of lipid oxidation in frozen meat may be illustrated by a short quotation from a paper published

by Lea (1931): "it is often the deterioration of the fat which limits the storage life—from the point of view at least of palatability—of the meat." This view has been reiterated many times since (Watts 1954; Love and Pearson 1971; Morrissey et al. 1998), and as freezing technology has improved, it is true to say that lipid oxidation remains the obstacle to very long term storage of frozen meat.

The reaction of oxygen with fat is an autocatalytic process (Enser 1974). Once the reaction starts, the products of the reaction stimulate it to go faster. The initial reaction is that between a molecule of oxygen and a fatty acid to form a peroxide. This is a slow reaction but, like any other chemical reaction, raising the temperature increases its rate. The type of fatty acid also influences the rate. Saturated fatty acids react slowly, but unsaturated fatty acids react more rapidly, and the more double bonds that a fatty acid contains, the more reactive it is. The presence of peroxides in fat does not change the flavor; it is the breakdown products of the peroxides that produce the rancid odor and flavor. The breakdown of peroxide is accelerated by heat, light, organic iron catalysts, and traces of metal ions, especially copper and iron. It is also the breakdown products of the peroxides that cause the oxygen to react more rapidly with the fatty acids, thus producing the autocatalytic effect.

The development of oxidative rancidity in meat is affected by many factors (Enser 1974; Morrissey et al. 1998; Pérez Chabela and Mateo-Oyague 2006), some intrinsic (such as species, muscle type, amount and type of fat in the diet, enzymes), others extrinsic (such as light, heat, damage to muscle structures caused by freezing, mincing, and the addition of sodium chloride). There is considerable evidence that dietary vitamin E supplementation reduces lipid oxidation (Morrissey et al. 1998). It is less clear what other components of the diet may beneficially effect lipid stability (Morrissey et al. 1998). This process can also be significantly slowed in frozen meat if oxygen is completely eliminated and the storage temperature is extremely low (i.e., under −60°C) (Pérez Chabela and Mateo-Oyague 2006).

Color and Appearance

The appearance of meat at its point of sale is the most important quality attribute governing its purchase. Changes in color of the muscle and blood pigments (myoglobin and hemoglobin, respectively) determine the attractiveness of fresh red meat, which in turn influences the consumer's acceptance of meat products (Pearson 1994). Consumers prefer bright red fresh meats, brown or gray cooked meats, and pink cured meats (Cornforth 1994).

The pigment concentration in meat that governs its color is certainly influenced by species. Beef and lamb contain substantially more myoglobin than pork and poultry meat, thus accounting for the difference between "red" (beef and lamb) and "white" (pork and poultry) meats. Pigment concentration (myoglobin content) also increases with age; for example, veal is brownish pink, while beef from three-year-old steers is bright, cherry red (Miller 2002). However, within a species, meat color can be adversely affected by a variety of factors, including postmortem handling, chilling, storage, and packaging (Miller 2002).

The color of frozen meat varies with the rate of freezing. There is a direct relationship between freezing rate and muscle lightness; the faster the rate, the lighter the product (MacDougall 1974). These differences in frozen meat lightness result from the dependence of ice crystal growth on the freezing rate. Small crystals formed by fast freezing scatter more light than large crystals formed by slow freezing, and hence fast frozen meat is opaque and pale and slow frozen meat is translucent and dark (MacDougall 1974).

"Freezer burn" is the main appearance problem that traditionally affected the appearance of meat in frozen storage. Desiccation from the surface tissues produces a dry, spongy layer that is unattractive and does not recover after thawing. This is commonly called freezer burn. It occurs in unwrapped or poorly wrapped meat. The problem is accentuated in areas exposed to low-humidity air at high velocities, and by poor temperature control. Since most meat is now wrapped and temperature control much improved, this is less of a problem than it once was commercially. Provided problems of freezer burn can be eliminated, the major appearance problem that affects frozen meat arises from the oxidation of oxymyoglobin to metmyoglobin.

Both temperature and illumination level affect the rate of discoloration during frozen storage, but light is by far the more serious factor. Lentz (1971) reported the progress of discoloration in the light (160–220 dekalux) and in the dark for frozen beef stored at a range of temperatures in terms of the Munsel color notation. At −18°C, a temperature typical of good commercial display, the color remained attractive for 3 months in the dark but only 3 days in the light.

There is an interaction between the color of meat after thawing and its freezing rate. Jakobsson and Bengtsson (1969, 1973) found that slowly frozen beef, which darkened on freezing, also showed considerable loss of redness after thawing. In contrast, meat frozen in liquid nitrogen and then defrosted was a light bright red. Little difference was also found between thawed beefsteaks that were frozen at $15\,cm\,h^{-1}$ in liquid nitrogen spray and those that were blast frozen at $4\,cm\,h^{-1}$ (Pap 1972). In thawed meat, the rate of pigment oxidation is increased (Cutting 1970), and therefore, the color will be less stable than in fresh. On prolonged frozen storage, a dark brown layer of metmyoglobin may form 1–2 mm beneath the surface so that, on thawing, the surface color will rapidly deteriorate. Meat that has lost its attractiveness during frozen storage because of oxidation of oxymyoglobin on the surface will remain brown after thawing.

Unwrapped meat thawed in high humidity air, water, or in steam under vacuum appears very white and milky after thawing. However, if then stored in a chill room for 10 to 24 hours, it will be almost indistinguishable from fresh meat. Unwrapped meat thawed in air at high temperatures and low humidities will take on a dark, dry, tired appearance. It will not recover its appearance during chilled storage and will often require extensive trimming before sale (James and James 2002).

The major problem in retail marketing of frozen meat is its appearance. The freezing process causes changes in the structure and color of the muscle, and the deterioration in appearance during frozen storage and display ultimately leads to rejection of the product by the consumer. Storage temperature, light intensity on the display area, and method of packaging all affect the rate of deterioration. The appearance of fresh meat is a primary factor in acceptability at retail level, and the same criteria of attractiveness will apply to frozen meat, retailed either frozen or after thawing. The poor color of the frozen product and the drip associated with it when it thaws have in the past both contributed to consumer resistance. The appearance of frozen meat is markedly improved if retail-sized portions are first packed in film to exclude air between the meat surface and the film and then rapidly frozen. With this product, however, the price differential between fresh and frozen would necessarily be small, and the consumer would have to be persuaded by the trade that such frozen meat was in no way inferior to fresh.

Freezing Systems for Meat

Heat transfer can only occur by four basic mechanisms: conduction, radiation,

convection, and evaporation/condensation. Conduction requires a good physical contact between the meat to be cooled and the cooling medium, and this is generally difficult to achieve with carcasses and other irregular meat cuts. Radiation does not require any physical contact, but a large temperature difference is required between the surface of the meat being cooled and that of surrounding surfaces to achieve significant heat flow. In primary freezing, radiation is only important in the initial stages of the process in a system where the meat is not surrounded by other product. Again, in the initial stages of the freezing of cooked meat products (e.g., pies, pasties, joints), radiant heat loss can be substantial if the products are surrounded by cold surfaces. Evaporation from a meat surface reduces yield and is not desirable in most meat refrigeration operations but can be useful again in the initial cooling of cooked meat products. Convection is by far the most important heat transfer mechanism employed in the majority of meat refrigeration systems. In most cases, refrigerated air is the transfer medium; however, in some cases water, brine, or a cryogenic gas can be used.

Air Freezing Methods

Air is by far the most widely used method of freezing meat, as it is economical, hygienic, and relatively noncorrosive to equipment. The big advantage of air systems is their versatility, especially when there is a requirement to freeze a variety of irregularly shaped products or individual products. However, relatively low rates of heat transfer are attained from product surfaces in air systems. Systems range from the most basic, in which a fan draws air through a refrigerated coil and blows the cooled air around an insulated room, to purpose-built conveyerized blast freezing tunnels or spirals. In a continuous system, meat is conveyed through a freezing tunnel or refrigerated room, usually by an overhead conveyor or on a belt. This overcomes the problem of uneven air distribution, since each item is subjected to the same velocity/time profile. Some meat products are frozen on racks of trays (2 m high), while pulled or pushed through a freezing tunnel by mechanical means. For larger operations, it is more satisfactory to feed meat on a continuous belt through linear tunnels or spiral freezers.

In the past decade, the use of impingement technology to increase the surface heat transfer in freezing systems has received attention (Newman 2001; Sundsten et al. 2001; Everington 2001). Impingement is the process of directing a jet or jets of fluid at a solid surface to effect a change. The very high velocity (20–30 $m\,s^{-1}$) impingement gas jets break up the static surface boundary layer of gas that surrounds a meat product. The resulting medium around the product is more turbulent, and the heat exchange through this zone becomes much more effective. Impingement freezing is best suited for products with high surface-area-to-weight ratios (i.e., hamburger patties or products with one small dimension). Testing has shown that products with a thickness less than 20 mm freeze most effectively in an impingement heat transfer environment. When freezing products thicker than 20 mm, the benefits of impingement freezing can still be achieved; however, the surface heat transfer coefficients later in the freezing process should be reduced to balance the overall process efficiency. The process is also very attractive for products that require very rapid surface freezing and chilling.

Contact Freezing Methods

Contact freezing methods are based on heat transfer by contact between products and metal surfaces (which in turn are cooled by either primary or secondary refrigerants) or direct immersion in a refrigerated liquid.

Modern plate cooling systems differ little in principle from the first contact freezer

patented in 1929 by Clarence Birdseye. Essentially, product is pressed between hollow metal plates containing a circulating refrigerant. A hydraulic cylinder is used to bring the freezing plates into pressure contact with the product. These plates can be either horizontal or vertical (Fig. 5.1). Good heat transfer is dependent on product thickness, good contact, and the conductivity of the product. Plate freezers are often limited to a maximum thickness of 50 to 70 mm. Good contact is a prime requirement. Air spaces in packaging and fouling of the plates can have a significant effect on cooling time; for example, a water droplet frozen on the plate can lengthen the freezing time in the concerned tray by as much as 30% to 60%.

An immersion freezer is made up of a tank with a cooled freezing liquid that can be any nontoxic salt, sugar, or alcohol solution in water, and a means of conveying the wrapped meat through the tank. The freezing process is often completed in an air blast system. Ice slurries are being considered as an alternative to conventional immersion liquids. Such binary systems are described in the scientific literature as flow ice, fluid ice, slush ice, or liquid ice. Such systems may achieve higher rates of heat transfer than the single-state liquids (Maria et al. 2005).

Contact freezing offers several advantages over air cooling—for example, much better heat transfer and significant energy savings. However, disadvantages are the need for regularly shaped products with large flat surfaces with plate systems, and the need to wrap and wash off the immersion liquid in immersion systems.

Cryogenic freezing is essentially a subset of immersion freezing, in that it directly uses cryogenic refrigerants, such as liquid nitrogen or solid carbon dioxide. The method of cooling is essentially similar to water-based evaporative cooling, where cooling is brought about by boiling off the refrigerant, the essential difference being the temperature required for boiling. As well as using the latent heat absorbed by the boiling liquid, sensible heat is absorbed by the resulting cold gas. Due to very low operating temperatures and high surface heat transfer coefficients between product and medium, cooling rates of cryogenic systems are often substantially higher than other refrigeration systems.

Figure 5.1. Vertical plate freezer for freezing of blocks of boned meat.

Frozen Storage Systems for Meat

Theoretically, there are clear differences between the environmental conditions required for cooling, which is a heat removal/temperature reduction process, and those required for storage, where the aim is to maintain a set product temperature. However, in many air-based systems, cooling and storage take place in the same chamber, and even where two separate facilities are used, in many cases not all the required heat is removed in the cooling phase. This failure to remove the required heat can be due to a number of causes:

1. Insufficient time allowed.
2. Insufficient refrigeration capacity to cater to high initial product load.
3. Overloading.
4. Variability in size of products.
5. Incorrect environmental conditions.

Three factors during storage—the storage temperature, the degree of fluctuation in the storage temperature, and the type of wrapping/packaging in which the meat is stored—are commonly believed to have the main influence on frozen storage life.

Storage Temperature

Extensive data are available on the optimum storage conditions and attainable frozen storage lives for many meats (IIR 2006; ASHRAE 2006), as shown in Table 5.1. It is generally accepted that lowering the frozen storage temperature of meat extends the storage life. However, there are surprisingly few articles where data are presented from experiments on the PSL of meat at different storage temperatures. Some researchers, such as Pérez Chabela and Mateo-Oyague (2006), have also questioned the validity of some of the data used, since much is "rather old, based on freezing conditions that nowadays are old-fashioned." Experimental data (James and Evans 1997) from many different publications have been plotted against the temperature of storage for beef (Fig 5.2), pork (Fig 5.3), and lamb (Fig 5.4). There is a clear effect of temperature on storage life, with lower temperatures resulting in extended

Table 5.1. Practical storage life (months) of meats and meat products at different storage temperatures

Product	−12°C	−15°C	−18°C	−22 to −24°C	−29 to −30°C	Reference
Beef	4 to 12		6 to 18	12 to 24	>12	ASHRAE 2006
Beef		6		12		Lawrie and Ledward 2006
Beef carcasses	8		15	24		IIR 2006
Beef steaks/cuts	8		18	24		IIR 2006
Ground beef	6		10	15		IIR 2006
Beef liver	2 to 3		2 to 4			ASHRAE 2006
Veal carcass	6		12	15		IIR, 2006
Veal steaks/cuts	6		12	15		IIR 2006
Lamb	3 to 8		6 to 16	12 to 18	>12	ASHRAE 2006
Lamb carcasses	18		24	>24		IIR 2006
Lamb steaks	12		18	24		IIR 2006
Pork	2 to 6		4 to 12	8 to 15	10	ASHRAE 2006
Pork		3		6	12	Lawrie and Ledward 2006
Pork carcasses	6		10	15		IIR 2006
Pork steaks/cuts	6		10	15		IIR 2006
Sliced bacon (vac.)	12		12	12		IIR 2006
Liver	4		12	18		IIR 2006

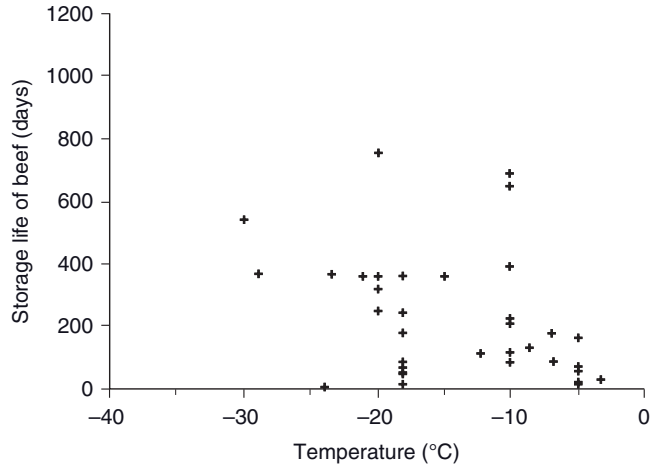

Figure 5.2. Experimental data on the frozen storage life of beef at different temperatures.

storage, but considerable scatter between results at any one temperature.

There is some evidence that consumer panels are often not very sensitive to quality changes. In a study on the quality of lamb stored at −5°C and −35°C, a consumer panel could not tell the difference between samples, although a trained taste panel could differentiate and scored the samples stored at −5°C as rancid (Winger 1984). Some researchers, such as Jul (1982), have questioned the wisdom of storage below −20°C and have asked whether there is any real economic advantage in very low temperature preservation.

Temperature Fluctuation

Generally, fluctuating temperatures in storage are considered to be detrimental to the product. However, it has been reported that repeated freeze-thaw cycles do not cause any essential change in the muscle ultrastructure (Carrol et al. 1981) and that several freeze-

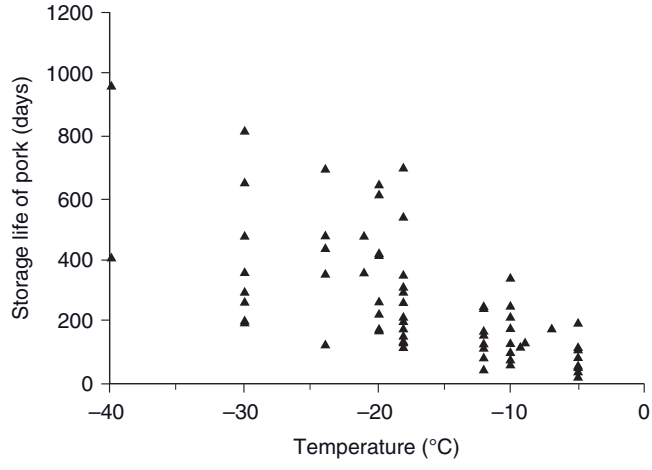

Figure 5.3. Experimental data on the frozen storage life of pork at different temperatures.

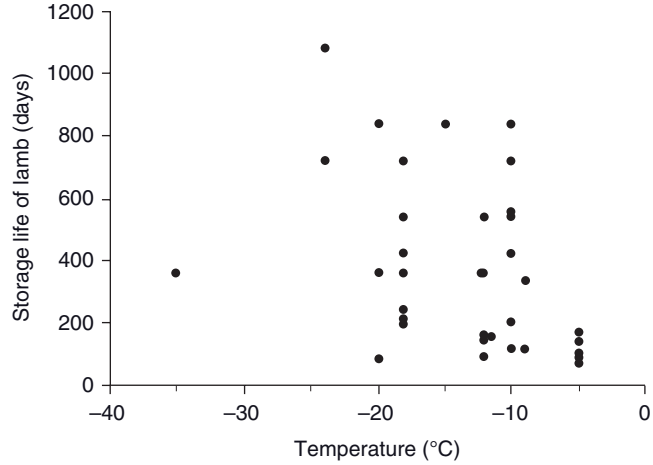

Figure 5.4. Experimental data on the frozen storage life of lamb at different temperatures.

thaw cycles during a product's life cause only small quality damage (Wirth 1979) or possibly no damage at all. In fact, a slight but significant improvement in samples that had been frozen and unfrozen several times was found by one taste panel (Jul 1982).

Minor temperature fluctuations in a stored product are generally considered unimportant, especially if they are below −18°C and are only of the magnitude of 1 to 2°C. Well-packed products and those that are tightly packed in palletized cartons are also less likely to show quality loss. However, poorly packed samples are severely affected by the temperature swings. There is disagreement on how much effect larger temperature fluctuations have on a product. Some authors consider temperature fluctuations to have the same effect on the quality of the product as storage at an average constant temperature (Dawson 1971); others consider that fluctuations may have an additive effect (Van Arsdel 1969; Bech-Jacobsen and Bøgh-Sørensen 1984). There is evidence that exposure to temperatures warmer than −18°C rather than temperature fluctuations may be the major factor influencing quality deterioration (Gortner et al. 1948).

Packaging

Packaging has a large direct effect on storage life, especially in fatty meats and meat products, and in extreme cases, indirectly due to substantially increasing the freezing time. A number of examples have occurred where large pallet loads of warm boxed meat have been frozen in storage rooms. In these cases, freezing times can be so great that bacterial and enzymic activity results in a reduction of storage life.

In most cases, it is the material and type of packaging that influence frozen storage life. Without wrapping, freezer burn may occur, causing extreme toughening and the development of lipid oxidation as the surface dries, allowing oxygen to reach subcutaneous fat in the affected area. Wrapping in a tightly fitting pack having a low water and oxygen permeability (such as a vacuum pack) can more than double the storage life of a product. Waterproof packing also helps to prevent freezer burn, and tight packing helps to prevent an ice buildup in the pack. When a product is breaded, packaging appears to have little effect, and in a trial where breaded pork chops and breaded ground pork were

packed in poor and very good packs, an effect of packing could not be found. Lighting, especially ultraviolet, can also increase lipid oxidation (Volz et al. 1949; Lentz, 1971). Exposure to the levels of light found in many retail frozen food display areas can cause appreciable color change within 1 to 3 days. Development of off flavor can be accelerated and may be noticeable within 1 to 2 months on display. Products kept in dark or opaque packages may therefore be expected to retain color longer than those exposed to the light.

Thawing and Tempering Systems for Meat

Frozen meat as supplied to the industry ranges in size and shape, although much of it is in blocks packed in boxes. Thawing is usually regarded as complete when the center of the block has reached 0°C, the minimum temperature at which the meat can be filleted or cut by hand. Lower temperatures (e.g., −5 to −2°C) are acceptable for meat that is destined for mechanical chopping, but such meat is "tempered" rather than thawed. The two processes should not be confused because tempering only constitutes the initial phase of a complete thawing process. In practice, tempering can be a process in which the temperature of the product is either raised or lowered to a value that is optimal for the next processing stage. In this section, methods of raising the product temperature will be discussed. Tempering systems where the temperature of frozen product is lowered will be covered in the tempering and crust-freezing section.

Thawing is often considered as simply the reversal of the freezing process. However, inherent in thawing is a major problem that does not occur in the freezing operation. The majority of the bacteria that cause spoilage or food poisoning are found on the surfaces of meat. During the freezing operation, surface temperatures are reduced rapidly, and bacterial multiplication is severely limited, with bacteria becoming completely dormant below −10°C. In the thawing operation, these same surface areas are the first to rise in temperature, and bacterial multiplication can recommence. On large objects subjected to long uncontrolled thawing cycles, surface spoilage can occur before the center regions have fully thawed.

Most systems supply heat to the surface and then rely on conduction to transfer that heat into the center of the meat. A few use electromagnetic radiation to generate heat within the meat. In selecting a thawing system for industrial use, a balance must be struck between thawing time, appearance, the bacteriological condition of the product, processing problems such as effluent disposal, and the capital and operating costs of the respective systems. Of these factors, thawing time is the principal criterion that governs selection of the system. Appearance, bacteriological condition, and weight loss are important if the material is to be sold in the thawed condition but are less so if the meat is for processing.

The design of any thawing system requires knowledge of the particular environmental or process conditions necessary to achieve a given thawing time, and the effect of these conditions on factors such as drip, evaporative losses, appearance, and bacteriological quality.

The process of freezing a high water-content material such as meat takes place over a range of temperatures rather than at an exact point, because as freezing proceeds, the concentration of solutes in the meat fluid steadily increases and progressively lowers the freezing temperature. Thawing simply reverses this process.

Thawing time depends on factors relating to the product and the environmental conditions and include:

1. dimensions and shape of the product, particularly the thickness,
2. change in enthalpy,
3. thermal conductivity of the product,
4. initial and final temperatures,
5. surface heat transfer coefficient, and
6. temperature of the thawing medium.

Thermal conductivity has an important effect in thawing. The conductivity of frozen meat muscle is three times that of the thawed material. When thawing commences, the surface rises above the initial freezing point. Subsequently, an increasing thickness of poorly conducting material extends from the surface into the foodstuff, reducing the rate of heat flow into the centre of the material. This substantially increases the time required for thawing.

There are two basic methods of thawing: thermal and electrical. Thermal methods are dependant upon conventional heat conduction through the surface. Electrical methods, on the other hand, employ heat generation inside the product. There is no simple guide to the choice of an optimum thawing system (Table 5.2). A thawing system should be considered as one operation in the production chain. It receives frozen material, hopefully, within a known temperature range and of specified microbiological condition. It is expected to deliver that same material in a given time in a totally thawed state. The weight loss and increase in bacterial numbers during thawing should be within acceptable limits, which will vary from process to process. In some circumstances (e.g., direct sale to the consumer), the appearance of the thawed product is crucial; in others, it may be irrelevant. Apart from these factors, the economics and overall practicality of the thawing operation, including the capital and running costs of the plant, the labor requirements, ease of cleaning, and the flexibility of the plant to handle different products, must be considered.

Table 5.2. Advantages and disadvantages of different thawing systems

		ADVANTAGES	DISADVANTAGES
Conduction systems	AIR	Easy to install: can be adapted from chill rooms. Low velocity systems retain good appearance.	Very slow, unless high velocities and high temperatures are used, when there can be weight loss, spoilage and appearance problems.
	WATER	Faster than air systems.	Effluent disposal. Deterioration in appearance and microbiological condition. Unsuitable for composite blocks.
	VACUUM-HEAT (VHT)	Fast. Low surface temperatures. Very controllable. Easily cleaned.	Deterioration in appearance. High cost. Batch size limited.
	HIGH PRESSURE	Fast. Reduces microorganisms.	Not commercially available at present.
Electrical systems	MICROWAVE/ INFRA RED	Very fast.	Problems of limited penetration and uneven energy absorption. Can cause localized 'cooking'. High cost.
	RESISTIVE	Fast.	Problems of contact on irregular surfaces.
	ULTRASONIC	Fast.	Not commercially available at present.

Thermal Thawing/Tempering Methods

Air

Air is used in the vast majority of thawing/tempering applications. Use of still air is limited to thin products; otherwise, thawing times are excessively long. Although little or no equipment is needed, considerable space is required to lay out individual items of product. Moving air is more commonly used, providing more rapid heat transfer as well as improved control of temperature and humidity. Two-stage air thawing with high initial air temperature followed by a second stage at an air temperature below 10°C has also been used. The duration of the high temperature stage is limited to 1 or 2 hours to avoid excessive bacterial growth, but the increase in heat input during this time considerably reduces the overall process time.

Immersion

Immersion in liquid media allows much more rapid heat transfer, especially if pumped or agitated to avoid temperature stratification in the liquid and grouping together of products. Thawing times are therefore greatly reduced. Practical limitations are that boxes and other packaging (unless vacuum pack or shrink wrap) must be removed before immersion, bulk blocks are liable to break up, leaching of product surfaces can lead to poor appearance, and frequent changing of water for hygiene reasons requires disposal or treatment of large quantities of effluent.

Plate

Plate thawing takes place between metal plates through which warm liquid is piped. The plates and product may also be immersed in water to improve thermal contact between them. Shape is important for reasonable contact with the flat plates, although immersion helps by filling gaps. If immersion is used, frequent water changes are required to prevent bacterial accumulation.

Vacuum

Vacuum thawing relies on the transfer of latent heat of condensation of steam onto product surfaces at low pressure and temperature. For example, if a pressure of $1704\,Nm^{-2}$ is maintained, steam can be generated at 15°C and will condense at this temperature onto the frozen product surfaces. This ensures that although large amounts of latent heat are added, the product will not rise above 15°C. The process is rapid, but evacuation to sub-atmospheric pressure restricts it to batch operation. It is more effective for thin products where the heat released at the surface is quickly conducted through the product.

High Pressure

High pressure decreases the phase change temperature of pure water (down to −21°C at 210 MPa). The lowering of the melting point allows the temperature gap between the heat source and the phase change front to increase, and thus enhances the rate of heat flux (LeBail et al. 2002; IIR 2006). The pressure is released when the food temperature is a little above 0°C. High pressure thawing has been applied experimentally to pork and beef (Suzuki et al. 2006). There is some evidence that the high pressure has the additional benefit of reducing the number of microorganisms (LeBail et al. 2002; IIR 2006).

Electrical Thawing/Tempering Methods

Resistance

Resistance to the passage of a current (50–60 Hz) creates heating effect (ohmic heating). Electrical contacts are required and product structure must be uniform and homogeneous;

otherwise the path of least resistance will be taken by the current, resulting in uneven temperatures and runaway heating. Frozen foods do not readily conduct electricity at low temperatures, but this improves at higher temperatures, so uniformity of initial temperature distribution is also important to avoid runaway heating.

Dielectric

This is split into two frequency bands: radio frequency and microwave. The first uses more typical electrical techniques, with conductors, electrodes, etc. The second relies more on electromagnetic wave technology, with waveguides to "beam" the waves into a cavity.

Radio Frequency

This uses the application of alternating electric e.m.f. (3–300 MHz), using electrodes. Product requirements are similar to resistance methods: uniform structure, homogeneity, and uniformity of temperature distribution. The field is created between two or more electrodes, but the product need not be in direct contact with them. Conveyorized systems have been applied to thawing of meat and offal, in some cases using water surrounding the material to aid temperature uniformity.

Microwave

Electromagnetic (900–3000 MHz) waves are directed at the product through waveguides without the use of conductors or electrodes. Potentially very rapid, the application is limited by thermal instability and penetration depth. Instability results from preferential absorption of energy by warmer sections and by different ingredients, such as fat. Warmer sections may be present at the start of the process; for example, the surface temperature may be warmer than the middle, or they may be produced during the process, such as when energy is absorbed at the surface rather than penetrating all of the product. In the extreme, such warming can lead to some parts of the food being cooked while others remain frozen. These problems, as well as the capital cost of equipment, have greatly limited commercial use. Attempts to avoid runaway heating have involved low-power (and hence longer duration) microwaving, cycling of power on and off to allow equalization periods, and cooling of surfaces with air or liquid nitrogen. Penetration depth depends upon temperature and frequency, being generally much greater at frozen temperatures and greater at lower frequencies.

Ultrasonic

In some work, ultrasound has been merely used to assist heat transfer during immersion thawing. However, research has shown that ultrasound is more highly attenuated in frozen meat than in unfrozen tissue, and that the attenuation increases markedly with temperature, reaching a maximum near the initial freezing point of the food (Miles et al. 1999). The ultrasound attenuation-temperature profile therefore appears to be better suited to producing stable rapid thawing than microwave. Miles et al. (1999) has demonstrated that using 300 kHz ultrasound at an intensity of 1 Wcm^{-2}, a 15 cm thick block of meat can be thawed in less than 1 hour.

Tempering and Crust-Freezing Systems for Meat

Tempering can be a process in which the temperature of the product is either raised or lowered to a value that is optimal for the next processing stage. Tempering systems where the temperature of frozen product is raised have been covered in the thawing and tempering section above. Tempering operations are used to produce the optimum texture in a

chilled product so that it is suitable for mechanical processing. Crust freezing is often used for the same purpose but is essentially a less controlled process where only the surface is frozen. In tempering, product is semifrozen so that it is stiff enough to be sliced, cubed, etc. without deformation. Reducing deformation during cutting improves the yield, by enabling faster cutting and reducing the number of misshapen slices. However, the process must be carefully controlled. The optimum tempering temperature is a function of the meat and the slicer. If too much of the water in the meat is frozen, the subsequent sliced, diced, or chopped meat is likely to show a large increase in the amount of drip released. Also, when the temperature is too low, the hard meat may shatter, and blade wear is excessive. When the temperature is too high, the soft meat will deform and may stick to the blade, and the fat may be torn away from the lean.

Methods for tempering or crust freezing are essentially the same as those used for freezing. A small number of operations use plate freezer, liquid immersion systems, and cryogenic tunnels to temper bacon for high-speed slicing. However, the majority of industrial systems employ air in a single or two-stage process. Since the temperature of a fully tempered product is critical, it can take a long time to be achieved in a single stage process.

Conclusions

Under commercial conditions, differences in freezing rates are unlikely to produce noticeable changes in the organoleptic quality of the meat produced. However, current legislation requires a minimum meat temperature of −12°C to be achieved before meat is moved from the freezing system. Freezing time is therefore of considerable economic importance.

Most unprocessed meat is either frozen in batch air systems as bone-in carcasses, sides, or quarters, or boned out in cartons. Freezing times in such systems are typically 25 to 72 hours. Some offal is frozen in plate freezers. Small processed items are typically frozen in continuous belt freezers or in cryogenic tunnels.

Crust freezing and tempering are increasingly being used to allow high-speed mechanical portioning or slicing of meat and meat products. The final temperature distribution produced by the freezing system is critical in such operations.

Although a great deal has been written on the frozen storage life of different meats, the underlying data are backed up by a relatively small number of controlled scientific experiments. Much of the scientific data date back to the time when meat was either stored unwrapped or in wrapping materials that are no longer used. It is not surprising when we consider the changes in packaging and handling methods over the last century that there is a considerable scatter in data on storage lives for similar products.

In recent years, energy conservation requirements have caused an increased interest in the possibility of using more efficient storage temperatures than have been used to date. Researchers, such as Jul, have questioned the wisdom of storage below −20°C and have asked whether there is any real economic advantage in very low temperature preservation. There is a growing realization that storage lives of several foods can be less dependent on temperature than previously thought. Since research has shown that red meats often produce nonlinear time-temperature curves, there is probably an optimum storage temperature for a particular product. Improved packing and preservation of products can also increase storage life and may allow higher storage temperatures to be used. One suggestion is that with storage at −18°C, low-stability meats such as mechanically recovered meat should be stored for 8 months or less, medium-stability meats such as pork and processed meats should be stored for

between 8 and 15 months, and high-stability meats, which include all red meat except pork, could be stored for more than 15 months.

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Chapter 6

Curing

Karl O. Honikel

Introduction

In its original meaning, curing means correcting, making better, that is, treating sick people, curing disease, or in our subject, preserving, drying, salting, pickling, or smoking a piece of meat (*The New Penguin Thesaurus* 2000). Curing is supposed to enhance the shelf life of meat by preserving and avoiding spoilage with the help of salt, acid (pickle), or drying and/or smoking. In a narrower sense today (and this is the meaning used in this chapter), we understand meat curing to be the addition of salt with or without nitrite and/or nitrate during the manufacture of meat products.

Originally, meat curing was the addition of rock salt, sea salt, or mined salt to an unheated piece or small cuts of meat in order to lower the water activity, prevent microbial growth and chemical spoilage, tenderize the meat, and add flavor to the product.

In the 19th century, meat processors realized that some salts preserved better than others and also some were better at enhancing and stabilizing the product's red color. Saltpeter (KNO_3) was recognized as the contaminant of these "better" salts. The chemical reactions behind it, however, remained unknown. Known for centuries for its oxidative power in gunpowder (carbon + KNO_3), saltpeter was known as an oxidative compound.

Thus the reaction of nitrate in preserving or in preventing oxidative changes (rancidity) remained a secret until Polenske (1891) from the German Imperial Health Office published experiments where he could prove that by adding nitrate (saltpeter) to a pickling solution, nitrite was formed, apparently due to the action of microorganisms in the brine. Lehmann (1899) and Kisskalt (1899) confirmed that nitrite was the agent producing meat's red color and heat stability. Haldane (1901) shed light on the chemistry of the curing process by showing that redox reactions occurred in meat on curing. He also extracted the NO-myoglobin as the substance responsible for the bright red color of cured meat. Hoagland (1910, 1914) showed that the nitrite anion was not the reactant; it was the nitrous acid (HNO_2) or a metabolite of it, such as NO, that reacted with the myoglobin, as shown in the equations in Figure 6.1.

The coloring of meat by nitrogen compounds was understood at the turn of the 20th century, but the antimicrobial and flavoring action was still thought to be mainly due to the salt (NaCl) concentration. Only in the last two decades was light shed on the action of nitrite on flavor and preservation (Grever and Ruiter 2001; EFSA 2003; Lücke 2008).

Nitrite or nitrous acid formed as shown by the first equation in Figure 6.1, when reduced by microbial action is able to sequester oxygen in a meat batter, chemically reacting in the reverse direction to form nitrate, thus preventing oxidative (rancidity) processes. (Honikel 2008; Fig. 6.2). Also, the formation of flavoring substances, mainly from fatty acids, during storage and preparation of meat

(KNO_3) nitrate → reduction by microorganisms → nitrite (KNO_2)

$$KNO_2 + H^+ \leftrightarrows HNO_2 + K^+$$
$$2HNO_2 \leftrightarrows N_2O_3 + H_2O$$
$$N_2O_3 \leftrightarrows NO + NO_2$$
$$NO + \text{myoglobin} \rightarrow \text{NO-myoglobin}$$

Figure 6.1. Scheme of the proposal of Hoagland (1910, 1914) for the action of nitrate in cured meat products.

in the presence of oxygen, does not take place in nitrite-cured meat, since nitrite has sequestered the oxygen. Commonly, this change in flavor is called curing flavor. Furthermore, nitrite or its derivatives bind to myoglobin (forming NO-myoglobin, responsible for the heat-stable red color of meat products), or they react with ascorbate, amino acids, and other compounds (Honikel 2008).

Additionally, NO_2^- and NO_3^- anions bind cations like Fe^{2+}/Fe^{3+}, which exist in cells in the form of free ions. There are microorganisms in which the binding to Fe ions by nitrite inhibits the growth of the microorganisms such as *Clostridium botulinum* (Grever and Ruiter 2001; Lücke 2008).

So we now understand more about the action of salt and nitrate/nitrite in curing. But due to the different modes of action, the curing with salt and salt plus nitrite or nitrate is discussed separately in this chapter.

Action of Salt in Meat Products

Chemically, salt is sodium chloride (NaCl), which dissociates in water into Na^+ and Cl^- ions. NaCl is soluble to 35.7 g/100 ml in cold water and 39.1 g/100 ml in hot water. Its molecular weight is 58.45 Dalton. A solution of 1% NaCl is equivalent to a concentration of 0.17 mol/litre (M); in sausage batters, the common 2% NaCl is equivalent to 0.34 M, while the final concentration of NaCl in a dry cured ham is around 5% ≙ 0.85 M.

	$2HNO_2$	⇆	$N_2O_3 + H_2O$
	N_2O_3	⇆	$NO + NO_2$
	$NO + ½\ O_2$	→	NO_2
	$2NO_2 + H_2O$	→	$HNO_2 + HNO_3$
overall:	$2HNO_2 + ½\ O_2$	→	$HNO_2 + HNO_3$

Figure 6.2. Oxidation of nitrous acid to nitric acid.

Microbial Inhibition

The ions go into solution by becoming surrounded by water molecules (Fig. 6.3). The polar water (H_2O) molecules, which are molecules with a 105° angle between the two hydrogen atoms and the oxygen atom, exhibit a partially negative load around the oxygen atom and a partially positive charge at the H atoms. Due to this polarity, the water molecules are immobilized (no free movement) around the ions in several layers and are no longer available for chemical/enzymatic reactions, neither in food nor in microorganisms. We call this immobilization: the water activity (a_w) is reduced. In pure water, the $a_w = 1.00$. In a totally dry product, $a_w = 0$. By freezing, the water molecules are also bound

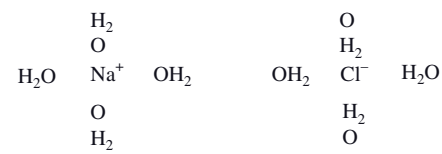


Figure 6.3. Action of water molecules on salt ions leading to solution of salt and immobilization of water molecules; the orientation of the H_2O molecules indicates their polarity.

in ice crystals and are not free in movement, and thus they are not available for chemical/enzymatic reactions. Ice has an a_w value below 1.00. In this way, freezing prevents one aspect of microorganism growth and food spoilage. In chilled fresh meat, the a_w = ca. 0.99. A 2% salt solution has an a_w value around 0.97. Adding 2% salt to a meat batter in emulsion type sausages or paté, the a_w is reduced to 0.96–0.97. For some microorganisms, this a_w is already too low for growth. In raw meat products with higher salt concentrations like salami or raw ham, a_w falls < 0.93. Bacteria do not grow any longer; only molds can cope with such low a_w values (Lawrie 1998).

Physical Action of Salt at Ambient and Chill Room Temperatures

Despite the water layers around salt ions that are dissolved in water, they are still attracted to each other by opposite charged ions, e.g., the charged side chains of amino acids like $-COO^-$ or $-NH_3^+$ in meat proteins.

The water-surrounded ions of a salt brine added to meat diffuse into the muscle cells, or when added into a batter with disrupted myofibers, they may penetrate between the fibrillar proteins. The salt ions themselves thus become immobilized together with their surrounding water layers (Fig. 6.4). But by moving the ions and water in between the protein chains of the myofibers, the attractive forces of the ions in the protein side chains themselves become weaker, and the myofibers swell by molecular movement. Figure 6.4 shows schematically that the myofibers' structure gets wider by salt diffusion.

This swelling allows more water molecules to move in between the protein chains. The volume of the myofibers increases (Fig. 6.5, from A to B). The disruption of the perpendicular and longitudinal fixed proteins of the myofibers caused by the knives in a bowl chopper is considerable, and in the course of swelling from salt, a part of the protein chains

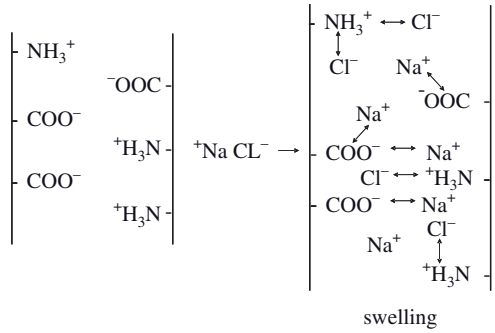


Figure 6.4. Diffusion and immobilization of water surrounded salt ions into myofibrillar structures. The width of myofibers increases through swelling.

is separated from the rest of the myofibers (Fig. 6.5, B to C). As these protein units become independent of the others in the salt solution, they are dissolved in a chemical sense. Dissolving means that the attractive forces between dissolved molecules or ions no longer exist, and the molecules/ions move freely in the solvent.

As the myofibrillar proteins are well coordinated and organized in the myofibers (Fig. 6.6) by cytoskeletal proteins, Z-line proteins, and the forces between the filaments such as the actomyosin complex, only a part of the proteins becomes dissolved. Under the physiological concentrations of a cell (equivalent to about 1% NaCl = 0.17 M), the myofibers are insoluble. Increasing the salt concentration to about 2%, as is the custom in cooked hams and emulsion type sausages, causes a majority of myofibers to swell and only a minority to dissolve.

Heating of Meat and the Addition of Salt

Heating means that the native structures of many constituents and membranes of cells (in our case, muscle and fat cells of meat) are denatured.

The lipid bilayers of meat's cellular membranes lose their integrity around 40°C at the pH of meat <5.8, as we see in the enhanced

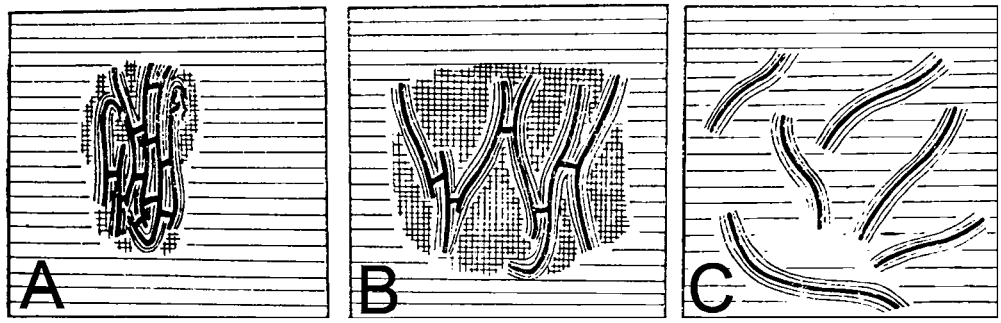


Figure 6.5. Scheme of myofibers and the action of salt. A shows the myofibers of meat with immobilized water (background net structures in an aqueous environment); B shows salt-swollen meat myofibers with more immobilized water within the myofibers; C shows that some myofibers are dissolved in the aqueous environment with only a few immobilized water layers (thin lines along the fibers).

drip loss of PSE pork (Honikel and Kim 1986). Between 40 and 50°C, some soluble proteins in the sarcoplasm denature and become insoluble, as can be seen also in the paleness of PSE pork (dispersion of light, as in milk). Whereas soluble proteins coagulate (denature) into a random structure by heating, fibrillar proteins shrink during denaturation (Fig. 6.7 left side).

Both denatured protein structures encounter less space. Myofibers consist of many proteins, as can be seen in Figure 6.6. They denature between 50°C (myosin) and 70°C (actin) (Quinn et al. 1980). Meat and fat cells are surrounded by connective tissue, which denatures and shrinks between 50–65°C, depending on age of the animal. The collagen in connective tissue starts to dissolve in this process between 55 and 85°C, again depending on the age of the animal.

By coagulation and shrinkage, water is pressed out of myofibrils or proteins in general and cooking loss occurs (Table 6.1). Cooking loss may increase to 45% or 60% of the total water (75%) in meat (45%/75% = 0.6).

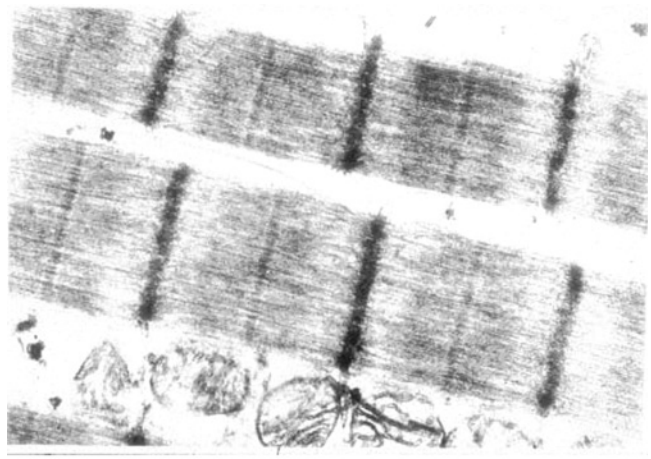
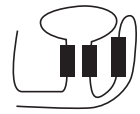


Figure 6.6. Electron microscope picture of myofibers in meat in their well-ordered structure.

e.g. amino acid compositon of myosin

hydrophilic amino acids:	66%
lipophilic or hydrophobic amino acids:	34%



Three dimensional schematic structure of a native protein (bold parts are lipophilic amino acid side chains, line parts are hydrophilic side chains.The surrounding environment is water or aqueous salt solution

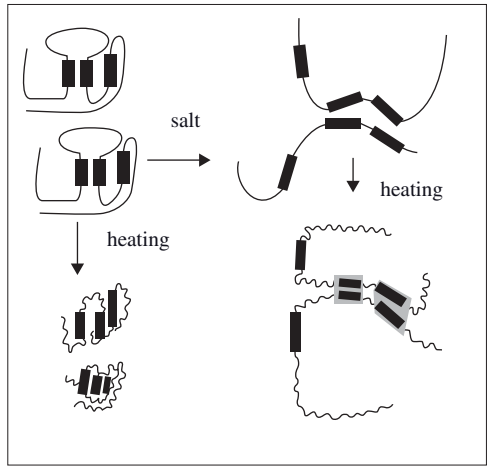


Figure 6.7. The effect of heating and salt on meat proteins.

Table 6.1. Cooking loss of lean beef in cubes of meat of about 100–120 g (75% water at pH 5.5) with a heating velocity of 2.5°C/min from 7°C to the temperatures indicated and kept there for 40 min

	% cooking loss
20°C	0
30°C	0
40°C	2
50°C	6
55°C	9
60°C	13
65°C	18
70°C	23
75°C	28
80°C	33
90°C	41
100°C	45

This water release is called cooking loss or cookout if fat is also released in fat-containing meat. Table 6.2 shows that when a lean meat (pork < 2% fat) is cooked, it loses 28% of its weight as cooking loss or 37.5% of the water in the meat (Table 6.2, A). Let us call this the "reference cooking loss." Mincing, without any further addition, reduces the cooking loss by a few percent due to the more open structure of the minced meat (Table 6.2, A to B).

Mixing salt into the minced meat (B to C) improves its water-holding capacity considerably (only 6% cooking loss). As can be expected, the addition of water (B to D), as is common in "emulsion type" sausages, enhances the cooking loss dramatically to

Table 6.2. Influence of the addition of salt, water, fat on cookout in meat and comminuted meat (B to G)

Heating was carried out from about 7°C with about 2.5°C/min until 80°C was reached. The size of meat in A was 3 cubes of 10 g each, from B-G, 30 g of mince or batter; lean pork contains 75% water					
Code	Composition	% water loss at 80°C[a]	water loss of total water %[b]	% fat loss at 80°C[a]	fat loss of total fat[b]
A reference	piece of lean pork (<2% fat)	28	37.5	0	—
B	minced lean pork (<2% fat)	24	32	0	—
C	B + 2% salt	6	8	0	—
D	B + 50% water	41	33	0	—
E	D + 2% salt	8	6.5	0	—
F	B + 25% water + 25% back fat	33	33	10	37
G	F + 2% salt	2	2	1	4

[a] % related to the material weight
[b] % related to total water/fat weight in the meat or mixture

41% from the reference value of 28%. But if 2% salt is added to the meat plus water (D) (Table 6.2 E), then the cooking loss is low again, at 8%, near to what it would be without water addition (C). The addition of water and fat (F) shows a cooking loss of 33%. In experiment G, 2% salt is added to the composition of F, and the cooking loss is the lowest of all experiments shown in Table 6.2.

If one relates the cooking loss to the total content of water/fat in the product, then a meat piece (reference) loses 37.5% of total water; mincing (B) with the addition of 50% water (D) or 25% water and 25% back fat releases about 32/33% of the total water in the product. The addition of 2% salt with or without added water reduces the cooking loss to 6.5/8% from 32/33% without salt; salt, water, and fat (experiment G) reduces it further to 2% of total water loss. The fat cookout is also reduced by salt.

In conclusion: mincing (comminuting) enhances the water-binding slightly; addition of salt reduces the cookout considerably even if water is added. The comminuting of additional fat and salt strongly improves the water- and fat-binding. The latter happens in emulsion type sausages, where salt and/or fat are needed for a sausage without cookout.

Heating of an Emulsion Type Sausage Batter (with Fat)

The addition of salt and water at low temperatures (0–10°C) causes a denaturation in myofibers by swelling and dissolving, as described earlier. If the meat is heated, the shrinkage is prevented, as shown in the experiments given in Table 6.2.

The proteins in the myofibers at physiological conditions (about 0.1 M salt, pH 7, and 37–38°C) are insoluble. They are kept together by chemical bonds like S–S bridges or (reduced) Schiff bases (such as those in collagen), by ionic attraction (Van-der-Waal forces), hydrogen bonds, or hydrophobic interactions of the lipid side chains of the amino acids. On denaturation with salt and heat, the native structure changes differently, as shown in Figure 6.7. The heat enhances the hydrophobic bonds of protein side chains, and the proteins become insoluble or shrink. With salt, the denaturation leads to a different structure, where the hydrophobicity is also enhanced (Fig. 6.7, right side), due to the weakening of ionic attraction and loss of hydrogen bonds. The swollen myofibrillar protein structure becomes wider, and the hydrophobic side chains are at the surface.

As already described, some of the proteins even dissolve and attract each other by the hydrophobic side chains. These swollen and dissolved proteins form a three-dimensional heat-stable network, as they do in emulsion-type sausages (scheme in Fig. 6.8). The hydrophobic bonds on the surface are able to interact with each other (Fig. 6.7) or interact with small "emulsified" fat particles of meat batters (Fig. 6.9) surrounding them, thus preventing their cohesion to larger fat droplets (Fig. 6.10) and the cookout of fat in a batter (Table 6.2)

When meat is heated without salt, the denatured shrunken protein (Fig. 6.7, left side) is no longer able to be dissolved or swollen by the addition of salt. This is why products like liver paté and blood sausages, which are cooked before salt is added, do not form a heat-stable batter. Paté, with liver as the only protein source, as well as blood sausages, do not form heat-stable batters because liver and blood proteins do not swell and dissolve like myofibrillar proteins. These products may be sliceable below ambient temperatures, but above 20 to 25°C they are usually spreadable.

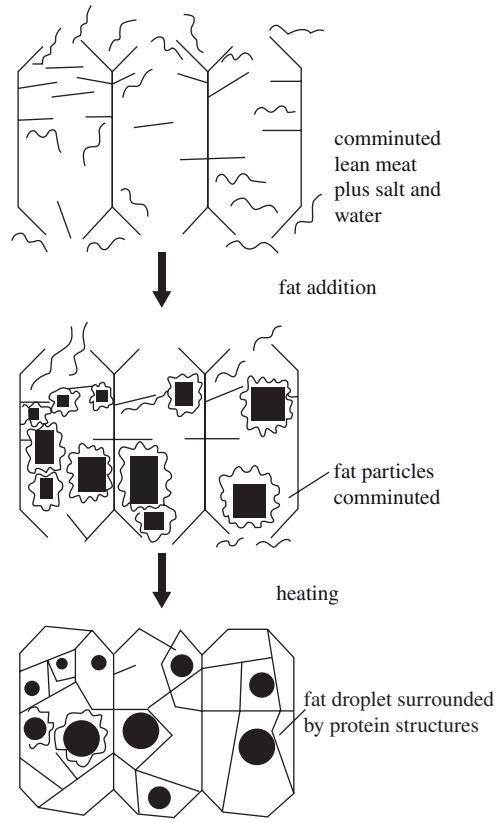


Figure 6.8. Formation of the three-dimensional meat batter network by comminution, salt, water, and heating. The myofibers are indicated by the honeycomb-like structures, solubilized proteins are shown by wave-like structure, fat particles are the black rectangular or circular structures.

Conclusion for the Meat Processors

Salt causes swelling of myofibers, and with the simultaneous addition of water, a partial dissolving of myofibrillar proteins takes

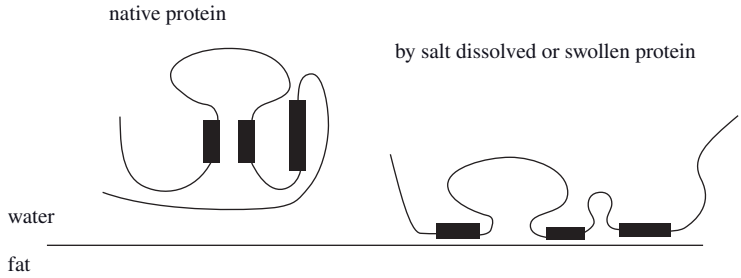


Figure 6.9. Covering of fat particles' surfaces by protein. For explanation of structures, see Figure 6.7.

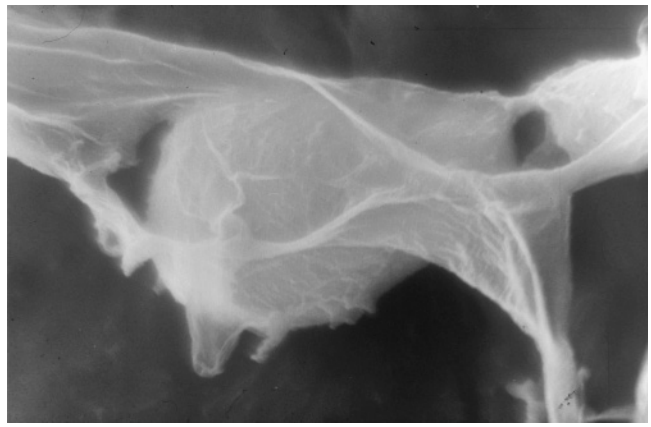


Figure 6.10. Electron microscope picture of a fat globule surrounded by protein sheets.

place. Through this process, water molecules are immobilized. Swelling and dissolving proteins at ambient temperatures and below enhances the tenderness of raw meat products. Salt also lowers the a_w values of meat, enhancing its microbial stability and shelf life. Finally, salt adds to flavor.

Upon heating, the swollen and dissolved proteins form three-dimensional networks in which "emulsified" small fat droplets are surrounded by the hydrophobic/lipophilic surface structures of proteins, causing a heat-stable structure in an emulsion-type sausage.

Principle Action of Salt in Various Types of Meat Products

Emulsion-Type Sausages

These are manufactured from raw meat plus fatty tissue. The meat plus fat is minced or comminuted with salt and water. Both salt and water are technologically necessary for swelling and dissolving of myofibrillar proteins by which the hydrophobic parts of the protein chains envelop small fat particles. Upon heating, they form three-dimensional stable networks.

Cooked Hams

Cooked hams are processed from raw meat (big or small pieces) with about 2% salt or brine given to the raw meat, which is usually mechanically revolved (tumbled). During this process, which generally lasts about a day, salt or brine penetrates into the meat cells and lets the myofibers swell. Due to the limited space for swelling by the more or less intact myofibers and connective tissue sheets and the usual use of smaller amounts of water than with emulsion type sausages, the dissolving of myofibrillar proteins is limited in cooked ham. On heating, the swollen myofibers get tender and the added water remains in the products, making them juicy.

Raw Hams

These are salted in principle in the same way as cooked hams. In many cases, pure salt, with no brine, is used. Due to the omission of a heating process, higher salt concentrations are necessary (lowering a_w value further) for preventing spoilage, and often the products also need chilled storage. The salt penetrates slowly into the meat and

during a drying process, reaches concentrations of 4 to 6% NaCl in the finished product. The salt lets the structures swell and become more tender. Salt enhances stability and shelf life.

Raw Sausages

Raw sausages like salami are manufactured by adding salt (ca. 3%) to a minced or comminuted meat plus fat batter without adding water. The fermentation (which lowers pH) and drying process (which enhances the salt concentration to 3.5–5%) make the product shelf stable, often without refrigeration. The swollen protein structures also "glue" the particles together at cool room or ambient temperatures and keep the products sliceable.

Liver Paté, Liver Sausages, and Blood Sausages

Meat products like these use either meat that is cooked without salt, or raw liver or blood, which contains no myofibrillar proteins. As discussed above, salt cannot swell heat-denatured myofibers, and liver and blood proteins do not form heat-stable networks above chill room temperatures. In some products, the sliceability at higher temperatures is obtained by the addition of gelatin.

Action of Nitrite and Nitrate Together with Salt in Meat Products

Nitrite and nitrate are not used as sole curing agents. Each is always applied with salt. The concentrations of nitrite and nitrate are in the range of 100 to 200 mg/kg, while the salt in meat products is 2000 mg/kg and more. As already mentioned, salt lowers the water activity and enhances food safety. Salt also changes the protein structures of meat. Nitrite and nitrate support the safety action of salt, and improve appearance and flavor.

Nitrogen exists in seven different states of oxidation, starting from ammonia (NH_3) with a formal electron load of −3, through nitrogen gas with a formal load of 0, to NO (load +2), nitrite (NO_2^-, formal load +3), NO_2 gas (load +4), to nitrate (NO_3^-, formal load +5). In the case of curing salts, the states of NO (load +2) to nitrate (load +5) mainly occur.

Figures 6.1 and 6.2 show the changes in nitrous acid occurring in meat systems. After nitrous acid is formed from nitrite in an acidic environment, it can form its anhydride (N_2O_3), which is in equilibrium with the oxides NO and NO_2. NO reacts with myoglobin or amino acids like cystein, or with glutathione, whereas NO_2 reacts with water, forming again one molecule of nitrous acid and one molecule of nitric acid. In this reaction sequence, nitrite or nitrous acid (oxidation state N^{3+}) is oxidized to nitric acid (nitrate), with oxidation state N^{5+}. When NO is the other reaction partner, it has a N^{2+} oxidation state and has been reduced.

In the past few decades, ascorbic acid or its salt, ascorbate resp. isoascorbate (erythorbate), has been used in cured meat batters. A reaction of ascorbate with oxygen forms dehydroascorbate, and thus prevents the oxidation of nitrite to nitrate (Andersen and Skibsted 1992; Skibsted 1992; Skibsted et al. 1994). On the other hand, ascorbate may react with nitrite (nitrous acid or NO) as Dahl et al. (1960), Fox and Ackerman (1968), and Izumi et al. (1989) have shown. Ascorbate reacts with "nitrite" by binding NO. The bound NO seems to be able to react as NO with other meat ingredients. Ascorbate is also added to reduce the formation of nitrosamines. The sequence of reactions of ascorbate preventing nitrosamine formation has not been fully elucidated. It may be due to the reduction by ascorbate of residual free nitrite in meat products (EFSA 2003) or the binding of NO to ascorbate and its retarded release.

Furthermore, ascorbate in batters reduces the toxin production by proteolytic *Clostridium botulinum* type A and B together with nitrite and salt (Robinson et al. 1982). The microbial action of nitrite has been discussed in the introduction and very thoroughly by EFSA (2003).

All this makes clear that nitrite is a very reactive substance that undergoes many reactions in meat products, and thus its use has to be controlled.

Legislative Requirements

Many countries have issued directives or other regulations for the use of nitrite and nitrate in meat products. The European Union (EU) has reconsidered its regulation, and in directive 2006/52/EC/ (Directive 2006), the use of nitrite and nitrate was limited as shown in Table 6.3.

In general, 150 mg nitrite/kg are allowed to be added to all meat products, plus 150 mg nitrate/kg for unheated meat products. That is a maximum of 300 mg nitrite plus nitrate/kg that may be added to a batter or a piece of raw ham. A large number of exceptions, such as Wiltshire or dry cured bacon, may have 175 mg residual nitrite/kg plus 250 mg residual nitrate/kg (i.e., 425 mg residual nitrite plus nitrate/kg are possible). This means that well above 500 mg nitrite plus nitrate have been added when the production starts.

Table 6.3. Directive (2006) of the European Union regarding nitrite and nitrate for meat products shortened in the list of specified products

E No	name	foodstuff	maximum amount that may be added during manufacturing (expressed as $NaNO_2$)	maximum residual level (expressed as $NaNO_2$
E249[a]	potassium nitrite	meat products	150 mg/kg	—
E 250[a]	sodium nitrite			—
		sterilized meat products ($F_O > 3.00$)[b]	100 mg/kg	
		traditional immersion cured meat products (number or products)	—	50–175 mg/kg
		traditional dry cured meat products (number of products)	—	50–175 mg/kg
		other traditionally cured meat products (number of products)	180 mg/kg	50 mg/kg
E 251[c]	potassium nitrate	non-heat-treated meat products	150 mg/kg	—
E 252[c]	sodium nitrate	traditional immersion cured meat products (number of products)	300 mg/kg	10–250 mg/kg (some without added)
		traditional dry cured meat products (number of products)	300 mg/kg	>50 mg/kg (some without nitrite added)
		other traditionally cured meat products	250–300 mg/kg (without nitrite added)	10–250 mg/kg

[a] When labelled "for food use" nitrite may be sold only in a mixture with salt or a salt substitute
[b] F_O-value 3 is equivalent to 3 minutes heating at 121°C
[c] Nitrates may be present in some heat-treated meat products resulting from natural conversion of nitrites to nitrates in a low acid environment

Many countries have similar regulations. For example, the regulations of the United States should be mentioned here. The U.S. Code of Federal Regulations (U.S. Government 2005) states that the food additive sodium nitrite may be safely used in or on specified foods in accordance with the following prescribed conditions:

> As a preservative and color fixative, with sodium nitrate, in meat curing preparations for the home curing of meat and meat products (including poultry and wild game), with directions for use which limit the amount of sodium nitrite to not more than 200 parts per million in the finished meat product, and the amount of sodium nitrate to not more than 500 parts per million in the finished meat product.

All regulations, directives, and laws take into account that nitrite is a toxic substance, and that unlike other additives, nitrite does not remain unchanged in the product during processing. The discoveries of the early 20th century are also taken into consideration. Nitrate is only effective after being reduced to nitrite. This is caused by microorganisms only in products that are not heat treated early after manufacturing, such as raw hams and raw sausages.

Chemical Changes of Nitrite and Nitrate in Meat

Sodium or potassium salts of nitrite (Table 6.3) are added to meat products. Both salts are very soluble (Honikel 2007) and dissociate into their ions. The mother compound, nitrous acid, is the actual reactive compound (Hoagland 1914). It, however, exists at meat's pH values mainly in its undissociated form. If 150 mg/kg nitrite are added at pH 5.7, nitrous acid is formed as shown in Table 6.4 in minute amounts of about 0.45 mg/kg HNO_2. This means that only 1/336 of the nitrite added is converted to nitrous acid. This small amount is in equilibrium with its anhydride N_2O_3 (Fig. 6.1), which again dissociates into NO + NO_2. The NO can react with myoglobin, or with oxygen, forming NO_2; the NO_2 reacts with water and nitrous and nitric acid (Fig. 6.2).

This means that only very small concentrations of NO are available in a batter to react with different compounds. If the pH of the batter is higher than 5.7, then even less nitrous acid is formed. At lower pH values, the concentration of nitrous acid increases. Thus, the nitrite concentration in a batter depends on its pH.

Table 6.5 shows that increasing batter pH from 5.3 to 6.3 increases the remaining nitrite

Table 6.4. Nitrous acid concentration in unheated meat batters

Assumption:	pH 5.7 of batter $= 2 \cdot 10^{-6}$ M H^+
Assumption:	addition of 150 mg $NaNO_2$/kg $= 2.17 \cdot 10^{-3}$ M $NaNO_2$
Facts:	pk_a of HNO_2 $= 3.35$; $k_a = 4.47 \cdot 10^{-4}$ M
	$NaNO_2$ (MW = 69 D)
	$Na^+ + NO_2^- + H^+ \rightleftarrows HNO_2 + Na^+$
	(MW = 46 D) $HNO_2 \rightleftarrows H^+ + NO_2^-$
Calculation:	
	$4.47 \cdot 10^{-4}\,\text{M} = \dfrac{C_{NO_2^-} \cdot C_{H^+}}{C_{HNO_2}}$
	$C_{HNO_2\,(M)} = \dfrac{2.17 \cdot 10^{-3} \cdot 2 \cdot 10^{-6}}{4.47 \cdot 10^{-4}}$ (M)
	$= 0.446$ mg/kg ~ 0.5 mg/kg (ppm)
	$\dfrac{0.446\,\text{ppm } HNO_2 \text{ present}}{150\,\text{ppm } NaNO_2 \text{ added}} = \dfrac{1}{336}$

Table 6.5. Nitrite breakdown and nitrate appearance after nitrite addition (100 mg/kg) to meat batters of various pH values after heating and storage (adapted from Đorđević et al. 1980)

pH of batter	days of storage	nitrite (mg/kg)	nitrate (mg/kg)	sum of nitrite + nitrate (mg/kg)
5.3	0	28	20	48
	12	5	9	14
5.8	0	45	30	75
	12	13	8	21
6.3	0	58	18	76
	12	31	10	41

and that nitrate also forms. The nitrite concentration at day 0 (immediately after preparation) with pH 6.3 is twice the concentration at pH 5.3; after twelve days in storage at pH 6.3, it is six times higher. The nitrate formed from nitrite by sequestering oxygen is less influenced by pH during manufacturing. It is reduced during storage but rather independently of pH. Immediately after heating, the measured sum of nitrite and nitrate at pH 5.3 amounts to 48%; at pH 5.8 and pH 6.3 it is 75/76%. During storage for twelve days, the amount of the sum is reduced to 14% at pH 5.3 and 41% at pH 6.3.

Table 6.6 shows that the disappearance of nitrite continues with storage. Heating reduces the nitrite to ca. 30% of its added amount, while 0.5 mg nitrite/kg remain after sixty days of chilled storage. When 75 mg nitrite/kg was added to the batter, a similar degree of breakdown was observed after the addition of 200 mg nitrite/kg.

Table 6.7 shows nitrite's possible reaction partners. The wide range of percentages is due to the various concentrations of nitrite, heating and storage conditions, and pH.

Table 6.6. Nitrite remaining (mg/kg) during chilled storage (2°C) after addition of 75 and 200 mg/kg after heating to 80°C (adapted from Kudryashow 2003)

time	nitrite 75 mg/kg added	nitrite 200 mg/kg added
after heating	22 (30%)	54 (27%)
20 days	75	15.4
60 days	0.5	5.8

The oxidation of nitrite to nitrate in meat is also the primary reason why nitrate will be found in considerable concentrations in meat products to which only nitrite has been added (see also footnotes to Table 6.3). In Figure 6.11 the nitrite concentrations of German meat products are shown. The emulsion type and cooked sausages and cooked hams are manufactured with nitrite only, but they contain a mean of 20–30 mg nitrate/kg, as is also shown in a very recent survey outlined in Table 6.8. Nitrite is in most cases lower than nitrate in the finished product, with concentrations below 20 mg nitrite/kg in the median value. Only a few samples of sausages and hams contain above 60 mg nitrite/kg (Fig. 6.11), and these also have higher nitrate concentrations. Nitrate may have been added to the raw meat products.

It can be assumed that the concentration of nitrate, in a sausage to which only nitrite

Table 6.7. Nitrite and metabolites in meat products (adapted and changed from Cassens et al. 1978)

bound to/or form	% of total	assumption Honikel
nitrite	5–20	
nitrate	1–10	10–40[a]
myoglobin	5–15	
bound to –SH	1–15	
bound to lipids	1–15	
bound to proteins	20–30	
gas	1–5	
sum	~70	90

[a] according to results presented in fig. 6.11 and tables 6.5 and 6.6

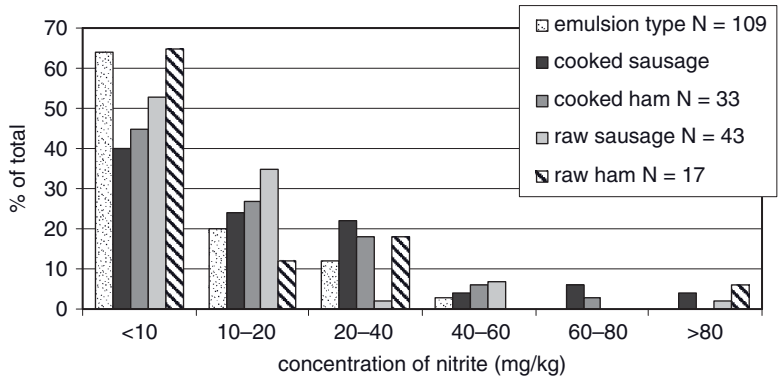


Figure 6.11. Nitrite concentrations in meat products. (Adapted from Dederer 2006.)

is added, is related to the nitrite content. Figure 6.12 shows that with emulsion-type sausages (only nitrite curing salt used), the residual amounts of nitrite and nitrate exhibit no relationship above 20 mg residual nitrite/kg. There is no generally recognizable increase of nitrate with increasing residual amounts of nitrite. Without nitrite addition, a residual amount of nitrate up to 30 mg/kg is probably due to the addition of drinking water to the batter (0–50 mg nitrate/l).

It is interesting to note that the application of ultra-high pressure does change the nitrite concentration, as the sum of nitrite plus nitrate is > 95% at control (no pressure, no heat *in vacuo*) up to 800 MPa (Table 6.9). Even after storage for twenty-one days (*in vacuo*), the sum of nitrite plus nitrate is around 90%. In products with access to oxygen, the remaining concentrations are by far lower than 90% (see Tables 6.5, 6.6, and 6.8).

Table 6.8. Nitrate and nitrite concentration in German meat products 2003–2005 (adapted from Dederer 2006)

sausage type	N	median	
		nitrite (mg/kg)	nitrate (mg/kg)
emulsion type sausages	91	13	24
non-heat treated sausages	15	18	59
non-heat treated ham	14	19	17
liver/blood sausages	16	12	43

Nitrosamine Formation in Meat Products

In the 1970s in the United States, a discussion arose about the formation of nitrosamines in cured meat products, especially fried bacon. Fiddler et al. (1978) showed that bacon and its cookout on frying contained considerable amounts of nitrosopyrrolidine.

According to the reactions shown in Figure 6.13, nitrosamines are formed by amines with nitrite at higher temperatures. But there are some prerequisites for the sequence of reactions to nitrosamines to occur.

- Amines must be present. In fresh meat there are very minute amounts of amines present, which are the decarboxylation products of amino acids. During aging and fermentation, amines will be formed.
- Only secondary amines form stable nitrosamines. Primary amines are immediately degraded to alcohol and nitrogen. Tertiary amines cannot react. Most amines in meat are primary amines derived from α-amino acids.

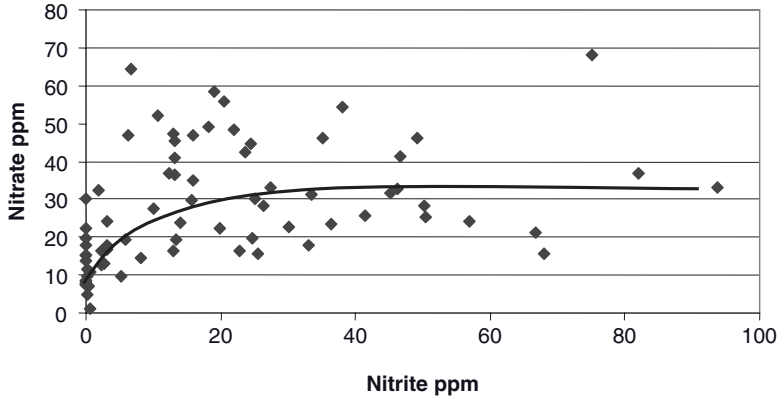


Figure 6.12. Relationship of nitrite and nitrate concentrations in emulsion-type sausage, N = 48. (Adapted from Dederer 2006.)

- The pH must be low enough to produce NO^+ or metal ions must be engaged to form NO^+.

As heated meat products are produced from fresh meat (chilled or frozen), no free amines are present. In dry-cured meat products with a long period of production, amines can be formed, but the nitrite concentration is rather low (see Fig. 6.11 and Table 6.8). Thus, the formation of NO^+ is rather unlikely. In products heated above 130°C, nitrosamines can be formed. Fried bacon, grilled cured sausage, and fried cured meat products such as pizza toppings may experience conditions that form nitrosamines. Table 6.10 shows the results of an investigation by Deierling et al. (1997) where, of the foods investigated, only beer and pizza exhibited dimethyl-nitrosamine in detectable amounts. Thus, nitrosamines occur only in small amounts and they are easily avoidable by proper frying, grilling, and pizza baking. Jakszyn et al. (2004) published a database for nitrosamines and other processing-related residues in foods. Besides amines, amides and unsaturated fatty acids or derivatives of the latter can react with nitrite or its derivatives. Fatty acids or their derivatives can form alkylnitrites. Very little is known about their presence and concentrations in meat products. (See the Jakszyn et al. 2004 database.)

In this context, it should be mentioned that nitrosamines can be present in elastic rubber nettings for meat products, which may contaminate the edible parts of meats such as cooked ham (Helmich and Fiddler 1994; Fiddler et al. 1998).

Table 6.9. Nitrite and nitrate concentration in emulsion type sausage (Bologna/Lyoner) (72 ppm nitrite added) after ultra-high-pressure application and storage of the unheated batter (adapted from Honikel 2007)

days	0	7	21	0	7	21	0	7	21
Treatment	nitrite (mg/kg)			nitrate (mg/kg)			nitrite + nitrate (mg/kg)		
control	54.3	47.1	39.3	15.15	19.5	26.2	69.45	66.55	65.5
400 MPa	53.3	46.4	37.7	15.8	22	26.95	69.05	68.4	64.6
600 MPa	53.0	44.8	37.2	16.15	23.85	29.1	69.1	68.65	66.25
800 MPa	52.3	44.7	37.7	17.5	23.95	26.6	69.75	68.65	64.25

	$NaNO_2$	$+ H^+ \rightarrow HNO_2 + Na^+$	
	HNO_2	$+ H^+ \rightarrow NO^+ + H_2O$	
		$2HNO_2 \rightarrow N_2O_3 + H_2O$	
		$N_2O_3 \rightarrow NO + NO_2$	
	NO	$+ M^+ \rightarrow NO^+ + M$	
primary amine	RNH_2	$+ NO^+ \rightarrow RNH\text{-}N = O + H^+ \rightarrow ROH + N_2$	
secondary amine	R_2NH	$+ NO^+ \rightarrow R_2N\text{-}N = O + H^+$	
tertiary amine	R_3N	$+ NO+ \rightarrow$ no nitrosamine formation	

Figure 6.13. Chemical reactions leading to possible nitrosamine formation; M/M⁺ are transition metal ions (like Fe^{2+}/Fe^{3+} and others).

Nitrite and the Color of Meat Products

For consumers, the red color of cured meat products is one of the important effects of nitrite in meat products. The red color develops in a number of complicated reaction steps until NO-myoglobin (Fe^{2+}) is formed.

Myoglobin exists in muscle in three states, in which the cofactor heme, a porphyrin ring with an iron ion in its center, binds different ligands or in which the iron exists in the Fe^{2+} or Fe^{3+} state. In the native myoglobin, the porphyrin moiety (Fig. 6.14) is supported in the ligand binding by amino acids of neighboring protein.

In its "original" state, myoglobin with Fe^{2+} in the porphyrin cofactor does not bind any ligand except water molecules. In the presence of oxygen, the porphyrin can bind an O_2 molecule, and it becomes bright red. The iron ion is in the Fe^{2+} state. But oxygen and other oxidizing agents such as nitrite can oxidize the Fe^{2+} to Fe^{3+}. The metmyoglobin (MetMb) that is formed is brown.

The "original" myoglobin (Mb), the oximyoglobin (MbO_2), and the metmyoglobin occur together in meat. In the muscle of a live animal there is very little metmyoglobin, but it increases postmortem with the disappearance of oxygen, except when meat is packed with high oxygen. In MAP-packs with about 70% oxygen, the color is bright red. Oximyoglobin is not heat or light stable, and

Table 6.10. NO-dimethylamine in foods (µg/kg); adapted from Deierling et al. (1997)

Food	N	>0.5	Content	
			min	max
Beer	195	3	0.5	1.2
Pizza	57	6	0.5	8.7
Meat products	17	0	0	0
Milk products	6	0	0	0

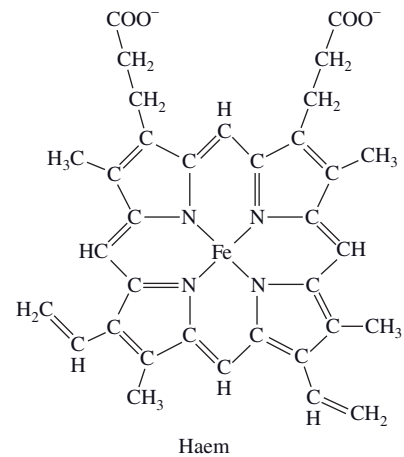


Figure 6.14. Haem/Fe-protoporphyrin (IX), cofactor of myo- and hemoglobin.

if oxygen is used up, it turns brown (because of the presence of metmyoglobin) or green (indicating spoilage from microbial action).

Reducing enzymes, or chemical reactions with a reducing agent like ascorbate, reduce the Fe^{3+} to Fe^{2+}. The NO formed from N_2O_3 can bind to the myoglobin (Fe^{2+}) and form a heat-stable NO-myoglobin. Oximyoglobin is not heat stable and dissociates. The meat turns grey or brown.

Oxygen, carbon monoxide (CO), and NO are biatomic molecules. Like NO, CO binds to myoglobin very tightly. In some countries, MAP packaging of fresh meat with 1–2% CO is permitted. CO and NO addition to fresh meat are not permitted in the EU.

The CO-myoglobin and the NO-myoglobin are heat stable. Heating denatures the protein moiety, but the red NO-porphyrin ring system (often called nitroso-hemochromogen) still exists and is found in meat products heated to 120°C. This heat-stable red color will change after bacterial spoilage, and it fades under UV light. The first is advantageous, since it allows the consumer to recognize spoilage, as fresh meat also changes color when it spoils. CO bound to myoglobin is light resistant.

In the last two years the riddle of the red color of cured raw hams, like Parma ham produced without nitrite or nitrate, has been solved. Various authors have proved that the Fe^{2+} in the porphyrin ring is exchanged with Zn^{2+}, which gives the hams a pleasant red color. Nitrite addition prevents the exchange (Møller et al. 2003; Parolari et al. 2003; Wakamatsu et al. 2004a, b; Adamsen et al. 2006).

Conclusions

Salt as the primary curing agent has several effects on meat. It lowers the water activity and retards or prevents microbial growth and spoilage. Salt enhances water binding in meat. Thus, it prevents the cookout of water on heating and also covers fat particles, preventing their release on heating. By dissolving and swelling the meat protein structure, salt also tenderizes meat and leads to heat-stable structures in "emulsion type" sausages.

The curing agents nitrite and nitrate react with meat ingredients due to the easily changeable oxidation status of nitrogen into many derivatives. Nitrite gives the products an esteemed and stable red color, acts as an antioxidant by sequestering oxygen, prevents or retards mircobial growth, and finally, adds a pleasant flavor. The positive effects are overwhelming compared to the small possibility of the formation of nitrosamines. The intake of curing agents (nitrite plus nitrate) through meat products is small (a few percent) in comparison with other foods (EFSA 2008).

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Chapter 7

Emulsification

Irene Allais

Definition

"Emulsified" meat products are generally defined according to their appearance: meat particles are so fine that they are not visually distinguishable on the smooth product surface.

"Emulsified" meat products are consumed all over the world. Hundreds of different products are available for consumers. They can be classified according to various criteria (Table 7.1). They are very convenient, and there is little or no waste from these products (Sebranek 2003).

From a processing point of view, comminuted meat products are generally classified according to the main stabilizing treatment used in the processing. "Emulsified" meat products can be either raw-cooked or precooked products (Heinz 2007). For meat products, it should be underlined that the emulsification step is always followed by a thermal treatment that aims to stabilize the multiphase batter and to attain the final sensory properties. Raw-cooked products are often named "cold emulsions." The product components are raw (i.e., uncooked) when they are finely comminuted. The resulting viscous batter is portioned and submitted to heat treatment (Figure 7.1a).

Precooked meat-products named "hot emulsions" are characterized by two heat treatment procedures. The first heat treatment is the precooking of some raw materials (fatty tissues), usually in the range of 80°C, and the second heat treatment is the cooking of the final product (Figure 7.1b).

Sensu stricto, an emulsion consists of two immiscible liquids with one of the liquids dispersed as small droplets in the other, called the continuous phase (McClements 1999). The term "meat emulsion" has been used as a general term to describe finely chopped meat mixture. Yet this term is questionable. In cold emulsions, the mixture is not a true emulsion but a multiphase media. The dispersed phase is itself a multiphase media containing solid fat particles with a size ranging from 1 to 50 μm, liquid fat droplets, and air bubbles. The continuous phase is a mixture of water, proteins, salt, carbohydrates, and many fibrous particles (Girad 1990). This media is structured but neither homogeneous nor an isotrope. Still, some controversy exists regarding the mechanism involved in meat emulsion's stabilization (Ruiz-Carrascal 2002). Traditionally, the most widely accepted theory viewed meat batters as behaving like a "classic" oil-in-water emulsion, where fat particles were dispersed in a continuous aqueous solution, and meat proteins formed an interfacial protein film around fat particles. On the other hand, some studies have indicated that the gel-forming ability of meat proteins is the main stabilizing factor (Regenstein 1988; Gordon and Barbut 1992). Micrographs taken in poultry meat batters showed that an organized matrix arrangement already exists prior to cooking (Barbut et al. 1996). Results from

Table 7.1. Classification and Examples of Emulsified Meat Products (From Heinz 2007 and Feiner 2006)

Classification criteria	Examples
Shape	Sausages, balls, loaves
Size	— Small-caliber: Frankfurters, Vienna, hot-dogs sausages — Large caliber: Bologna, Lyoner sausages
Composition	Pork, beef, poultry mixed or pure, cereals, vegetables, various seasoning and flavorings
Geographical origin	— mortadella (Italy) — Lyoner sausage, foam or emulsion or block of fattened duck liver (France) — Chicken sausage with oil — Frankfurter, Cooked Bratwurst, Bockwurst, Weisswurst, Fine liver sausage, … (Germany) — Krakowska Sausage (Poland) — Yor sausages (Thailand) — Luncheon or "Devon" or "Polony" (Australia) — Wiener Fine Veal Liver Sausage, Fine liver sausage (Austria) — Fine liver sausage (Russia, South Africa) — …
Thermal treatment	Cooking, sterilization, smoking
Way of consuming	Cooked, cold or reheated
Final product or ingredient	— Final product used alone : Frankfurters or liver pate — Ingredient as a basic mix containing coarse particles product: Buffalo, coarse ham or Krakow sausages
Main stabilizing treatment	— Raw-cooked meat products : Cold emulsions — Precooked-cooked meat products : Hot emulsions (Fine liver sausages/pates)

a recent work (Ruiz-Carrascal 2002) support the hypothesis that meat emulsions are most likely stabilized (at least in part) by trapping the oil, rather than by surface coating the oil droplets with emulsifying agents.

In "hot emulsions," due to the temperature (>35°C), the major part of the fat is liquid and the term "emulsion" seems to be more appropriate than for cold emulsions. Still, some small elements remain solid (collagen), and the proteins do not equally and completely coat the fat particles. Therefore, it might be better to use the term meat "batter" or "matrix" instead of meat "emulsion" and the term "fine emulsion-like products" or "finely comminuted meat products" instead of "emulsified" products. Nevertheless, similar basic phenomena are encountered for structuration of finely chopped meat mixtures and for true emulsions.

Theory of Emulsion

During emulsification, the interfacial area between phases drastically increases and a high amount of mechanical energy is required. The higher the interfacial tension, the more energy is required to achieve emulsification (Wilson 1981).

Emulsifiers are conducive to emulsion formation by reducing the interfacial tension. They also enhance emulsion stability. Myosin is the main emulsifier in comminuted meat products; it orients itself with the heavy meromyosin head facing the hydrophobic phase, and the light meromyosin tail oriented toward the aqueous phase (Mandigo 2004). Emulsions are by definition thermodynamically unstable. Therefore, a stable emulsion is only kinetically stable (Bergenstahl 1995). Different mechanisms are involved in

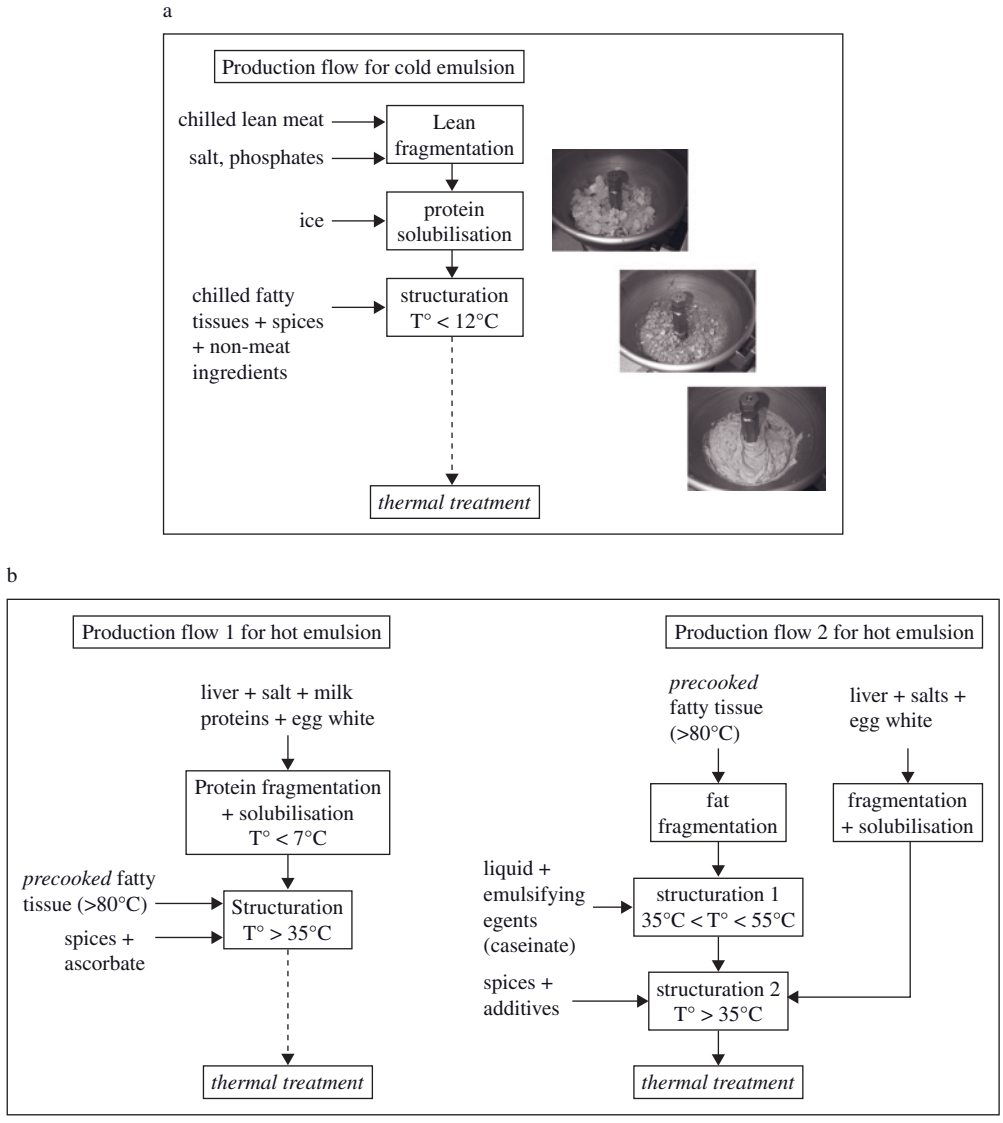

Figure 7.1. a. Processing diagrams for "cold" emulsions. b. Processing diagrams for "hot" emulsions.

emulsion instability: creaming or sedimentation; Ostwald ripening, which is a diffusion transport of the dispersed phase in small droplets into larger ones; coalescence, which is the process in which two droplets combine to form a single droplet; and flocculation, which is the aggregation of droplets due to collisions. The droplet size is the key parameter determining the destabilization kinetics of emulsion. Large droplets are prone to sedimentation and coalescence, whereas finely dispersed emulsions are more sensitive to flocculation and Ostwald ripening (McClements 1999).

The bulk physicochemical and organoleptic properties of emulsions depend on molec-

ular and colloidal interactions, which govern whether emulsion droplets aggregate or remain as separate entities (McClements 1999). These interactions affect protein functionality attributes such as emulsification, gelation, and water binding (Gordon and Barbut 1997). Main molecular forces are repulsive electrostatic forces, attractive van der Waals forces, and steric overlap. Hydrogen bonding and hydrophobic interaction are higher-order interactions that occur between specific chemical groups commonly found in food molecules (McClements 1999). Hydrogen bonds and electrostatic attractions appear to have some importance and seem to participate in the binding of the interfacial protein film (IPF) to the protein matrix (Mandigo 2004). Hydrophobic interactions are strong attractive forces that act between nonpolar groups separated by water (McClements 1999). They happen when surfaces are nonpolar, either because they are not completely covered by emulsifier (during homogenization or at low emulsifier concentration) or because the emulsifier has some hydrophobic regions exposed to aqueous phase (e.g., adsorbed proteins) (McClements 1999). Protein hydrophobicity is important for effective formation of the IPF and stabilization of the protein matrix (Mandigo 2004). During heating, myofibrillar proteins start to denature, leaving several hydrophobic domains exposed. Hydrophobic interactions are then more likely, as is enhanced protein aggregation that immobilizes the fat globules by physical entrapment (Mandigo 2004).

Aims of Emulsification

The emulsification unit operation has three specific aims. First, it has to ensure the physicochemical stability of the product. Emulsification determines the characteristic structure of the batter, which greatly influences fat and moisture separation from the product during cooking. Second, it creates typical sensory properties such as appearance, texture, flavor, or noise. Finely comminuted products are defined by their smooth surface. The "knackwurst," which contains pork, beef, spices, and some garlic, is named for the familiar popping noise when bitten ("Knack!"). Generally, "cold emulsions" give sliceable products, whereas "hot emulsions" give spreadable products. Third, it is a means to create "value-added" meat: relatively low-value meat offcuts can be used, including trimmings or parts of the animal that are less acceptable in their whole state due to a high content of connective tissue or fat (Sebranek 2003; Wilson 1981). Emulsification operation also has side effects: it improves keepability and safety by the use of ingredients such as salt and by thermal treatment. It is also a means to improve nutritional properties through low-fat and low-salt products or through products enriched with fibers and micronutrients. Relevant properties are obtained by a proper combination of ingredients and processing procedures.

Formulation

Basic meat batter ingredients are lean meat, fat, water, and sodium chloride, but various nonmeat ingredients are often used.

Meat Muscles and Meat Derivatives

Structure of Muscles

High-quality lean meat shows very good functional properties. It is well recognized that solubilized muscle proteins are a natural emulsifying agent due to the nature of the amino-acid side-chains situated along their lengths, some of which are lyophilic and others hydrophilic (Wilson 1981). Meat proteins have very different properties, depending on their functional role in the muscle. They can be divided into three groups, based on their solubility characteristics (Nakai and Li-Chan 1988; Zayas 1997). Proteins that are

insoluble even in solutions of high salt concentration constitute the stromal fraction. The main ones are elastine and collagen contained in the connective tissue. Proteins that are soluble in water or dilute salt solutions constitute the sarcoplasmatic fraction. There are around fifty sarcoplasmatic proteins, which represent 30% to 35% of the total muscle proteins. They have very weak binding properties, but they participate to stabilize the emulsion by lowering the interfacial tension between aqueous phase and lipid (Calderon 1984). Among them, myoglobin is the most abundant. Proteins that are soluble in more concentrated salt solutions constitute the myofibrillar fraction. They play the most critical role during meat processing, as this fraction is responsible for the structure build-up and the resulting texture of meat products (Culioli et al. 1993; Xiong 1997; Zayas 1997). They represent more than 50% of the muscle proteins (Cheftel 1985). Their functionality is based on their ability to form three-dimensional viscoelastic gels, bind water, and form cohesive membranes at the oil/water interface of emulsions or flexible films around air bubbles. Actin, myosin, and actomyosin are responsible for most of the functional properties of meat; they contribute to approximately 95% of total water-holding capacity of the meat tissue and 75%–90% of the emulsifying capacity (Li-Chan et al. 1985). Among them, myosin is the most important for fat emulsification and water-holding capacity (Galluzzo and Regenstein 1978; Xiong 2000). The superior emulsifying properties of myosin are believed to be the result of the concentration of hydrophobic residues in the head region, the hydrophilic groups in the tail region, and the high length-to-diameter ratio promoting protein-protein interaction and molecular flexibility at the interface (Xiong 1997). Meat functional properties depend on the type of meat muscle, species, and pH. Binding properties decrease with the decreasing skeletal muscle content of the meat used. Low pH meats have less binding properties. Different kinds of meat—beef, pork, veal, poultry—can be used in finely comminuted products. Beef has the highest binding properties compared with pork or poultry, but pork is often used (Heinz 2007).

Influence of Processing Treatment

High pH or the addition of NaCl or polyphosphates prerigor decrease the actine-myosine interaction and thus increase the swelling capacity. Prerigor meat is known to have higher water-holding capacity (WHC) and better fat emulsifying properties than postrigor meat; it is thus better suited to make comminuted meat products such as sausages (Hamm 1982; Calderon 1984; Bentley et al. 1988; Claus and Sorheim 2006). This is due to higher pH and ATP level (Pisula and Tyburcy 1996).

The high level of ATP in prerigor muscle inhibits the interactions between actin and myosin and creates a loose network able to absorb water. During rigor, the ATP degradation releases divalent ions (Ca++ and Mg++), which favor bridges between proteins, leading to a tight network. During the later maturation, bridges between actin and myosin can be broken and WHC can increase (Calderon 1984).

At high pH, above the isoelectric point, the WHC is maximum. After 24 hours postmortem, pH decreases due to lactic acid formation's tendency toward the isoelectric point. Due to the decrease of electrostatic repulsion, the protein network tightens, and WHC drastically decreases. Moreover, it was found that emulsion stability increases at high pH, particularly for myosin (Denoyer 1978). HLB (Hydrophile Lipophile Balance) meat protein values increase with muscle pH: HLB equals 14.5 for proteins in the prerigor state, whereas HLB equals 13 to 13.5 for proteins in the postrigor state (Girard 1990).

An alternative method to keep a high water-holding capacity is to use freezing or

salting (Calderon 1984). Adding salt (NaCl) before the concentration of ATP has fallen to a level where rigor is initiated can maintain the high binding properties of the meat. Preventing bonds from forming between actin and myosin causes the myofibrils to swell (Sorheim et al. 2006). The pH in the meat should be at a level above 5.9–6.0 at the time of salting to fully utilize the benefits of pre-salting of prerigor meat (Farouk and Swan 1997; Honikel et al. 1981). During frozen storage at temperatures of –20°C or lower the ATP concentration remains almost unchanged. If beef frozen in this way is processed before thawing, sausages of excellent quality are obtained (Hamm 1972). It is better to salt the prerigor meat before freezing than to add the salt during the preparation of the sausage emulsion (Honikel 1978).

Mechanically Deboned Meat (MDM)

MDM is obtained by mechanically separating remaining muscle tissue from the carcasses (Heinz 2007). Mechanically deboned poultry meat (MDPM) is frequently used in the formulation of comminuted meat products due to its fine consistency and relatively low cost (Harding Thomsen 1988; Mielnik et al. 2002; Daros et al. 2005; Sariçoban et al. 2008). Proteins from mechanically deboned chicken meat show high gelling properties compared with egg white and beef plasma (Selmane et al. 2008). MDM contains about ten times more polyunsaturated fatty acids (PUFAs) and also more hemoproteins than HDM and is essentially more susceptible to both chemical and biochemical oxidation, which results in off-flavors and off-odors. This is why MDM addition is limited, as it affects physical, microbiological, and sensorial properties of the products and may result in products that are not in line with national food regulations in some countries (Püssa et al. 2008).

Water

Water has several functions. It accelerates curing and helps to extract water-soluble meat proteins influencing most of the functional properties and yield. It is also a means to control batter temperature by adding ice in cold emulsions or hot water during paté manufacturing. It reduces the per-unit cost and lowers the product energy value, and it has a direct impact on product texture by increasing juiciness (Ockerman 2004).

Fat

For finely comminuted meat products, fats are major ingredients (in the range of 20%), and they are essential for texture, taste, flavor, and physical-chemical stability. Traditionally, fats came from fatty animal tissues directly incorporated into meat batters as ingredients. Fat emulsion or vegetable oils can also be used. The four main factors influencing the firmness of fat tissues are the water and lipid contents, the extent of connective tissue, and the fatty acid composition (Lebret et al. 1996). Fat tissues are less firm when they contain few lipids and a lot of water. When fat tissues contain a lot of connective tissue, they are relatively soft at ambient temperature, but when temperature increases, the connective tissue contracts and gels, trapping lipids in a network that prevents lipid flow (Lebret et al. 1996). At a given temperature, a fat containing fewer unsaturated acids will be firmer than one containing more. Highly unsaturated fats have a lower melting point compared with saturated fats. Animal fats are principally triglycerides, but the fatty acid composition of adipose tissues (and consequently their firmness and their melting temperature) depend greatly on their origin (Lebret and Mourot 1998; Mourot and Hermier 2001) and on the feeding regime. Recent works were targeted to increase the ratio of PUFA (polyunsaturated fatty acids)

to SFA (saturated fatty acids) and to get a more favorable balance between n-6 and n-3 PUFA in meat products by selecting appropriate dietary fats. This has consequences on technological quality by lowering the fat melting temperature, increasing carcass fat softness, and increasing oxidation sensitivity (Mourot 2001). Due to temperature increase during grinding, highly unsaturated fats can start to melt and form a fat coating on the product, which is visually unattractive (Carr et al. 2005).

The taste and flavor of fat varies between animal species. Chicken fat is neutral in taste and well suited as a fat component for pure chicken products (Heinz 2007). It contains a lot of linoleic acid; it melts at a low temperature; and it becomes oxidized easily (Solignat 2003). Beef fat is considered less suitable for further processing than pork fat, due to its firmer texture, yellowish color and more intense flavor (Heinz 2007). Its melting behavior is comparable to pork kidney fat due to its low content of collagen tissues and saturated fat (Solignat 2003). Beef and mutton fats are used for specific processed meat products when pork fats are excluded for sociocultural or religious reasons. Spices can be added to mask their strong flavor and taste. It is usual to use pork fat as it is almost odor- and flavorless and largely available.

The combination of the four main factors described above can explain the behavior of the different pork fat tissues used to manufacture "emulsified" meat products (Table 7.2).

Knowing the fatty tissue composition and understanding the crystalization and melting of lipids in food is important to create food emulsions with desirable properties. Traditionally, the rules for making meat emulsions were based on fat choice and temperature control. To obtain sliceable products, backfat is the more appropriate, even if jowl and belly fat can also be used. The end-point chopping temperatures should remain below 18°C, 12°C, and 8°C for beef, pork, and poultry fats respectively to avoid fat melting (Mandigo 2004). To make spreadable products, fat must be dispersed in the liquid state at "hot" temperatures. The end-point chopping temperatures should be above the fat melting point (i.e., 35°C) (Solignat 2003). To achieve this final temperature, fats are usually poached in water at temperatures above 80°C before being mixed with the proteins (liver or lean meat). The objective is to reach a final internal temperature between 50°C and 60°C for ham fat and between 70°C and 75°C for jowl fat. Fat poaching also causes contraction of the connective tissues, which will facilitate the grinding; it eliminates low-melting fats, which can cause weight losses during cooking, and it lowers the microbial content. Thus, for hot emulsions, low-melting fats are preferred, such as ham and jowl fats that remain firm during cooking at high temperatures.

Increasing concerns about the potential health risks related to the consumption of high-fat foods has led the food industry to develop new formulations or modify traditional products to make them healthier. The most common of these modifications has been fat reduction (Papadima and Bloukas 1999). This is achieved by the use of leaner meat raw materials and by substituting fat with water and other ingredients such as inulin (Jimenez-Colmenero 2007). Significant cholesterol reduction (20%–50%) can be achieved by replacing animal fat with vegetable oils and by adding various plant-based proteins (Jimenez-Colmenero 2007). Fatty acid profile can be improved by using vegetable oils or fish oil (Caceres 2008) or by incorporating vegetable ingredients such as walnuts (Jimenez-Colmenero et al. 2005). Fatty acid profiles have also been improved by the direct addition of CLA to meat batters. Adding oat, rye, or wheat bran contributed to trans-fatty acid reduction (Yilmaz 2004).

Table 7.2. Pig Adipose Tissues: Composition and Technological Properties

	Jowl fat	Belly fat	Ham fat	Backfat	Kidney fat
Water content (%)	15–20 [5]		11–14 [3]	10 [3]	7.5 [3]
Protein content (%)	10–15 [5]		6–7 [3]	6–7 [3]	3.5 [3]
Fat content (%)	70–75 [5]		79–80 [3]	81.3–83 [3]	89 [3]
Saturated fatty acids (% fat content)	34.3 [2]	35.1 [2]		39.3 [1] 36.0 [2] 41.4–43.1 [3] 38.9 [6] 32.3–36.9 [7] C12:0 trace [1] C14:0 1.60 [1] C16:0 23.7 [1] C16:0 24.8 [6] C18:0 15.0 [1] C18:0 13.7 [3] C18:0 12.2 [6]	52.5 [3] 49.5 [6] C16:0 28.8 [6] C18:0 19.4 [3] C18:0 18.6 [6]
Mono-unsaturated fatty acids (% fat content)	49.9 [2]	49.1 [2]	C18:0 14.5 [3]	39.7 [1] 48.0 [2] 46.9–47.9 [3] 40.3 [6] 52.5–57.3 [7] C16:1 4.0 [1] C17:1 trace [1] C18:1 34.7 [1] C18:1 n-9 36.8 [6]	40.2 [3] 36.1 [6] C18:1 n-9 33.6 [6]

Polyunsaturated fatty acids (% fat content)	15.8 [2]	15.8 [2]	C18:2 7.4 [3]	21.0 [1] 16.0 [2] 10.7–10.0 [3] 20.7 [6] 8.7–11.4 [7] C18:2 17.1 [1] C18:2 9.2 [3] C18:2 n-6 18.5 [6] C18:3 2.0 [1] C18:3 n-6 0.8 [6] C20:2 0.55 [1]	7.3 [3] C18:2 6.0 [3] C18:2 14.4 [6] C18:2 n-6 13.0 [6] C18:3 n-6 0.6 [6]
UFA:SFA ratio	1.92 [2]	1.85 [2]	1.15 [3]	1.54 [1] 1.78 [2] 1.18 [3]	1.16 [3]
Firmness at T° < 30°C	Soft	Firm	Soft	Firm	Firm
Melting at T° > 30°C	Low	Average	Low	High	Very high
% melted fat	10°C: 20.9 [4] 40°C: 73.9 [4]	10°C: 20.1 [4] 40°C: 76.4 [4]	10°C: 17.4 [4] 40°C: 64.5 [4]	10°C: 17.2 [4] 40°C: 73.7 [4]	10°C: 9.1 [4] 40°C: 64.5 [4]
Recommended use	Hot emulsion	Cold emulsion	Hot emulsion	Cold emulsion	Lard Fat emulsion

Sources: [1] Al-Rashood et al. 1996, Egyptian pig, [2] Benz et al. 2008, PIC pig, [3] Bucharles et al 1987, Large White pig, [4] Favreau 1981, unknown pig, [5] Solignat 2003, unknown pig, [6] Renaudeau 2007, Large White pig, [7] Ninoles et al. 2007, Iberian pig.

Sodium Chloride

Sodium chloride (NaCl) is involved in water holding, firmness, taste, and flavor, as well as the microbiological safety of meat products (Puolanne et al. 2001). NaCl usually ranges from 0% in salt-free products to 4% in sterilized products. In meat processing, typically 2%–3% salt is incorporated in the product formulation (Claus et al. 1994). Sodium chloride increases water binding in meat linearly from 0 to 0.8–1.0 ionic strengths in the water phase (Hamm 1972; Offer and Knight 1988). This corresponds to less than 5% NaCl in lean meat, provided that the water content is about 75% (Ruusunen and Puolanne 2005). Salt induces important changes in myofibrils. Negative protein charges are increased because chloride ions are more strongly bound to the proteins than sodium ions. According to Hamm (1972), this causes repulsion between the myofibrillar proteins (myofilaments), which results in a swelling of myofibrils or even a partial solubilization of filaments. Offer and Knight (1988) indicate that the selective binding of chloride ions to the myofibrillar proteins causes a loosening of the myofibrillar lattice, due to a repulsion between the molecules of myosin filaments breaking down the shaft of the filament. Moreover, sodium ions are pulled very close to the filament surfaces by the proteins' electrical forces. This increases osmotic pressure within the myofibrils, causing the filament lattice to swell. The factors inhibiting the unlimited swelling are the actomyosin cross-bridges between the filaments and Z-lines. In sausage meat, the sarcomere alteration depends on the interaction of the ionic strength with the processing conditions, particularly of the mincing and mixing conditions (Ripoche et al. 2001). Comminution alone enhances the meat-setting properties, in that the thermal stabilities of myosin and actin are modified and protein salt-solubility is increased (Fernández-Martín et al. 2002).

The swelling depends both on pH and NaCl content (Hamm 1972; Offer and Knight 1988). Without salt, there is a maximum at pH 3.0, a minimum (the average isoelectric point of meat proteins) at pH 5.0, and from there a constant increase within the physiological pH range (Rusunen and Puolonne 2005). Due to the selective binding of ions, salts move the isoelectric point. In cooked pork and beef sausages, approximately the same water holding as with 2.5% NaCl in pH 5.7 can be reached with 1.5% NaCl in pH 6.1 and above (Puolanne et al. 2001).

Since sodium intake generally exceeds nutritional recommendations in industrialized countries and approximately 20%–30% of common salt intake comes from meat products, there is increasing interest among consumers and processors in reducing the use of NaCl content in meat processing (Jimenez-Colmenero et al. 2005). In cooked sausages, it can be concluded that without phosphate, the NaCl content can be lowered to 1.5%–1.7%, and with phosphate, to 1.4% without jeopardizing the technological quality and yield approaches (Ruusunen and Puolanne 2005). However, lowering salt content raises several problems. In low-salt meat products, the increased meat protein content (i.e., lean meat content) reduces perceived saltiness and the intensity of the characteristic flavor decreases (Ruusunen and Puolanne 2005). The functionality of the traditional myosin heat-set matrix may be limited due to low ionic strength (Pietrasik and Li-Chan 2002). This can lead to a decrease in textural characteristics: low-salt batters produce gels that are less hard and chewy, and they have poorer binding properties than gels produced with higher salt (Pietrasik and Li-Chan 2002). Excessive loss of water can lead to a mushy texture. Depending on their origin (beef, pork, or poultry), meat proteins show different gelation patterns and different responses to salt (Barbut and Mittal 1989).

The simultaneous reduction of both salt content and fat content is not easily achieved.

Indeed, to maintain the same NaCl ionic strength, the NaCl content must increase when the fat content is decreased (Ruusunen and Puolanne 2005).

A variety of approaches to reduce sodium content of meat products has been reported: (1) lowering the level of sodium chloride (NaCl) added; (2) replacing all or part of the NaCl with other chloride salts (KCl, $CaCl_2$, and $MgCl_2$); (3) replacing part of the NaCl with nonchloride salts, such as phosphates or with new processing techniques or process modifications; and (4) combinations of any of the above approaches (Ruusunen and Puolanne 2005).

The sodium chloride content of meat can be reduced when using prerigor meat without detrimentally affecting the physical, chemical, or sensory properties of frankfurter-type sausages (Puolanne and Terrell 1983). At lowered salt additions, it appears important to keep the pH of raw materials high enough to ensure a high level of water holding and firmness in cooked sausages, irrespective of how the high pH has been obtained (Puolanne et al. 2001). This would then mean, for example, that high-pH phosphates could be utilized to raise the batter pH. A sausage of normal gel-forming capacity can be made with about 0.3%–0.5% units lower sodium chloride content when phosphates are used, compared with a sausage made without added phosphates (Ruusunen and Puolanne 2005).

Another way to reduce the required amount of salt and phosphate is to use microbial transglutaminase (MTG). It is a calcium-independent enzyme that catalyzes the polymerization and crosslinking of proteins through the formation of covalent bonds between protein molecules (Carballo et al. 2006; Heinz 2007). Although the addition of MTG had beneficial effects on reducing cook loss and increasing hardness and chewiness, Pietrasik and Li-Chan (2002) found that the detrimental effects of salt reduction on these properties were not overcome by the addition of MTG at the levels studied (0%–0.6%).

It was also suggested to use seaweeds, which contain a high concentration of mineral elements, to reduce the amount of added NaCl in meat processing (Cofrades et al. 2008).

Nonmeat Components

Nonmeat components play an important role in "emulsified" meat products, since they influence nutritional, sensory, and functional properties. They were used from very ancient times in comminuted products: the Egyptians used colors and flavorings; and the Romans used saltpeter (potassium nitrate), spices, and colors for preservation and to improve the appearance of foods. More recently, in addition to meat proteins, a variety of nonmeat ingredients have been used as fillers, binders, and extenders to reduce cook shrink and formulation costs. The use of nonmeat components is changing due to several reasons: new components or new forms of them (i.e., nano-ingredients having specific properties) are available; and knowledge about their role has increased, while nutritional concerns have become increasingly important, leading manufacturers to develop new formulations or to modify traditional products to make them healthier.

The use of some nonmeat components can be submitted to food regulation (e.g., additives defined in the Council Directive 89/107/EEC 1989). Most food additives are considered safe. However, some are known to be carcinogenic or toxic. Allergic reactions to colorings and hyperactivity from phosphates have been reported for sensitive individuals. Moreover, some nonmeat ingredients, such as vegetables, egg, or milk proteins, contain substances that cause allergic reactions in some consumers (e.g., gluten enteropathy or lactose intolerance) (Jimenez-Colmenero 2000).

There is a gap between theoretical knowledge of nonmeat ingredients in model foods and their behavior in real food systems. A systematic approach to the study of nonmeat ingredients' effects in meat products is missing. Thus, synergetic or antagonist effects between several ingredients have to be studied for each meat product individually.

Meat products are generally recognized as contributing to nutrition in that they constitute an important source of high biological value proteins, group B vitamins, minerals, trace elements, and other bioactive compounds. However, a negative image often attaches to meat products as a source of fat, saturated fatty acids, cholesterol, sodium, and other substances that in inappropriate amounts may produce negative physiological effects (Cofrades et al. 2008). Numerous researchers are working to optimize meat product composition in order to achieve a composition that is better suited to nutrient intake goals. To achieve this, nonmeat ingredients play a crucial role (Cofrades et al. 2008).

Only nonmeat ingredients having a role in the emulsification process will be detailed here. In the following, we will present nonmeat ingredients through their chemical structures that can explain their functional roles.

Mineral Salts

The main salts having a role in the emulsification of comminuted meat products are NaCl and phosphates. Phosphates have a wide application in the meat-processing industry and improve binding and texture in processed meat products. For meat preparations such as sausage mixes, where phosphates are added as dry powder, phosphates with moderate alkaline effect are preferred, in particular di-phosphates (pH 7.3). Diphosphates have a low water solubility, but they are the most effective form of increasing water binding (Heinz and Hautzinger 2007). They stabilize color by chelating free divalent cations (Fe and Cu). They can indirectly increase shelf life because higher temperatures or longer cooking times can be used without increasing weight loss. Phosphates are believed to act on muscle proteins by increasing the pH and ionic strength (Fernandez-Martin 2002). They affect meat fibers in a similar way as ATP. The simultaneous addition of NaCl and phosphate to meat, therefore, yields considerable modification of the physicochemical features of the myofibrillar proteins (Kijowski and Mast 1988; Findlay and Barbut 1992). The interest in phosphate addition to maintain water binding and gel strength in low-salt products is well known. Phosphate usage is limited to 0.5% in countries such as the United States and Canada, and totally prohibited in Germany for meat products (Trespalacios et al. 2007).

Phospholipids

Surfactants are amphiphilic molecules that have a hydrophilic head group, which has a high affinity for water, and a lipophilic tail group, which has a high affinity for oil. Their principal role is to enhance emulsion formation and stability (McClements 1999). A typical example of such a molecule is lecithin, which in comminuted products is often from eggs or soybeans.

Proteins

Proteins are polymers of amino acids; they have a high proportion of nonpolar groups, and they are surface active. They must rapidly absorb to the surface (McClements 1999). Nonmeat proteins are mainly used for their emulsifying and thickening properties (Delaitre et al. 1988). They have been used in meat products for technological purposes (e.g., protein isolates as binders) and to lower costs (e.g., soy flour as meat extenders). They also provide nutritional benefits (e.g., soy

protein has a positive impact on blood cholesterol content, and whey proteins contain bioactive compounds that may have a positive effect on cardiovascular disease) (Jimenez-Colmenero et al. 2006). Their functional properties are determined by their molecular weight, conformation, flexibility, polarity, and interactions (McClements 1999). Three typical configurations were defined for proteins in aqueous solution: globular, rod-like, and random-coil. Membranes formed by globular proteins tend to be more resistant to rupture than those formed from random-coil proteins. In practice, many biopolymers have some regions that are random coil, rod-like, or globular, and they can change from one conformation to another if their environment is altered (McClements 1999).

Globular proteins form relatively thin but dense interfacial layers that have high viscoelasticities. When globular proteins unfold, they expose amino acids capable of forming disulfide bonds with their neighbors and thus an interfacial membrane that is partly stabilized by covalent bonds. This occurs when emulsion ages or when proteins are heated (i.e., when β-Lactoglobulin is heated to 70°C). Examples of globular proteins used in comminuted meat products are plasma or lactoserum proteins (α-lactalbumin, β-Lactoglobulin, lysozyme), which mainly have a stabilizing role when they form a gelified network during heat treatment (Delaitre 1988).

Native and modified dairy proteins are known for their stabilizing role (Barbut 2006). Different fractions can be extracted. They have various protein compositions and thus different functional properties. Caseins are amphiphilic and unfolding molecules that mainly play a role in emulsifying and viscosity. They rapidly adsorb and stabilize a newly formed oil/water interface. Because the caseins exist in open structures, they are not as sensitive to structural alterations; for example, the caseins are very stable to heating. Casein can impart a pale color and soft texture to meat products. In intensively heated products, this disadvantage is outweighed by the good binding properties, and prevention of jelly and fat separation (Heinz 2007). Barbut (2006) compared the effects of adding dry caseinate, whole milk, skim milk, and regular and modified whey protein powders in emulsified chicken meat batters. Caseinate and modified whey contributed more to enhancing the textural properties of the meat batters compared with the other dairy proteins. Overall, the most cost-effective ingredient appeared to be the modified whey, which also provided the best moisture retention.

Blood plasma is rich in proteins (8%–9%) and these proteins have a higher water-binding capacity than meat proteins. Moreover, the pH of blood plasma is slightly alkaline (7.5–7.8), which is also beneficial to the water-binding capacity. Flakes of plasma ice are particularly suitable for raw-cooked meat products where water or ice has to be added (Heinz 2007).

Polysaccharides

Polysaccharides are polymers of monosaccharides. They are mainly used for their thickening and gelifying properties due to their high molecular weights and their extended structure. Indeed, large highly extended linear biopolymers increase the viscosity more effectively than small compact branched biopolymers. Most polysaccharides are predominantly hydrophilic and are therefore not particularly surface active, except a small number of polysaccharides (gum Arabic) or some modified starches (McClements 1999). Polysaccharides increase water binding and fat binding, thus improving products' juiciness and texture. Plant products rich in polysaccharides are used as fillers for cost reduction and volume addition. Previous studies have reported that emulsion stability was increased due to the

addition of various polysaccharides in different meat emulsion products such as frankfurters and bologna-type sausages (Lee et al. 2008). Polysaccharides from various origins were tested in low-fat meat products: xanthan (Wallingford and Labuza 1983; Pearson and Gillett 1996), carrageenan (Trius and Sebranek 1996), carboxymethylcellulose, beta-glucan, guar gum, gellan, locust bean gum, and starch (Pietrasik 1999; Chattong et al. 2007; García-García and Totosaus 2008). These polysaccharides are available under purified form and are generally considered as additives. Interactions between several polysaccharides and between polysaccharides and salts or nonmeat proteins were often studied: iota-carrageenan, xanthan, and guar gum (Solheim and Ellekjær 1993), potato starch, locust bean gum, and kappa-carrageenan (García-García and Totosaus 2008), blood plasma, microbial transglutaminase, and kappa-carrageenan (Jarmoluk and Pietrasik 2003).

More recently, several studies were dedicated to the use of dietary fibers from different sources (Cofrades et al. 2008). Using raw materials directly instead of purified extracts has several advantages: (a) it reduces formulation costs; (b) it enhances meat products' potential health-beneficial properties by providing not only dietary fiber but also other bioactive components such as polyphenols or carotenoids; and (c) raw materials are not considered as additives for labeling (Cofrades et al. 2000; Cofrades et al. 2008).

Various types of fiber additives such as soy fiber (Cofrades et al. 2000); citrus fiber (Fernandez-Lopez et al. 2004); oat fiber (Chang and Carpenter 1997; Desmond et al. 1998; Steenblock et al. 2001); edible seaweeds such as sea Spaghetti, Wakame, and Nori (Cofrades et al. 2008); pea fiber (Claus and Hunt 1991); peach dietary fiber (Grigelmo-Miguel et al. 1999); and kimchi powder (Lee et al. 2008) have been used alone or combined with other ingredients in the formulation of meat products. Inulins were used to replace fat and to reduce energy intake in breakfat sausages (Archer et al. 2004), bologna sausages (Nowak et al. 2007), and mortadella sausages (10% fat) (Garcia et al. 2006).

Process

Three steps are required to manufacture finely comminuted meat products: lean fragmentation, protein solubilization, and structuration. The order of these steps depends on the emulsion type (cold or hot) and the process used (Figure 7.1). For cold emulsions, these three steps can be achieved either successively in different apparatus (e.g., grinder, mixer, colloid mill) or simultaneously in a unique chopper (Figure 7.2).

For hot emulsions, when a traditional process is used, liver fragmentation and protein solubilization are first achieved simultaneously, then poached fats are fragmented, and the emulsion is formed (Figure

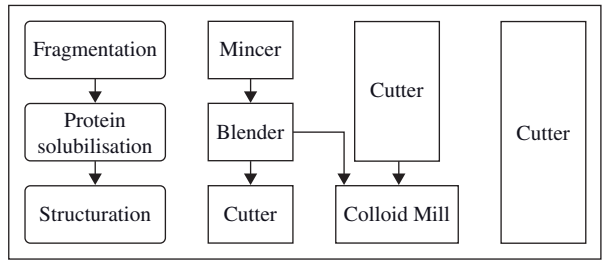

Figure 7.2. Different technology combinations to manufacture "cold" emulsions.

7.1b). Another method is to start with poached fats fragmentation, and then caseinate and hot liquid are added. Caseinate proteins solubilizate, and the structuration starts. Then fragmented liver is added, and the structuration is achieved. The emulsification step is always followed by a cooking step.

Lean Fragmentation

Fragmentation is very specific to emulsified meat products. Contrary to true emulsions made from immiscible liquids, muscles are structured media that have to be disorganized to enable emulsification. During fragmentation, fiber bundles are separated and their membranes are broken. Sarcoleme is broken and myofibrils are liberated, actomyosin swells, and water can be captured (Schut 1976). The collapse of the myofibrillar structure is promoted by mechanical action such as comminution, mixing, tumbling, and massaging (Fernandez-Martin et al. 2002). The disruption of the myofibrillar structure is also achieved by the addition of salt and phosphates (Pearson and Tauber 1984) or by an increase in pH (Samejima et al. 1985; Wang and Smith 1992; Xiong 1992, 1997; Zayas 1997).

Solubilization of Meat Proteins

Many of the functional properties of biopolymers in food emulsions are governed by their interactions with water, and they are only exhibited when they are fully dissolved and evenly distributed throughout the aqueous phase (McClements 1999). Protein solubility is, for instance, a prerequisite step for emulsification, gelation, and water retention (Xiong 1994). Myofibrillar proteins' solubilization requires a minimal ionic strength of 0.5 M; this condition is usually met in processed meats (Offer and Trinick 1983; Xiong 1993). Main factors acting on meat protein solubilization are salt content and pH. After meat protein solubilization is achieved, a colloidal structure is obtained: a solid phase containing nonsoluble proteins, muscle particles, and connective tissue dispersed in a liquid aqueous phase containing salts, soluble proteins, and carbohydrates (Schut 1976).

Structuration

During this step, similar phenomena as in true emulsions occur: fat particles are fragmented to reduce their size, and solubilized proteins in salts must surround the finely chopped fat particles. Fat stabilization during chopping is due both to the formation of an interfacial film surrounding the fat globules and to the physical entrapping of the fat globules within the protein matrix (Barbut 1998). As fat particles' size decreases, the emulsion stability will increase, provided there is sufficient protein to coat all the fat particles. A lack of emulsifier will result in insufficient binding, leading to a soft texture or excessive losses. Excessive amounts of emulsifier could result in a hard texture, often characterized as tough and rubber-like (Mandigo 2004). Like other mixing processes, chopping is characterized by an optimum state where the separation of water and fat from the product is minimal (Brown and Toledo 1975; Girard 1981). Generally, this optimum point is determined according to the final chopping temperature. "Underchopping" results in interfacial surfaces with thick layers of myofibrillar segments around the fat globules and without efficient distribution of proteins and/or fat throughout the interface (Mandigo 2004). "Overchopping" leads to a thin protein film having a low mechanical strength unable to stop fat droplets' migration to the product surface, where they form small pockets of fat called "fat caps." Temperature increases during chopping cause the melting of part of the fat and a decrease in the surface tension of the fat particles. For cold-emulsions, in conventional cutters, the knives can reach local peak tem-

peratures of up to 75°C. This causes denaturation of the proteins, which then form unwanted small lumps in the sausage meat and partly lose their ability to bind water.

Cooking

The final texture of comminuted meat products is primarily the result of the protein gel network that is formed upon processing (Lavelle and Foegeding 1993). For example, in poultry meat batters prepared with salt and phosphate, the structure existing in the raw state (i.e., 20°C) is reinforced by protein gelation during cooking (Barbut et al. 1996). A failure to form the gel can produce an excessive loss of water and fat, producing a mushy and mealy texture (Whiting 1987). Heat processing produces a sol-gel transition, causing protein unfolding and the formation of an ordered, three-dimensional network stabilized by hydrophobic interactions and hydrogen bonding (Whiting 1988). Heat-induced protein aggregation is usually an irreversible process, meaning that the aggregates cannot be broken down by physical means. This results in an immobilization of the fat, water, and other constituents (Mandigo 2004). During cooking, fat separation is due to creaming, flocculation, and coalescence, whereas water exudation depends on polyphosphates, sodium chloride concentration, and water content.

Technology

Mechanical Energy

Lean and fat fragmentation and structuration require a high level of energy from mechanical energy. Mincers are dedicated to meat fragmentation. Mixers achieved protein solubilization and ingredient mixing. Cutters are very polyvalent devices, as they can achieve fragmentation, protein solubilization, and structuration. Colloid mills are dedicated to structuration; they are suitable for homogeneizing intermediate- and high-viscosity fluids. The coarse batter flows through a narrow gap between two disks, a rotative disk (the rotor) and a static disk (the stator). Intense shear stress in the gap is due to the high rotation speed (from about 1000 to 2000 rpm) and the narrowness of the gap (50 to 1000 µm). Many of the factors that increase the effectiveness of droplet disruption also increase the manufacturing costs. Typically, colloid mills with droplet diameters between 1 to 5 µm can be used to produce emulsion (McClements 1999). Compared with cutters, finer and more regular batters are obtained in colloid mills. These devices can be combined in different ways, depending on the amount of production (Figure 7.2). Although chopping technology has been used for a long time, it is still used to develop new products. For example, a process for manufacturing very low-fat sausages (maximum fat content of 4%, i.e., 60%–80% fewer calories than traditional sausage products) was recently performed in Germany (Pointner 2007, patent DE102005026752 2007).

High Pressure

High-pressure application has been shown to act on myofibrillar proteins in a similar manner to salts, so both sodium chloride and phosphates can be reduced (Cheftel and Culioli 1997; Fernández-Martín et al. 2002). The effect of pressure on meat products' binding properties depends on various factors, such as animal species, type of muscle, pH and ionic strength, level of fat and protein, treatment conditions (i.e., pressure level 100 to 700 MPa), time, and temperature (Iwasaki et al. 2006; Trespalacios et al. 2007).

For comminuted meat products, high pressure can be used at several levels during manufacturing: (1) at low temperature (0–5°C) prior to chopping (MacFarlane et al. 1986; Crehan et al. 2000), (2) on comminuted batter prior to heating (MacFarlane

et al. 1984; Fernández-Martín et al. 2002; Hong 2008), (3) during heating (Fernandez-Martin et al. 1997; Yuste et al. 1999a; Chattong 2007; Supavititpatana and Apichartsrangkoon 2007), or (4) after heating to increase shelf life (Yuste et al. 1999b; Ruiz-Capillas et al. 2007).

The effects of high pressure on comminuted meat are difficult to compare, due to the diversity of meat matrixes and the various ways high pressure is applied. Moreover, high-pressure conditions are not precisely enough described (Jiménez-Colmenero 2002).

High-pressure treatment applied to beef muscles for manufacturing frankfurters successfully reduced salt level to 1.5% without any noticeable change in cook loss, and emulsion stability of the frankfurters could be improved with salt reduction, independently of the applied pressure level (Crehan et al. 2000). Main limitations are due to texture modifications (MacFarlane et al. 1984, 1986; Crehan et al. 2000). Pressure-induced protein gels differ from those induced by heat, being glossier, smoother, and softer, and having greater elasticity (Supavititpatana and Apichartsrangkoon 2007). Heat-induced gels and pressure-induced gels show different properties. Heating (>40°C) under high-pressure conditions limits the gelling process of meat systems (Jimenez-Colmenero 2002). When pressure was applied to frankfurters after cooking, pressurized sausages showed different texture attributes (less hard) than heat-treated sausages (Mor-Mur and Yuste 2003; Ruiz-Capillas et al. 2007). High-pressure treatment was combined with microbial transglutaminase to enhance the binding properties, textural parameters, microstructure, and color in low-fat and low-salt chicken gels (Trespalacios et al. 2007).

Control

Like every food product, the quality of finely comminuted meat products is assessed through sensory, chemical, and physical measurements. They can be performed on batter or on the final product. Most of them are off-line measurements performed in a laboratory. Rapid measurements can be performed at-line (e.g., water and fat content), but few on-line measurements are available.

Off-Line Measurements

In the following, only measurements directly related to the emulsification process (i.e., water and fat binding and microstructure) will be discussed.

Microstructure Characterization

Microscopical techniques are useful to characterize the structure of comminuted meat products. Light microscopy achieved the observation of fat globules' distribution and protein gel in emulsion-type buffalo meat sausages (Krishnan and Sharma 1990). It revealed that caseinate and modified whey form distinct dairy protein gel regions within meat batters, and this could explain their ability to enhance the textural properties of the meat batters compared with the other dairy proteins (Barbut 2006). In minced ostrich meat batter, confocal microscopy suggested that the size of the fat droplets varied with gum type (Chattong et al. 2007). In chicken meat gels, it showed that low-fat protein gels obtained by pressure and containing microbial transglutaminase had a more compact and homogeneous microstructure compared with controls that were pressurized but contained no MTGase (Trespalacios et al. 2007). Scanning electron microscopy was useful to show structure differences in low-fat sausages (Morin et al. 2004; Cáceres et al. 2008).

Differential scanning calorimetry (DSC) showed that sausages with a higher gum-to-protein ratio required additional energy for protein denaturation to occur (Morin et al. 2004). It was used to study structural changes

during heating and to trace the gelling process when meat batter was submitted to pressure/heat processing (Fernández-Martín et al. 1997, 2002; Supavititpatana and Apichartsrangkoon 2007). It helps to explain the differences between the functional properties of pressure/heated and heated-only gels (Fernández-Martín et al. 2002).

Texture measurement is a macroscopic assessment related to the product microstructure. Texture profile analysis and the recording of the maximum force required to move a blade through the sample using the Warner Bratzler Shear Blade Set are generally used (Mittal and Barbut 1994; Hughes et al. 1997, 1998; Grigelmo-Miguel et al. 1999; Cáceres et al. 2008).

Water and Fat Binding

In finely comminuted meat products, properties characterizing the degree of water and fat binding are particularly relevant to measure. The main ones are water-holding capacity (WHC), emulsion stability, cooking loss, processing yield, jelly and fat separation, and purge accumulation (Table 7.3). The measurements are generally performed off-line or at-line.

On-Line and At-Line Measurements

There is a lack of on-line sensors to control chopping and to stop the process in order to avoid "overchopping." Only temperature sensors are commonly integrated on cutters and mixers. It is useful to detect the endpoint chopping temperature to determine the optimal state of the batter. In the absence of relevant sensors, most of the time operators also use their know-how to decide when to stop the chopping process. Curt et al. (2004a, b) proposed a method to assess the batter state at the end of chopping using operator knowledge. At-line sensory measurements performed by the expert (fat particle size, size homogeneity, firmness, and adhesiveness) were collected and formalized. They were merged with temperature measurement using the theory of fuzzy sets to define a global index called "chopping degree."

Few studies have dealt with the use of on-line instrumental devices to control the chopping process. Barbut (1998) used a fiber optic probe to detect, at an early stage, meat emulsion breakdown (i.e., the response of the probe); L^* value was correlated to the cook loss. Conductimetry has been used to measure stability of meat emulsions (Morrison et al. 1971; Haq et al. 1973; Kato et al. 1985; Koolmes et al. 1993), but rarely to control chopping (Curt 1995). Recently, temperature and light reflection measurements made during emulsification were used as potential indicators of cooking losses and gel texture in pork sausages (Álvarez et al. 2007; Bañón et al. 2008). Fluorescence spectroscopy was assessed for characterizing meat emulsions and frankfurters manufactured at various fat/lean ratios, chopping speeds, and chopping times. Multidimensionnal data analysis showed that batter fluorescence spectra were correlated to batter and frankfurters' texture attributes (Allais et al. 2004).

Process Control

Manufacturers face an important decision: the optimal combination of raw materials, ingredients, and process parameters to achieve a high-quality product with low production costs fullfilling legal restrictions. Several studies applied optimization methods to finely comminuted products. Good quality models are required to describe the relationship between formulation variables and end-product quality. This is still difficult to achieve because different raw materials (i.e., meat trimmings and animal fat) show a large variability in their biochemical and functional properties (Gunvor et al. 2005).

Most of the studies aimed to optimize emulsion quality through formulation. Different optimization methods were used.

Table 7.3. Methods to Assess Meat Emulsion Properties

	Principle	Observations and sources
Water holding capacity (WHC)	— Force is applied, by centrifugation without any heating (at 4°C or 15°C) or by compression, to remove unbound or loosely bound water. — WHC expressed in g H_2O absorbed/g meat	Mittal & Barbut 1994; Candogan & Kolsarici 2003; Desmond and Kenny 1998; Lin & Huang 2003.
Emulsion stability	— A destabilizing treatment combining heating at 70°C during a given time and centrifugation is applied. — % total expressible fluid (TEF) = [(weight of centrifuge tube and sample) − (weight of centrifuge tube and pellet)]/ sample weight ×100	Centrifugation can be applied before heating (Crehan et al. 2000; Jiménez Colmenero et al. 2005) or after heating (Hughes et al. 1998) for a few minutes (2 to 5 min) at various intensities (e.g. 2600 to 3600 g).
Water and fat released	— Calculated after the supernatant was dried for 16 h at 103°C and expressed as a % of sample weight — Fat released: weight on heating the exudate. — Water released: difference between total fluid released and fat released.	Jiménez Colmenero et al. 2005
Cooking loss	— calculated as weight loss during "standard" heat processing and eventually smoking. — expressed as % of initial sample weight	It is a quasi systematic measurement on comminuted meat products (Mittal and Barbut 1994; Jiménez Colmenero et al. 2005).
Processing yield	calculated after heat processing and cold storage, according to the same way as cooking loss	Paneras et al. 1996
Jelly and fat separation	— Determined after batter samples were heated (35 min, core temperature about 90°C), cooled (4°C for 24 h) and reheated in cans (45°C for 1 h). — The fluid jelly and fat, separated in a volumetric cylinder, are measured in ml — calculated as % of the original weight of batter	It is often measured according the procedure described by Bloukas and Honikel 1992; Lurueña-Martínez et al. 2004; Paneras et al. 1996
Purge accumulation	— Determined on cooked product during cold storage — Calculated as weight difference between the beginning and the end of the storage (at least 7 days at 4°C)	Paneras et al. 1996; Bloukas et al. 1997; Candogan and Kolsarici, 2003; Bishop et al., 1993; Andres et al., 2006

Mixture design was used to optimize emulsion characteristics in a model system containing beef, chicken, and turkey meat (Zorba and Kurt 2006). Plackett–Burman design was used to select compatible ingredients from eleven alternates to optimize the sensory quality of extended meat cubes (Modi and Prakash 2008). Response surface methodol-

ogy was used to determine the optimum salt level (1.3%–2.1%) and pectin level (0.25%–1.0%) when olive oil replaced pork backfat (0%–100%) for the production of highly acceptable low-fat frankfurters (9% fat, 13% protein) (Pappa et al. 2000). Gunvor et al. (2005) used a cross-mixture design to construct the sensory attributes model for sausages' firmness and color. The color and firmness were instrumentally measured and modeled as mathematical functions of biochemical composition (protein, connective tissue, and fat) and muscle content. These models were constrained by acceptability limits found through a consumer test. Constraints were then applied in a nonlinear least-cost optimization model. The objective function to be minimized was the cost function of the meat ingredients, which were varied. Constraints for protein, fat, and connective tissue contents were also made according to legal restrictions. Three optimal solutions were compared. A least-cost solution was found fulfilling consumer acceptability, without fulfilling the legal restrictions. In the second optimal solution, a bit more expensive solution fulfilling the legal restriction without fulfilling the consumer acceptability was found. In the third optimal solution, the biochemical composition (legal restrictions) and linear sensory attributes were restricted but the total cost became significantly higher compared to the previous solutions. These results illustrate the difficulty in fulfilling several quality requirements (legal, sensory, and cost) using only formulation parameters (quantities of the biochemical components and protein sources).

Few studies deal with process optimization. One difficulty that has been encountered in the optimization of processing conditions is the measurement of certain food product properties and the lack of suitable on-line sensors. Curt et al. (2004a) used the Simplex method to determine the value of two process parameters, mixing duration and mixer rotation speed, to obtain a product with desired sensory characteristics at the end of the chopping step. These characteristics were evaluated as a global index called the chopping degree (CD). The processing conditions established at the end of the Simplex algorithm (six trials only) were 3 minutes and 2000 rpm. They achieved a high value for the CD (4.8/5; 5 being the maximum value) (Curt et al. 2004a). This result was confirmed by another study using response surface methodology, where the effects of four process parameters—chopping duration, speed, temperature, and pressure—on the chopping degree were studied (Curt et al. 2004b).

Chopping is often performed as a batch operation. Another strategy to determine the optimal processing conditions is to use the repetitive nature of batch processes in batch-to-batch methodologies. Curt et al. (2007) showed that a batch-to-batch algorithm using human knowledge was able to control the process to obtain the desired sensory properties at the end of the chopping process. Ten runs were carried out independently from each other to validate the algorithm in various processing situations. For each of the ten run tests, only one batch was necessary to achieve the targeted chopping degree.

Conclusion

Emulsification control is based on smart combinations between ingredients' choice and processing definition. Although comminuted meat products are traditional products and their manufacturing follows ancient rules of thumb, new combinations have to be invented to face changing requirements, such as lowering cost, improving nutritional balance, and decreasing energy consumption. To achieve this, a better knowledge of ingredients' properties and their behavior in comminuted meat products is required; the use of new ingredients and technologies can be useful; and the development of on-line sensors and control strategies is necessary.

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Chapter 8

Thermal Processing

Jane Ann Boles

Introduction

The origin of meat cookery is older than civilization itself, and, like meat curing, it probably first occurred by accident when fresh meat was exposed to fire and/or heat. This theory has some support in the classic poem entitled "Dissertation upon Roast Pig" by Charles Lamb. According to Lamb's humorous account, the ancient Chinese kept their pigs in the houses as pets, and after the accidental burning of one house along with the pigs, they learned that "roast pig" was indeed a delicacy. In fact, Lamb satirically suggests that it became a custom to purposely set fire to their houses as a means of preparing roast pork.

Regardless of the origin of meat cookery, it not only improves palatability but also reduces the incidence of spoilage by partial destruction of bacteria. Thus, cooking meat improves the keeping qualities and extends storage life. Cooking not only contributes to the stability of meat products, but also plays an important role in providing a variety of meat products, which can be achieved solely by modifying cooking procedures. Therefore, meat cookery has contributed greatly to advances in civilization.

Cooking has the following effects on meat and meat products:

1. destroys considerable numbers of microorganisms and improves the storage life of meat products if not contaminated post processing
2. improves meat palatability by intensifying the flavor and altering the texture
3. develops color
4. decreases the water content of raw meat, especially on the surface, which in turn lowers the water activity and improves the peelability of casings on cured meats products and extends their shelf life
5. modifies the texture or tenderness of meat and meat products
6. coagulates and denatures the meat proteins, at the same time altering their solubility and stabilizing the cured meat color
7. inactivates endogenous proteolytic enzymes and prevents development of off-flavors due to proteolysis

Importance of Cooking

Destruction of Bacteria and Improving Stability

Cooking performs a most important function by causing destruction of spoilage and pathogenic organisms. The number of organisms destroyed will depend on the temperature to which the product is cooked, how long the product is held at that temperature, and the type of bacteria on the product. Meat is not sterilized under normal cooking conditions, and the net effect is merely a reduction in total bacterial load and an extension of storage life. By proper handling to avoid

recontamination of the product, along with refrigeration to slow down multiplication of bacteria, the storage life is extended. The length of storage will depend largely on the care taken to prevent recontamination and minimize conditions favorable to growth of organisms still present in the meat. Different pathogens can be a problem. *Listeria monocytogenes* is the current focus of much research on ready-to-eat meat products. The bacteria are not greatly heat resistant but like cold temperatures. If a ready-to-eat product is contaminated with *Listeria* after processing, during packaging or slicing, and no post packaging treatments are used to destroy the bacteria, this can be a problem. Many recalls have been conducted because of this pathogen. Specific internal temperatures of cooked products must be met to control other pathogens such as *Salmonella* spp., *Campylobacter jejuni*, and *Escherichia coli*. Time and temperature combinations can be used to control different pathogens (Table 8.1).

In the manufacture of smoked meats, cooking is done primarily to produce a table-ready product. However, cooking also plays a major role in extending the shelf life of such products. Although raw meat is subject to spoilage within a few days, finished cured meat products can normally be stored for several weeks after cooking, with proper packaging and refrigeration.

Improvement in Palatability

Cooking is an important factor in developing the palatability of meat products. Although some people like to eat raw meat, most prefer the flavor and aroma of cooked meat. Cooking intensifies the flavor of meat and changes the "blood-like" or "serumy" taste of fresh meat to pronounced cooked flavor and aroma.

Aromatic compounds become a bigger contributor to the palatability of meat after cooking; prior to cooking, the basic tastes (sour, salty, sweet, bitter) are the major flavor components identified in raw meat (Seideman and Durland 1984). Hamm and Hofmann (1965) observed that the evolution of meat flavors happens as temperatures exceeding 70°C are reached and the oxidation of sulfhydryl groups to disulfide groups occurs. Although the aroma of cooked meat has a characteristic sulfury note, there appears to be a number of other components that make important contributions to the odor (Table 8.2). Taste becomes relatively less important as the aroma develops during cooking, but the overall impact of the taste of cooked meat is still a combination of taste and aroma.

Methods of cooking can profoundly influence the flavor of meat; in fact, it is questionable if any other factor is as important. Browning of meat, various flavor additives used in cooking, and a variety of modifications during cooking markedly affect the flavor of the end product (Seideman and Durland 1984).

Both the amount and kinds of fat present have an influence on the flavor of meat products. Since fat is believed to impart the characteristic species flavor, not only the kind but also the percentage of fat will have a great influence on the characteristic flavor of various meat products. Fat is also important in carrying added flavors from seasonings.

Texture of meat is affected greatly by cooking (Table 8.3). Meat samples become harder and drier as the internal temperature of the meat increases (Ritchey and Hostetler 1965; Bertola et al. 1994). The greatest differences seen are between 74°C and 80°C internal temperature. However, as the internal temperature increases, mealiness scores have also been reported to increase (Bertola et al. 1994). Bertola et al. (1994) observed an interesting phenomenon; at an intermediate range of temperatures 66–68°C, hardness decreased very quickly, reached a minimum value, and then increased until maximum value was reached above 80°C. Bertola et al. (1994) attributed the increased toughening associated with increased cooking tempera-

Table 8.1. Effect of cooking on selected pathogenic bacteria

Author	Product	Bacteria	Cooking Process	Effect
Pepe et al. 2006	Breaded chicken cutlet	Staphylococcus aureus	Baking	Absent from 3.5×10^7
Whyte et al., 2006	Chicken livers	Campylobacter	Pan fry to internal temperature 70°C	No positive samples
Yilmaz et al, 2005	Veal meatballs	Stapylococcus aureus	Grill 160°C for 3 min	1.02 log reduction
			Conventional oven 160°C 6 min	1.08 log reduction
		Escherichia coli O157:H7	Grill 160°C for 3 min	4 log reduction
			Conventional oven 160°C 6 min	4 log reduction
Murphy et al 2004a	Franks, pork	Escherichia coli O157:H7	55°C	33.44 min D-value[a]
			57°C	10.37 min D-value
			60°C	3.22 min D-value
			62.5°C	3.22 min D-value
			65°C	0.80 min D-value
			67.5°C	0.077 min D-value
			70°C	0.048 min D-value
Murphy et al 2004a	Franks, pork	Salmonella	55°C	45.87 min D-value
			57°C	26.67 min D-value
			60°C	5.07 min D-value
			62.5°C	2.56 min D-value
			65°C	1.91 min D-value
			67.5°C	0.36 min D-value
			70°C	0.083 min D-value
Murphy et al 2004a	Franks, pork	Listeria monocytogenes	55°C	47.17 min D-value
			57°C	22.32 min D-value
			60°C	5.61 min D-value
			62.5°C	2.87 min D-value
			65°C	1.56 min D-value
			67.5°C	0.44 min D-value
			70°C	0.085 min D-value
Murphy et al 2004b	Beef/Turkey link	Escherichia coli O157:H7	55°C	23.23 min D-value
			57°C	7.43 min D-value
			60°C	2.38 min D-value
			62.5°C	0.76 min D-value
			65°C	0.24 min D-value
			67.5°C	0.08 min D-value
			70°C	0.03 min D-value
Murphy et al 2004b	Beef/Turkey link	Listeria monocytogenes	55°C	50.35 min D-value
			57°C	18.60 min D-value
			60°C	6.87 min D-value
			62.5°C	2.54 min D-value
			65°C	0.84 min D-value
			67.5°C	0.35 min D-value
			70°C	0.13 min D-value

(continued)

Table 8.1. Effect of cooking on selected pathogenic bacteria *(cont.)*

Author	Product	Bacteria	Cooking Process	Effect
Murphy et al 2004a	Beef/Turkey link	Salmonella	55°C	41.02 min D-value
			57°C	15.15 min D-value
			60°C	5.6 min D-value
			62.5°C	2.07 min D-value
			65°C	0.76 min D-value
			67.5°C	0.28 min D-value
			70°C	0.10 min D-value
Guo et al 2006	Ground beef	Escherichia coli O157:H7	Radio Frequency Cook 72°C IT	7 log reduction
			Water bath 72°C IT	7 log reduction
Patel et al 2005	Blade tenderized steaks	Escherichia coli O157:H7	54.4°C	2.71 log reduction
			62.8°C	3.59 log reduction
			71.1°C	5.21 log reduction
Mukherjee et al., 2008	Restructured roast beef	Escherichia coli O157:H7	60°C	1.9 log reduction
			65°C	2.5 log reduction
Singh et al., 2006	Ground beef	Escherichia coli O157:H7	62°C	1.97 min d-value
			65°C	1.58 min D-value
		Salmonella	62°C	1.93 min d-value
			65°C	1.15 min D-value
Stopforth et al., 2008	Ground beef patties	Salmonella	48.9°C	0.9 log reduction
			54.4°C	1.1 log reduction
			60°C	1.7 log reduction
			65.6	3.8 log reduction
			71.1°C	6.3 log reduction
Sallami et al., 2006	Bologna batter	Listeria monocytogenes	50	25.21 min D-value
			55°C	17.3 min D-value
			60°C	5.57 min D-value
			65°C	0.93 min D-value
			70°C	0.08 min D-value
		Salmonella	50	10.11 min D-value
			55°C	3.49 min D-value
			60°C	1.47 min D-value
			65°C	0.28 min D-value
			70°C	0.04 min D-value

[a] D-value is the time required to reduce a given bacterial population by 90%

ture to the contraction and likely hardening of filamentous materials present within the meat. These results showed that two reactions with opposite effects were taking place, one producing tenderization and the other increasing hardness of the samples.

Many researchers have observed an increased shear force with increasing internal temperature, followed by a decrease in shear force after internal temperature reach 70° (Ritchey and Hostetler 1965; Bouton et al. 1976, 1982; Leander et al. 1980). However, other researchers have reported a decrease in shear force values at temperatures between 50°C and 60°C (Davey and Neiderer 1977; Hearne et al. 1978). The different observations could be associated with temperatures being evaluated. Bouton et al. (1976) reported

Table 8.2. Compounds contributing to flavor and odor of cooked meat

Author	Compound	Flavor or Odor
Prescott et al., 2001	Branched chain fatty acids	Barnyard, milky, sour, sheepmeat flavor
Calkins and Hodgen, 2007	n-caprioc acid	Goaty
	Cyclobutanol	Roasted
	2-decenal	Tallow, orange
	2,4-decadienal	Deep fat flavor, chicken flavor
	1,3-bis(1,1-dimethylethyl)benzene	Cooked beef
	Hexanal	Fatty-green, grassy, strong green, tallow, fat
	2-acetyl-1-pyrroline	Roasty beef note
	2 acetylthazole	Roasty beef note
	12-methyltridecanal	Tallow beef like
Gasser and Grosch, 1988	2-methyl-3-furanthiol	Beef aroma
	Bis(2-methyl-3-furyl)disulphide	Beef aroma
Young and Baumeister, 1999	4 hetanal	Fat yucky, sweaty feet odor
	Heptanal	Fat yucky, sweaty feet odor
	1-octen-3-ol	Mushroom odor
	Nonanal	Fatty odor
	Nonanoic acid	Fat/cheese odor
	2-decenal	Fat/cheese odor
	2.4-decadienal	Fat odor
Farmer and Patterson, 1991	Bis(2-methyl-3-furyl)disulphide	Meat, roasted, burnt odor
	2-furfuryl 2-methyl-3-furyl disulphide	Meaty, roasted, burnt odor
	Bis(2-furfuryl) disulphide	Roasted burnt odor
Werkhoff et al., 1990	2 methyl-3-[(cis-2-methyltetrahydor-3-thienyl)thio] furan	Meaty, grilled, mushroom-like, grilled odor
	2 methyl-3-[(trans-2-methyltetrahydor-3-thienyl)thio] furan	Roasted notes, vegetable-like, mushroom-like, meaty odor
	2-methyl-2-[(2-methyl)-3-thienyl)thio] tetrahydrothiopene	Typical meat note, characteristic, roast meat odor
	2-methyl-3-[(2methyltetrahydro-2-thienyl) thio]furan	Roasted, meaty, typical meat note odor

Table 8.3. Effect of internal temperature on tenderness of cooked meat

Author	Temperature	Effect
Bertola et al., 1994	66–68°C	Hardness decreased quickly thin
	80°C	increase to a maximum
Ritchey and Hostetler, 1965;	Increased temperature to 70°C	Increased shear force
Bouton et al., 1976; 1982;	>70°C	Decreased shear force
Leander et al., 1980		
Davey and Neiderer, 1977;	50–60°C	Decreased shear force
Hearne et al., 1978		
Bouton et al., 1976	Ambient–60°C	Increased shear force
Laakkonen et al., 1970	50–60°C	Major decrease in shear force
Leander et al., 1980	63–73°C	Shear force increased
Hearne et al., 1978	40–50°C	Small decrease in shear force
	50–60°C	Greater decrease in shear force
	60–70°C	No further change
Ritchey and Hostetler, 1965	61–80°C	No difference in ease to fragment, increased connective tissue, increase softness of connective tissue
Boles et al., 1991	71–77°C	Reduced initial and sustained tenderness at lower temperature

an increase in shear force upon cooking occurred between ambient temperature and 60°. Laakkonen et al. (1970), however, reported a major decrease in shear values between 50 and 60°C, and Leander et al. (1980) observed an increase in shear force as the internal temperature increased from 63°C to 73°C. Hearne et al. (1978) reported a small decrease in shear values when the internal temperature was between 40°C and 50°C, with a greater decrease in shear value taking place between 50°C and 60°C, and no difference seen between 60°C and 70°C. Alterations in the collagen and meat microstructure could explain some of the differences in the observations reported.

Davey and Neiderer (1977) suggested that heat tenderizes meat in three distinct stages. The first stage, up to 65°C, was from increased proteolytic breakdown of myofibrillar elements; the second stage, between 70°C and 100°C, was through the destruction or solubilization of collagen with little loss of myofibrillar strength; and the third stage, beyond 100°C, was from a combination of collagen and myofibril breakdown. These researchers concluded that cooking in the range of 70° to 100°C halved shear force values and were as effective as aging in increasing tenderness.

Sensory evaluation of meat cooked to different internal temperatures has been reported. Some of the information does not agree with what has been reported for shear force values. Ritchey and Hostetler (1965) reported no difference in ease to fragment a sample when steaks were cooked to an internal temperature between 61°C and 80°C, and scores for amount of connective tissue and softness of connective tissue increased as the internal temperature increased. Boles et al. (1991) however reported reduced initial and sustained tenderness when pork chops were cooked to 77°C compared with 71°C. Fjelkner-Modig (1986) also reported reduced tenderness when chops were cooked to higher internal temperatures. The hardening observed by other researchers is usually a surface phenomenon. Shear force values are measured in a way to avoid the surface hardening. Sensory panels, however, evaluate all of the cooked meat, including the surface. This may explain the differences seen between shear force measurements and sensory evaluation.

Cooking temperatures are important to the tenderness of meat from older animals. The increased cross-linking of collagen due to age reduces tenderness. Beilken et al. (1986) reported peak shear force values were not influenced by animal age until heating temperatures reached 50°C. Peak shear force values of veal decreased above 50°C, and at 55°C, animal age differences became significant. Peak shear force values decreased above 55°C and 60°C for intermediate and oldest age groups. At temperatures above 65°C, peak shear force values increased up to 80°C before decreasing. The initial force values increased steadily, with heating temperatures up to 70–80°C before declining.

Final internal temperature also impacts the juiciness of meat products. Fjelkner-Modig (1986) reported pork fried to an internal temperature of 60°C was much more juicy than that fried to 80°C. Boles et al. (1991) also reported improved sensory scores for pork chops cooked to 71°C compared with chops cooked to 77°C. This difference in juiciness could be related to the increased cook yields seen with lower final internal temperatures (Boles et al. 1991).

Color Development

Cooking has an important function in stabilizing cured meat pigment formed by the action of nitric oxide with myoglobin (Vösgen 1992). Without cooking of the product, the color is more red than pink and is less stable than it is after cooking. This is one of the important functions of cooking for cured meat production. End-point tempera-

ture can affect the development of color in cured-meat products. Tauber and Simon (1963) reported that cured-meat color developed more rapidly in frankfurters as the temperature was raised from 76.7°C to 98.9°C. Wirth (1986) observed that if the temperature was applied for too short a time or if the temperature was not high enough, then the proper cured color would not be reached. Any products cooked to lower temperatures will have a less stable color. Fox et al. (1967) reported samples cooked to 68.9°C had a more stable pigment than those cooked to 54.4°C.

Fresh meat color is also affected by cooking. The extent of denaturation of the globular portion of myoglobin affects how consistent the brown color is in cooked meat. Protection of the myoglobin pigment by high pH results in a redder appearance of the cooked meat at the same internal temperature (Swan and Boles 2002). This phenomenon is sometimes called the "hard to cook" defect and is often seen in high pH meat products. Meat with normal pH will appear redder at lower final internal temperatures than at higher internal temperatures. Lyon et al. (1986) reported as final internal temperature increased, subjective color scores increased, indicating less redness and a more apparent degree of doneness. Objective measurements supported this observation. Hunter L values increased, while both Hunter a and b values decreased, with increasing internal temperature. Boles and Swan (2002b) reported similar results. The final internal temperature increased lightness, and decreased redness and yellowness of cooked beef roasts.

Types of Cooking

Many small processors utilize smokehouses to cook their processed products. These houses can be very complicated with computer controls or more simple with manual controls. Small batch ovens (Fig. 8.1) are often used by small processors because of space restraints as well as versatility. Larger processors may utilize continuous cooking ovens to increase output. These ovens have stages that allow for different cooking rates, as well as smoke application. Other options that are used for cooking of processed meat products are steam jacketed kettles and water baths. Products cooked in this equipment are submerged in the heated water to cook and

Figure 8.1. View of a smokehouse.

are usually vacuum packaged prior to cooking.

The rate of heat penetration depends on the type of cookery used (Seideman and Durland 1984). For example steam cookery or other moist heat methods of cookery will result in faster heat penetration (McCrae and Paul 1974) than dry cooking methods (Buck et al. 1979). Drummond and Sun (2006) reported moist heat methods of cooking resulted in more rapid surface temperature increases compared with dry heat cooking. However, surface browning, which contributes to the aroma of cooked meat, did not develop the same when moist heat cookery was used.

For a product to reach a specific internal temperature, the cooking apparatus must be set at a higher temperature than the target temperature. Evaporative cooling prevents the product from being the same temperature as the set point. Therefore, cooking temperatures must be greater than the desired internal temperature. Boles and Swan (2002a) reported cooking time was faster when roasts were cooked at a constant temperature as compared with roast cooked by a step-up (maintain 10°C differential between the internal temperature and the cooking temperature) or delta T process. This shows that cooking temperature does affect the rate of cooking. Bengtsson et al. (1976) reported that increasing the oven temperature from 175° to 225°C resulted in steeper temperature gradients, shorter cooking times, and lower yields.

Microwave or radio frequency cooking are newer methods that have been introduced to the meat industry. Welke et al (1986) reported roasts cooked by microwave took less time to reach endpoint temperatures than did roast cooked by convection or conventional methods. The shorter cooking times associated with microwave cooking doesn't give enough time for the browning reaction. Meat cooked with microwaves does not have the typical browned surface associated with other methods of cookery. Radio frequency heating is another rapid cooking alternative that is regarded as a volumetric form of heating in which heat is generated within the product, which reduces cooking times and could potentially lead to a more uniform heating (Zhang et al. 2006). Zhang et al. (2006) reported that radio frequency cooking resulted in a significant reduction in cooking times for leg and shoulder hams. However, a number of quality attributes of the radio frequency cooked samples differed from those of their steam-cooked counterparts. Radio frequency cooked hams had significantly lower water-holding capacity and higher yields than their steam cooked counterparts. Additionally, radio frequency cooking resulted in a less well-done coloration with higher Hunter a* values (Zhang 2006) than the steam-cooked product.

Contact cooking, as seen with pan frying or clamshell grills, is a popular method of cooking meat patties. Oroszvári et al. (2005b) showed that the higher heating temperature resulted in shorter total frying times to reach the same internal temperature. Oroszvári et al. (2005a) also found that the longer the thawing time in the frying pan, the less total water was lost. These researchers reported that the thawing time was the longest part of the frying time for beef burgers cooked from the frozen state. For heating of the core of a hamburger from 0°C to 72°C, lower losses were favored with quick heat transfer. The interconnections of all these parameters show the interaction between heat and mass transfer. Oroszvári et al. (2005a) reported that the time required to reach the final temperature in pan-fried beef burgers was controlled mainly by the water content of the product.

Major Effects of Cooking

Dimensional Changes

During cooking, meat products change size and shape. This is especially obvious in

products like hamburger patties or fresh pork sausage patties. Cooking has a lesser effect on whole-muscle products, but it still happens. The change in dimension is caused by moisture loss and changes at the myofibrillar level. Boles and Shand (2008) reported dimensional changes of stir-fry slices were affected by both meat cut used and slice thickness. The greatest dimensional changes (esp. shrinkage of length and width) were observed in slices made from the inside and outside round. Samples that had intact connective tissue around the slices had less dimensional changes, suggesting that intact connective tissue may have some impact on the dimensional changes observed. Bouton et al. (1976) reported that connective tissue had a major impact on the dimensional changes in meat. Collagen shrinkage with increased cooking temperature will contribute to dimensional changes seen in a meat product. As slice thickness increased (2, 4, or 8 mm), the changes in length and width were reduced. As the slices became thinner, there would be less water available to migrate to the surface, causing fibers to become drier and shrink more on heating, increasing cook loss and dimensional change (Boles and Shand 2008). Bouton et al. (1976) reported increased myofibrillar contraction as endpoint temperature increased. This change in contraction was associated with increased cooking loss. Bouton et al. (1976) suggested that changes in meat fibers' length happened in three zones. Temperatures between 40°C and 45°C resulted from changes in the myofibrillar structure, while changes between 55°C and 60°C were primarily caused by changes in collagen, and above 70°C were both from myofibrillar and connective tissue changes.

Cooking Losses

Cooking losses or cook yields are very important in processed products as well as fresh products. Maintenance of moisture in the product during cooking helps improve juiciness (Ritchey and Hostetler 1965) but also helps with the profitability of producing cooked, further processed products. Cooking temperature (Loucks et al. 1984; Shin et al. 1992), cooking rate (Hearne et al. 1978; Boles and Swan 2002a), and final internal temperature (Ritchey and Hostetler 1965; Laakkonen et al. 1970; Beilken et al. 1986; Fjelkner-Modig 1986) all affect cook yields.

Increased cooking temperatures result in higher temperature gradients from the outside to the inside of the processed products. Increased surface temperature during frying resulted in increased evaporative losses but had little effect on the water drip loss from the product (Oroszvári et al. 2005b). Hearne et al. (1978) however found greater evaporative and total cooking losses when meat cores were heated at a slow rate compared with a faster rate of cooking.

Laakkonen et al. (1970) reported weight loss of meat pieces increased almost linearly to the seventh hour of cooking and remained relatively constant after that as the samples were held at 60°C. Ritchey and Hostetler (1965) observed that as internal temperature of steaks increased, the cooking losses also increased. Fjelkner-Modig (1986) reported increased cooking losses from 15% to 25%–30% as internal temperature of pork increased from 68°C to 80°C. Internal product temperature can affect the type of losses seen from meat. Hearne et al. (1978) reported evaporative losses were greater when cores were cooked to higher internal temperatures. Bengtsson et al. (1976) reported increased cooking loss as the time of cooking and internal temperature increased. These researchers found evaporative losses created an almost straight line, indicating that evaporation occurred from the wet surface of the meat for the entire cooking time and that surface temperature was therefore below the oven temperature. Additionally, these researchers reported that most of the losses occurring between 65°C and 70°C were evaporative

losses. Beyond 70°C, drip losses rose rapidly. These researchers suggest that drip loss can be minimized if internal temperatures can be kept below 65°C. Furthermore, evaporative losses could be kept to a minimum by increasing the relative humidity in the cooking environment.

Hamm and Deatherage (1960) suggested that the changes in water-holding capacity during cooking, and thus cooking losses, are due to changes in charges and unfolding of proteins. This results in the shifting of the isoelectric point to a more basic pH.

Palka (2003) reported increased cooking losses when starting meat was aged for 7 days compared with 12 days postmortem. Boles and Swan (2002b) also reported cook yields decreased as refrigerated storage of inside rounds and flats increased to 8 weeks of storage. Furthermore, an increase in pH was found during refrigerated storage of inside rounds and flats, and was related to a decrease in cook yield.

Cooking is responsible for the setting of gels that make it possible to manufacture sausages and restructured products. Increasing internal temperature results in increased gel strength (Barbut et al. 1996). Increased gel strength is paralleled by a decrease in soluble protein. Changes in gel structure can be related to changes in protein solubility. The matrix density decreases as temperature is raised (Barbut et al. 1996). The major changes in microstructure could be related to changes observed in gel rigidity and the reduction in extractable proteins.

Effect of Heat on Proteins and Protein Structure

Although the exact nature of denaturation and coagulation is not fully understood, there are distinct and easily recognized physical changes in meat proteins during cooking (Table 8.4). Solidification of the muscle and color changes are readily observed and are closely associated with the reduction in solubility. Heating meat up to 45°C internal temperature resulted in a slight decrease in the amount of water-soluble fraction extracted from meat (Laakkonen et al. 1970). Between the temperatures of 45°C and 58.5°C, the water-soluble fraction decreased rapidly, and only a slight decrease in water-soluble fraction is seen during holding at 60°C (Laakkonen et al. 1970). Solubility of the myofibrillar fraction also decreases with increasing temperature (Hamm and Deatherage 1960; Lyon et al. 1986; Barbut et al 1996). The decrease in solubility is great-

Table 8.4. Effect of cooking on meat proteins

Authors	Temperature	Effect on proteins
Laakkonen et al., 1970	45–58.5°C Hold at 60°C	Rapid decrease in water soluble fraction of meat. No further decrease in water soluble fraction when held.
Hamm and Deatherage, 1960; Lyon et al., 1986; Barbut et al., 1996	40–60°C	Reduction in solubility of myofibrillar fraction
Leander et al., 1980	63°C	Slight disfigurement of the myofibril, some swelling of the perimysial connective tissue
	68°C	Swelling of the A-band, connective tissue coagulated
	73°C	Sarcomeres contraction and breakage at Z-line, Coagualtion of sarcolemma, increased loss of sarcomeric structure
Bendall and Restall, 1983	40–90°C	Decreased myofiber diameter
Martens et al., 1982	53–63°C	Denaturation of collagen, breaking up of fibrous structure
Welke et al., 1986		Increased epimysial connective tissue with cooking

est between 40°C and 60°C, with the proteins being essentially insoluble above 60°C (Hamm and Deatherage 1960; Lyon et al. 1986). Hamm and Deatherage (1960) reported denaturation occurred in different steps. The first reaction was the unfolding of the tertiary structure of the protein. The second was the aggregation of protein chains, resulting in the coagulation of proteins. The initial changes are confined to the surface, but as time and temperature increase, the action penetrates further into the interior of the meat.

Changes in the muscle structure are seen during cooking. Leander et al. (1980) reported slight disfigurement of the myofibrils after cooking to an internal temperature of 63°C, with some evidence of induced swelling of the perimysial connective tissue. Increased temperatures to 68°C resulted in more swelling in the A-band due to thermally induced contraction of the sarcomeres. Muscle fibers remained intact, while connective tissue sheaths underwent coagulation and appeared granular. These researchers reported the greatest effects were observed in samples heated to 73°C. Sarcomeres exhibited thermally induced contraction and breakage at the Z-line, while some transverse lines remained intact. Coagulation of the sarcolemma and exposure of myofibrils were also observed. Increased loss of sarcomeric structure was observed, with increased final internal temperature. Hearne et al. (1978) also observed greater fiber disintegration, with increased final internal temperature. Furthermore, these researchers found faster cooking rates to result in greater fiber disintegration compared with slow rates of cooking to an endpoint of 60°C.

Bendall and Restall (1983) reported no change in sarcomere length when fibers were heated, but diameter of fibers changed markedly. Myofibers heated in aqueous medium to final temperatures of 40°C to 90°C resulted in a decrease in diameter of myofibers but no change in length. These researchers concluded that the change in volume was mostly due to moisture loss. Expulsion of water from the myofiber was slow and incomplete from 40°C to 52.5°C but accelerated markedly to maximal rate between 57.5°C and 60°C. The acceleration of moisture lost was attributed to collagen shrinkage.

Changes in connective tissue are also seen when meat is cooked. Welke et al. (1986) observed increased weights of epimysial connective tissue after cooking, indicating hydration and hydrolysis of the collagen. Martens et al (1982) reported collagen denatured between 53–63°. The denaturation of collagen involved the breaking up of the fibrous structure, probably first by the breakage of hydrogen bonds. If collagen is not stabilized by intermolecular bonds, it will dissolve and form gelatin on further heating (Tornberg 2005), especially when meat is cooked with moisture.

Surface Drying

Reduction of moisture at the surface of meat and meat products serves several purposes. Lowering surface moisture reduces the water activity on the surface and thus reduces microbial growth. The reduced surface moisture plays a key role in preventing not only the growth of surviving bacteria, but also the growth of any bacteria that may recontaminate the surface of the product.

Surface drying during cooking is also responsible for skin formation in production of hams and other similar products. Coagulation of the surface proteins results in the formation of an outer layer that serves as a “skin” when the casings are removed. The skin formed during cooking is a function of the temperature the product reached during cooking and the time it was held at that temperature. The nature of the skin is most important for peelability or removal of casing or netting. Drying of the surface also aids in giving the skin a dense texture and imparts the characteristic appearance of skinless

products. Although the ingredients have some influence on peelability, proper cooking without excess weight loss and wrinkling are important in imparting good peelability. The cooking process must be carefully controlled to make the product readily peelable and of good appearance.

Lipids

The changes that occur during cooking also affect lipids. Oxidation of lipids occurs when oxygen is present and will occur at a faster rate as the temperature increases. These changes in lipids help to give the characteristic odor and flavor of cooked meat. Cooked meat exposed to oxygen results in further lipid oxidation, which can cause off-flavors and odors to appear in just a few hours. This off-flavor development has been traditionally referred to as "warmed-over" flavor. More recently, some researchers have referred to the process as cooked-meat flavor deterioration.

Oxidation of lipids in whole-muscle products occurs relatively slowly unless a catalyst is present. In processed products, salt acts as a catalyst and can result in rapid lipid oxidation. For many years, researchers have investigated what catalyzes the oxidation of lipids in fresh cooked meats. During cooking, the muscle cells are broken, allowing high molecular weight iron sources to be released (Morrissey et al. 1998). Denatured heme iron from myoglobin is one possible candidate for catalysis, along with free iron (Fox and Benedict 1987).

The stability of the lipids during cooking is affected by the fatty acid makeup of the lipids and dietary compounds that can function to reduce oxidation. Increasing the degree of unsaturated fatty acids will reduce lipid stability. Also, altering diet to include rapeseed oil, corn oil, or oil seed meals will increase the unsaturated fats in the meat. This is especially true in pork and poultry (Romans et al. 1995; Corino et al. 2002; Rey et al. 2004). Increased levels of oil or oilseed seeds in the diet can also increase the levels of α-tocopherol found in the meat (Leskanich et al. 1997). Increased levels of α-tocopherol or Vitamin E fed to pigs can result in lower TBARS (Corino et al. 1999; Hasty et al., 2002)) and thus less oxidation of the lipids (Corino et al. 1999).

Warmed-over flavor (WOF) occurs in uncured products after they are cooked. Nitrites in cured products function as a potent inhibitor of WOF development. As little as 50 ppm of nitrites effectively prevents lipid oxidation (Sato and Hegarty 1971). However, one of the most noticeable reactions in meat when nitrites are first added is the oxidation of the heme pigments to the ferric form (Fox and Benedict 1987). Nitrite is readily reduced by endogenous reductants in the meat to form nitric oxide, which combines with myoglobin to form the cured-meat pigment (Fox and Benedict 1987). The nitric oxide is an efficient radical chain terminator that slows the propagation of lipid oxidation (Fox and Benedict 1987). Other antioxidant properties have been suggested for nitrite.

Vitamins

Most of the effects cooking has on meat are positive: improved palatability, reduction of bacteria, and flavor development. However, cooking does have a negative impact on the vitamin content of meat, especially water-soluble vitamins such as thiamine, riboflavin, and niacin (Al-Khalifa et al. 1993; Lombardi-Boccia et al. 2005; Riccio et al. 2006). Reported retention of vitamins, however, is much more variable, with retention of thiamin being reported as low as 39% (Al-Khalifa et al. 1993) and as high as 66% (Rhee et al. 1993).

Severity of heating has a major impact on the retention of water-soluble vitamins (Riccio et al. 2006). Kumar and Aalbersberg (2006) reported that microwave oven cooking tended to retain higher amounts of vitamins.

Low retention of retinol, thiamin, and riboflavin was seen in earth-oven-cooked meat compared with microwave-cooked meat. The difference in vitamin retention could be due to a higher cooking temperature of earth-oven cooking compared with microwave cooking. Riccio et al. (2006) observed that cooking, in general, produced a decrease in the B vitamins content, both under mild (70–90°C) and severe (120°C) conditions. These researchers found that after 5 minutes of cooking at 100°C, all B vitamins analyzed were not detectable in the homogenized boiled ham without fortification.

Rhee et al. (1993) reported that samples lower in fat had a lower retention level of thiamin, riboflavin, and B_{12}. These samples lost more of their weight in water, which resulted in the loss and destruction of more B vitamins then in ground meat that possessed higher fat contents.

Overall mineral retention is better than vitamin retention (Rhee et al. 1993). The overall retention of minerals in cooked meat has been reported to be between 84% and 90% (Rhee et al. 1993).

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Chapter 9

Fermentation: Microbiology and Biochemistry

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Introduction

Meat fermentation has been the subject of intensive study over the last decades. It has been early recognized that the development of a proper ecosystem is a prerequisite in order to address safety and organoleptic, as well as nutrition-related, issues. The establishment and application of new molecular tools has provided new insights that have led researchers to more comprehensive understanding regarding both the microbial dynamics and the biochemical changes that occur during the development of this ecosystem. In this chapter, an update concerning the ecosystem of spontaneously fermented sausages, the biochemical changes during ripening, and the development of starter cultures is provided, as well as nutritional and public health aspects.

The Spontaneously Fermented Sausage Ecosystem

Spontaneously fermented sausage production includes mixing of minced meat and fatty tissue with curing agents, carbohydrates, and spices; stuffing into casings; and ripening, which can be further subdivided into fermentation and aging. Variations in the type and amount of raw materials, fermentation, and drying conditions lead to an extended diversity concerning the dominating microbiota, giving rise to a wide range of products with unique sensorial traits.

The microbiota of batter freshly stuffed into casings is dominated mainly by microorganisms present in the raw materials, with raw meat and casings being their major source (Comi et al. 2005; Lebert et al. 2007). As a result, pseudomonads and members of the *Enterobacteriaceae* family may reach levels as high as 10^5 and 10^4 cfu g^{-1}, respectively (Drosinos et al. 2005; Lebert et al. 2007). The presence of pathogens, such as *Escherichia coli*, aerobic spore formers, *Staphylococcus aureus*, and *Listeria monocytogenes*, has also been reported (Samelis et al. 1998; Comi et al. 2005; Chevallier et al. 2006). On the other hand, lactic acid bacteria, Gram-positive catalase-positive cocci, enterococci, and yeasts-molds are usually present at populations lower than 10^5 cfu g^{-1}. During ripening and as the water activity is reduced to 0.96–0.97 and the oxygen consumed, a shift in the microbiota composition toward lactic acid bacteria and Micrococcaceae takes place (Luecke 1998). By the end of ripening—depending upon the raw materials, ripening conditions, and hygienic parameters—the microecosystem usually consists of 10^7–10^9 cfu g^{-1} lactic acid bacteria, 10^4–10^6 cfu g^{-1} Micrococcaceae and enterococci, and 10^2–10^4 cfu g^{-1} yeasts-molds.

Apart from composition, microbial dynamics has also been a field of extensive study, especially over the last decade with the advancement of new molecular tools, in particular the ones based on the polymerase chain reaction (PCR). The availability of

new, powerful, and reliable techniques has enabled the detailed study of this complex ecosystem. Table 9.1 lists techniques used for the identification of technological microbiota in fermented sausages throughout the world. The phenotypic approach (i.e., the identification based on assimilation–fermentation–growth challenges) has been the first to be applied and is still in use, despite its drawbacks regarding reliability and accuracy, even at species level. The most frequently applied and most reliable technique is sequencing of the 16S-rRNA gene. This technique has been applied either in combination with other techniques, such as SDS-PAGE of whole cell proteins, RAPD-PCR, or PFGE, or directly to the isolated microorganism. Although this approach provides accurate identification at strain level, an equally important part of the microbiota, namely the viable but not culturable fraction, is not taken into consideration. This weakness can be addressed by the application of techniques such as PCR-DGGE, which instead of relying on culturing of the bacteria, incorporates the direct extraction of the DNA from the food sample. Furthermore, another technique that has not yet been applied in fermented sausages is fluorescence in situ hybridization (FISH). In this technique, the direct detection of a microorganism in a food sample is achieved by using specific probes that allow spatial distribution studies to take place.

The microorganisms that are most frequently encountered are *Lactobacillus curvatus*, *Lb. plantarum*, *Lb. sakei*, *Staphylococcus carnosus*, *St. saprophyticus*, and *St. xylosus* (Table 9.1). These microorganisms seem to be autochthonous in this ecosystem and have the capacity to prevail during fermentation. The competitiveness of *Lb. sakei* has been

Table 9.1. Microbial diversity in spontaneously fermented sausages throughout the world

Species	Origin of spontaneously fermented sausages	Identification approach
Lb. alimentarius	Greece	Sequencing of 16S-rRNA gene[5]
	Hungary	PCR-DGGE[7]
		Phenotypic[10]
Lb. bavaricus	Hungary	Phenotypic[10]
Lb. brevis	Greece	Species specific PCR[1]
	Croatia	Phenotypic[10]
	Italy	PCR-DGGE[1, 16]
	Spain	SDS-PAGE-sequencing of 16S-rRNA gene[20]
Lb. casei	Italy	Species specific PCR[1]
		PCR-DGGE[1]
		RAPD-PCR—sequencing of 16S-rRNA gene[21]
Lb. casei/paracasei	Greece	Sequencing of 16S-rRNA gene[5]
		PCR-DGGE[7]
Lb. cellobiosus	Serbia	Phenotypic[10]
Lb. collinoides	Serbia	Phenotypic[10]
Lb. curvatus	Italy	Species specific PCR[1, 12]
	Greece	PCR-DGGE[1, 3, 7, 14, 15, 16, 22, 23]
	Hungary	Sequencing of 16S-rRNA gene[5]
	Croatia	Phenotypic[10]
	Bosnia and Herzegovina	RAPD-PCR-Species specific PCR[13]
	Spain	RAPD-PCR-sequencing of 16S-rRNA gene[14, 21]
	Argentina	SDS-PAGE-sequencing of 16S-rRNA gene[20]
Lb. delbrueckii spp. *bulgaricus*	Serbia	Phenotypic[10]

Table 9.1. Microbial diversity in spontaneously fermented sausages throughout the world *(cont.)*

Species	Origin of spontaneously fermented sausages	Identification approach
Lb. fermentum	Croatia Italy Serbia	Phenotypic[10]
Lb. paracasei	Italy	Phenotypic[10] RAPD-PCR-Species specific PCR[13]
Lb. paraplantarum	Italy Greece	Species specific PCR[1] PCR-DGGE[1, 7, 16]
Lb. paraplantarum/ pentosus	Italy	PCR-DGGE[16]
Lb. paraplantarum/ plantarum	Greece Hungary	Sequencing of 16S-rRNA gene[5] PCR-DGGE[7]
Lb. pentosus	Greece Croatia Bosnia and Herzegovina Argentina	Phenotypic[10] PCR-DGGE[15]
Lb. plantarum	Italy Greece Hungary Croatia Bosnia and Herzegovina Argentina Spain	Species specific PCR[1] PCR-DGGE[1, 3, 7, 14, 15, 16, 22] Phenotypic[4, 10, 24] RAPD-PCR-sequencing of 16S-rRNA gene[4, 14] Sequencing of 16S-rRNA gene[5] RAPD-PCR-Species specific PCR[13] SDS-PAGE-sequencing of 16S-rRNA gene[20]
Lb. plantarum/pentosus	Greece Italy	Sequencing of 16S-rRNA gene[5] PCR-DGGE[7]
Lb. rhamnosus	Greece	Phenotypic[10, 24]
Lb. sakei	Italy Greece Hungary Bosnia and Herzegovina Spain Argentina	Species specific PCR[1, 12] PCR-DGGE[1, 3, 7,14, 15, 16, 23] Phenotypic[4, 10, 24] RAPD-PCR-sequencing of 16S-rRNA gene[4, 14, 21] sequencing of 16S-rRNA gene[5] RAPD-PCR-Species specific PCR[13] SDS-PAGE-sequencing of 16S-rRNA gene[20]
Lb. sanfranciscensis	Hungary	Phenotypic[10]
Lc. garvieae	Italy	Species specific PCR[1] PCR-DGGE[1]
Lc. lactis	Italy	Species specific PCR[1] PCR-DGGE[1] Phenotypic[10]
Lc. lactis ssp. *lactis*	Italy Greece	PCR-DGGE[3, 7, 16] Phenotypic[10]
Ln. carnosum	Italy	Species specific PCR[1] PCR-DGGE[1]
Ln. citreum	Hungary Italy	PCR-DGGE[7, 16]
Ln. mesenteroides	Italy Hungary Spain	Species specific PCR[1] PCR-DGGE[1, 7, 16] Phenotypic[10] Sequencing of 16S-rRNA gene[12]
Ln. mesenteroides mesenteroides	Hungary Serbia	Phenotypic[10]

(continued)

Table 9.1. Microbial diversity in spontaneously fermented sausages throughout the world *(cont.)*

Species	Origin of spontaneously fermented sausages	Identification approach
Pd. acidilactici	Italy Argentina Spain	PCR-DGGE[3, 15] Phenotypic[10] SDS-PAGE-sequencing of 16S-rRNA gene[20]
Pd. pentosaceus	Italy	Phenotypic[10]
Ws. hellenica	Italy	Species specific PCR[1] PCR-DGGE[1]
Ws. paramesenteroides	Italy Greece	Species specific PCR[1] PCR-DGGE[1] Sequencing of 16S-rRNA gene[5]
Ws. paramesenteroides/ hellenica	Greece Hungary Italy	Sequencing of 16S-rRNA gene[5] PCR-DGGE[7, 16]
Ws. viridescens	Greece Hungary	Sequencing of 16S-rRNA gene[5] PCR-DGGE[7]
St. aureus	Spain	SDS-PAGE-sequencing of 16S-rRNA gene[18]
St. auricularis	serbian	Phenotypic[10]
St. capitis	Serbia Croatia	Phenotypic[10]
St. caprae	Greece Bosnia-Herzegovina	Phenotypic[10]
St. carnosus	Slovakia Spain Croatia Italy	Species specific PCR[2,11] 16S–23S rDNA intergenic region amplification—species specific PCR[9] Phenotypic[10]
St. cohnii	Italy	PCR-DGGE[11]
St. cohnii cohnii	Greece	Phenotypic[24]
St. cohnii urealyticum	Greece	Phenotypic[10]
St. epidermidis	Spain Bosnia-Herzegovina Italy	16S–23S rRNA intergenic region amplification-species specific PCR[9] Phenotypic[10] Species specific PCR[11] SDS-PAGE-sequencing of 16S-rRNA gene[18]
St. equorum	Italy France Argentina Spain	PCR-DGGE[6,11,14, 15, 23] PFGE-Sequencing of $sodA_{int}$ gene[8] SDS-PAGE-sequencing of 16S-rRNA gene[18] RAPD-PCR-sequencing of 16S-rRNA gene[21]
St. gallinarum	Greece	Phenotypic[24]
St. haemolyticus	Italy	PCR-DGGE[6]
St. hominis	Greece Italy	Phenotypic[10]
St. lentus	Hungary Italy	Phenotypic[10]
St. pasteuri	Italy	PCR-DGGE[6, 11]
St. saprophyticus	Italy Greece Boznia-Herzegovina Croatia Argentina Spain	PCR-DGGE[6, 14, 15] Phenotypic[10, 24] RAPD-PCR-sequencing of 16S-rRNA gene[14] SDS-PAGE-sequencing of 16S-rRNA gene[18]

Table 9.1. Microbial diversity in spontaneously fermented sausages throughout the world *(cont.)*

Species	Origin of spontaneously fermented sausages	Identification approach
St. sciuri	Italy Boznia-Herzegovina	Phenotypic[4, 10] RAPD-PCR-sequencing of 16S-rRNA gene[4]
St. simulans	Greece Bosnia-Herzegovina	Phenotypic[10, 24]
St. succinus	Italy France	PCR-DGGE[6, 23] PFGE-Sequencing of $sodA_{int}$ gene[8] RAPD-PCR-sequencing of 16S-rRNA gene[21]
St. vitulus	Spain	SDS-PAGE-sequencing of 16S-rRNA gene[18]
St. warneri	Italy France Spain Serbia	PCR-DGGE[6] PFGE-Sequencing of $sodA_{int}$ gene[8] 16S–23S Rdna intergenic region amplification-species specific PCR[9] Phenotypic[10] Species specific PCR[11]
St. xylosus	Slovakia Italy Spain Greece Croatia Hungary	Species specific PCR[2, 11] Phenotypic[4, 10, 24] RAPD-PCR-sequencing of 16S-rRNA gene[4] PCR-DGGE[6, 18,22, 23] 16S–23S rDNA intergenic region amplification-species specific PCR[9] SDS-PAGE-sequencing of 16S-rRNA gene[18]
Mc. caseolyticus	Italy	PCR-DGGE[11]
Bc. subtilis	Italy	RAPD-PCR-sequencing of 16S-rRNA gene[21]
Kc. varians	Spain	16S–23S rDNA intergenic region amplification-species specific PCR[9]
En. faecalis	Greece Serbia Argentina	Sequencing of 16S-rRNA gene[5] PCR-DGGE[15]
En. faecium	Greece	PFGE-sequencing of 16S-rRNA gene[25]
En. faecium/durans	Greece	Sequencing of 16S-rRNA gene[5] PCR-DGGE[7]
En. flavescens	Argentina	PCR-DGGE[15]
En. mundtii	Argentina	PCR-DGGE[15]
En. pseudoavium	Italy	PCR-DGGE[7, 16]
En. durans	Argentina	PCR-DGGE[15]
Db. hansenii	Italy	PCR-DGGE[3, 17, 22, 23] RAPD-PCR-sequencing of 16S-rRNA gene[21]
Cd. psychrophila	Italy	PCR-DGGE[3]
Sc. barnettii	Italy	PCR-DGGE[3]
Pn. hirsutum	Italy	PCR-DGGE[3]
Mt. pulcherrima	Italy	PCR-DGGE[17]

Lc.: Lactococcus; Lb.: Lactobacillus; Ln.: Leuconostoc; Pd.: Pediococcus; Ws.: Weissella; St.: Staphylococcus; Mc.: Macrococcus; Bc.: Bacillus; Kc.: Kocuria; En.: Enterococcus; Db.: Debaryomyces; Cd.: Candida; Sc.: Saccharomyces; Pn.: Penicillium; Mt.: Metschnikowia

[1] Urso et al. 2006; [2] Simonova et al. 2006; [3] Silvestri et al. 2007; [4] Rebecchi et al. 1998; [5] Rantsiou et al. 2006; [6] Rantsiou et al. 2005a; [7] Rantsiou et al. 2005b; [8] Morot-Bizot et al. 2006; [9] Martin et al. 2006; [10] Kozacinski et al. 2008; [11] Iacumin et al. 2006; [12] Aymerich et al. 2006; [13] Andrighetto et al. 2001; [14] Fontana et al. 2005a; [15] Fontana et al. 2005b; [16] Comi et al. 2005; [17] Cocolin et al. 2006; [18] Cocolin et al. 2001; [19] Benito et al. 2008a; [20] Benito et al. 2008b; [21] Baruzzi et al. 2006; [22] Aquilanti et al. 2007; [23] Villani et al. 2007; [24] Drosinos et al. 2007; [25] Paramithiotis et al. 2008

studied in detail and has been attributed partly to the presence of genes involved in the energetic catabolism of nucleosides, such as adenosine and inosine that are abundant in meat (Chaillou et al. 2005), and partly to its mode of arginine catabolism (Champomier-Verges et al. 1999; Zuriga et al. 2002; Chaillou et al. 2005). Other species, such as the ones mentioned in Table 9.1, are also likely to be sporadically present.

Biochemical Changes during Ripening

The main biochemical changes that occur during ripening, affecting appearance, organoleptic quality, and safety of fermented sausages, are shown in Figure 9.1. These biochemical reactions lead to the formation of a variety of metabolic end products, which are summarized in Table 9.2.

Carbohydrates serve as carbon and energy sources for the native microbiota or the added starter culture. Microbial fermentation results in the production of lactic acid, the configuration of which depends upon the dominant species. The lactic acid results in a decrease of the pH value that has a manifold effect on the quality of the product. This pH drop, apart from contributing to the inhibition of spoilage and pathogenic microorganisms, favors water release through protein coagulation, as well as the hydrolytic action of both cathepsin D and lysosomal acid lipase. Addition of heterofermentative lactic acid bacteria results in the production of additional compounds, such as acetoin and diacetyl. On the other hand, addition of sugars, apart from being the decisive parameter on the final pH, means that their residual amount will inevitably contribute to taste development, given that they are present in levels above their sensory threshold.

As a general rule, proteolysis, at least at its early stages, is primarily a function of the muscle proteinases (Luecke 2000), especially cathepsin D. Complete hydrolysis into free amino acids takes place by bacterial peptidases, along with endogenous ones (Sanz et al. 1999a). The proteolytic capacity of several lactic acid bacteria and staphylococci strains isolated from fermented meat products has been investigated (Fadda et al. 1998, 1999a, b; Sanz et al. 1999a, b; Mauriello et al. 2002; Drosinos et al. 2007), and a rather rare proteolytic capacity of lactic acid bacteria has been stated, as well as a comparatively common one of staphylococci. In both

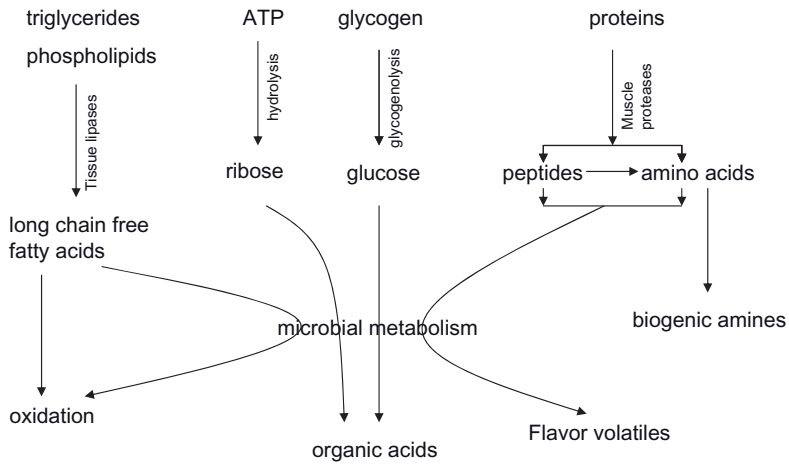

Figure 9.1. Main biochemical changes occurring during sausage fermentation.

Table 9.2. Metabolic end products formed during fermentation and ripening of dry fermented sausages

End product	Natural flora	Starter culture	*Kocuria* added	Yeast-molds added
Lactic acid	x	x	x	
Acetic acid	x	x	x	
Butyric acid	x	x		
Oxalic acid	x			
Citric acid	x			
Pyruvic acid	x			
Malic acid	x			
Formic acid	x			
Fumaric acid	x			
Propionic acid	x	x		
Diacetyl	x	x	x	
Acetoin	x	x	x	
2,3-butyleneglycol	x			
Ethanol	x	x	x	
Free fatty acids	x	x		x
Peptides	x	x		x
Free amino acids	x	x	x	x
Amines	x	x		x
Ammonia	x	x	x	x
Aldehydes, ketones	x			x

cases, it seems that the mode of proteolysis is a strain-dependent property. Drosinos et al. (2007) reported that six *Lb. sakei* strains were found proteolytic only against the myofibrillar protein fraction and by a mode quite different from the one already described by Fadda et al. (1999a) referring to *Lb. plantarum* strain CRL 681. In the former case, a complete decomposition of myosin, actin, and all myofibrillar proteins ranging in molecular weight from 200 to 12 kDa was observed, compared with the partial hydrolysis of only actin and myosin that was observed by *Lb. plantarum* strain CRL 681 (Fadda et al. 1999a). Comparable differences were observed in the proteolysis of sarcoplasmic and myofibrillar protein fractions by staphylococci (Drosinos et al. 2007). The results obtained in that study were not in accordance with the ones obtained by Mauriello et al. (2002) referring to the decomposition of sarcoplasmic proteins. In the latter study, the decrease in intensity of protein bands at approximately 48.4, 41.6, 22.4, and 20.3 kDa (*St. xylosus* strains BS5 and ES1) or their complete hydrolysis (*St. xylosus* strain AS27) has been reported, whereas in the study by Drosinos et al. (2007), seven *Staphylococcus* sp. strains hydrolyzed the sarcoplasmic protein fraction by the same mode and only one *St. xylosus* strain LQC 5401 by a different mode. As far as the myofibrillar protein fraction was concerned, Mauriello et al. (2002) reported that strains ES2 and BS5 resulted in a complete decomposition of myosin and actin, and the appearance of bands at about 100 and 25 kDa. On the other hand, Drosinos et al. (2007) reported that all 53 *Staphylococcus* sp. strains found to be proteolytic were able to completely hydrolyze the myofibrillar fraction to polypeptides with molecular weight less than 12 kDa.

Lipolysis in fermented sausages has been attributed partly to the microbiota and partly to tissue lipases. It has been estimated that muscle lipases contribute at 60%–80%, with the rest being due to microbial ones (Molly et al. 1996, 1997). Several authors have studied the lipolytic activities of both lactic acid bacteria and staphylococci in pork fat. In general, lactic acid bacteria hydrolyze

mono-, di-, and triacylglycerols at a lower rate (Sanz et al. 1998), stating their weak lipolytic system (El Soda et al. 1986; Montel et al. 1998; Drosinos et al. 2007), whereas the production of lipolytic enzymes among staphylococci seems to be a common characteristic (Miralles et al. 1996; Coppola et al. 1997; Kenneally et al. 1998; Mauriello et al. 2004; Casaburi et al. 2007). Once free fatty acids are released, they are subjected to oxidative reactions that give rise mainly to aliphatic hydrocarbons, alcohols, aldehydes, ketones, and esters, with the latter being produced in the absence of nitrite in the recipe. Excessive oxidation results in the formation of off-flavors such as rancidity. The microbiota, through the consumption of oxygen, negatively affects rancidity formation.

Development of Starter Cultures

The concept of starter cultures for fermented sausages is nearly as old as the product itself. The rationale is to minimize the variability quelling from spontaneous fermentation and to enrich organoleptic quality and safety. Toward this direction, a huge amount of research has taken place. Desirable technological features include acidification, catalase, protease, and lipase activity, as well as avoidance of possible discoloration phenomena through the production of peroxides according to Figure 9.2. Regarding the safety of the product, bacteriocin production is involved in antibiotic resistance, as well as the absence of amino acid decarboxylase activity and transferable genes.

Acidification possesses a key role in fermented sausage manufacture, as it enables control of spoilage and pathogenic microbiota and affects flavor, color, and texture development. It has also been reported that a rapid decrease of the pH value can prevent biogenic amine accumulation (Maijala et al. 1993). Catalase activity is important, as it hydrolyses the hydrogen peroxide (produced

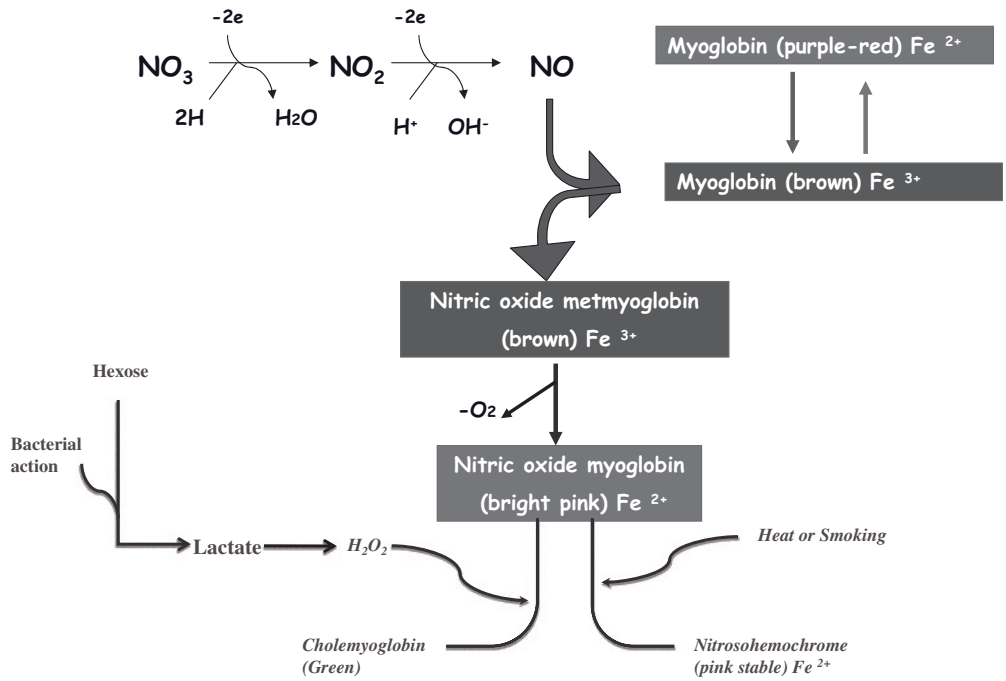

Figure 9.2. Color development in fermented sausages.

by most lactobacilli), which increases rancidity and discoloration of the final product. Despite the fact that catalase production is a constitutive characteristic of coagulase-negative staphylococci, it is still regarded as a desirable property for lactic acid bacteria as well, and therefore its presence and activity in lactic acid bacteria has been studied (Abriouel et al. 2004; Noonpakdee et al. 2004; Ammor et al. 2005).

The utilization of bacteriocinogenic strains, either as starter or as protective cultures, has drawn special attention. Several autochthonous meat lactic acid bacteria, among them *Lactococcus lactis* (Rodriguez et al. 1995; Noonpakdee et al. 2003), *Lb. sakei* (Mortvedt et al. 1991; Aymerich et al. 2000), *Pediococcus acidilactici* (Cintas et al. 1995; Albano et al. 2007), *Lb. curvatus* (Mataragas et al. 2003; Messens et al. 2003), *Enterococcus faecium* (Cintas et al. 1997, 1998), and *Leuconostoc mesenteroides* (Mataragas et al. 2003; Drosinos et al. 2006) strains have been screened for bacteriocin production against several food-borne pathogens. Bacteriocin production has been in many cases optimized, and mathematical models have been created in order to predict its production under various conditions (Drosinos et al. 2008). Increased attention has also been given to the production and concomitant accumulation of biogenic amines, due to their potential toxic effects on consumption. The biogenic amine content of a variety of meat products has been studied (Ruiz-Capillas and Jimenez-Colmenero 2004). In the case of fermented sausages, the microorganisms present possess a key role in their formation, and thus absence of amino acid decarbolyxase activity has become a key requirement in the selection of a starter culture (Ammor and Mayo 2007). Finally, the increased concern regarding the transmission of antibiotic resistance genes, along with the ability of several food-associated lactic acid bacteria to survive passage through the human gastrointestinal tract, inevitably led to the assessment of such a potential in strains involved in meat fermentation. In has been shown that several *Lb. alimentarius*, *Lb. curvatus*, *Lb. plantarum*, and *Lb. sakei* strains harbor such genes, making horizontal gene transfer possible (Gevers et al. 2003).

Whole-genome sequencing of bacteria and, more accurately, of bacteria capable of serving as starter cultures has provided new tools in the quest for the suitable starter culture. Genome analysis of *Lb. sakei* 23K revealed a lack of main aroma-production pathways, as well as genes responsible for amino acid decarboxylation (Chaillou et al. 2005). Similarly, genome analysis of *St. carnosus* TM 300 has revealed that the genetic background was present for encoding a series of desired technological properties, such as branched-chain amino acid aminotransferase producing flavor compounds, superoxide dismutase, and catalase contributing to the control of lipid oxidation (Barriere et al. 2001; Madsen et al. 2002). The presence of the required genes does not necessarily mean a functional biochemical pathway, but once the mechanisms that influence their transcription and translation are understood, it will be possible to assess the presence of desired properties, merely by the use of specific probes bypassing classical microbiological techniques.

Nutritional Aspects

Generally, lactic acid fermentation can have multiple effects on food nutritional value, either by modifying the level and bioavailability of nutrients or by interacting with the gut microbiota and even the human immune system. The nutrients that determine the nutritional value of meat are the high biological value proteins and micronutrients such as vitamins B1 and B12, niacin equivalents, zinc, and iron, with the latter being mainly in the heme form that can be efficiently absorbed by humans (Hambraeus 1999; Mann 2000). Currently, there is no data available concern-

ing the effect of fermentation on the level of the above-mentioned nutrients.

The substitution of NaCl and fat and the concomitant re-formulation of fermented sausage recipes has also been the subject of extensive research, the former due to its relation to the development of hypertension in sensitive individuals, and the latter due to the high saturated fatty acid and cholesterol content and their relation to cardiovascular disease. Since both ingredients possess a specific role in the manufacture of dry fermented sausages, this task seems to be quite challenging. Potassium chloride, potassium lactate, glycine, manganese chloride, calcium chloride, and calcium ascorbate (Ibanez et al. 1995, 1996, 1997; Gou et al. 1996; Gimeno et al. 1998, 1999, 2001) have been examined for their potential to substitute sodium chloride, and the difference regarding the sensorial quality of the end product has been pointed out, without, however, studying the effect on safety from a microbiological point of view. On the other hand, fat reduction with the addition of compounds such as inulin (Mendoza et al. 2001) and dietary fiber (Garcia et al. 2002) and even substitution by olive oil (Bloukas et al. 1997; Muguerza et al. 2001, 2002) or soy oil (Muguerza et al. 2004) has been studied with interesting and promising results.

Since the idea of using probiotic starter cultures in sausage fermentation has developed, several lactic acid bacteria have been screened for their capacity to survive their passage through the human gastrointestinal tract and their possible in-site actions. *Lb. curvatus* strain RM10 and *Pd. acidilactici* strain P2, isolated from freeze-dried commercial meat starter cultures, exhibited the strongest capacity for surviving acidic conditions and 0.30% bile salts (Erkkila and Petaja 2000). The suitability of three probiotic *Lb. rhamnosus* strains (GG, E-97800, and LC-705) to produce dry sausage has been demonstrated by Erkkila et al. (2001). A very effective screening procedure has been suggested by Pennacchia et al. (2004), leading to the isolation of twenty potentially probiotic *Lactobacillus* strains, eleven of which exhibited good adhesion capability to Caco-2 cell layers, most of them belonging to *Lb. plantarum* group (Pennacchia et al. 2006). Klingberg and Budde (2006) demonstrated the capacity of two *Lb. plantarum* strains to survive the passage through the human GIT either as freeze-dried culture or embedded in a sausage matrix. Microencapsulation has also been proposed as an alternative for the incorporation of either probiotic or bacteriocinogenic strains. In the latter case, though, the inhibitory action of reuterin, producing *Lb. reuteri* against *E. coli* O157:H7, was found to be reduced during sausage fermentation compared with that of the free microorganism (Muthukumarasamy and Holley 2006, 2007).

Public Health Aspects

The ability of pathogens (e.g., *Salmonella* spp., *E. coli*, *L. monocytogenes*) to survive in many low-acid as well as low-water activity meat products, such as fermented meat products, makes it unlikely that complete suppression can be achieved by the application of control measures at a single source (Skandamis and Nychas 2007). Thus, effective control strategies must consider the multiple points at which pathogens can gain access to the human food chain. The persistence and the ability of very small numbers of these organisms to establish life-threatening infections with serious long-term clinical consequences, particularly among at-risk sections of the human population, mean that many elements of our food safety strategies have to be improved. Measures to control pathogens during fermented meat production, processing, and distribution, at the retail level and during commercial/domestic preparation, should be considered in detail. Therefore, the best approach to control pathogens in fermented meat products is to

implement HACCP principles into the food safety management systems at all stages of meat and meat product production and distribution. However, due to their unusual tolerance to low pH (i.e., *L. monocytogenes*, *E. coli*, and *Salmonella* spp.), some pathogens should be considered at higher risk than other pathogens (e.g., *St. aureus*) in meat products (sausages) that have a low pH and are minimally processed or are not cooked before consumption. The basic control measures should include the following:

- A quantitative microbiological hazard analysis or quantitative microbial risk assessment on current practices should be carried out for products in these categories.
- Training of personnel working in food preparation and food service industries coupled with consumer education on hygienic handling and adequate cooking of food can play a role in reducing the incidence of pathogenic infection.

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Chapter 10

Starter Cultures for Meat Fermentation

Pier Sandro Cocconcelli and Cecilia Fontana

Introduction

Dry sausages are made from a mixture of frozen pork, beef, and pork fat. In addition, these fermented products contain sugars, salt, nitrite and/or nitrate, and spices. Fermentation is a crucial phase of sausage production, since the major physical, biochemical, and microbiological transformations take place at this stage (Lizaso et al. 1999; Villani et al. 2007). All these transformations, which are influenced by the microbiota, the ripening conditions, and the ingredients, have a considerable effect on the sensorial quality of fermented meat products. These changes can be summarized as follows: decreases in pH; changes in the microbial populations; reduction of nitrates to nitrites a; formation of nitrosomyoglobin; solubilization and gelification of myofibrillar and sarcoplasmic proteins; proteolytic, lipolytic, and oxidative phenomena; and dehydration (Casaburi et al. 2007).

Knowledge and control of the bacteria present in the batter and involved in the fermentation are essential in terms of the microbiological quality, sensory characteristics, and food safety. In the last decade, numerous studies on the ecology of traditional fermented sausages confirmed and extended the knowledge, especially in terms of technological lactic acid bacteria (LAB) and Gram-positive catalase positive cocci (GCC+), (Aymerich et al. 2003; Corbière Morot-Bizot et al. 2004; Giammarinaro et al. 2005; Rantsiou and Cocolin 2006; Urso et al. 2006). The LAB species most commonly identified in traditional fermented sausages are *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum* (Fontana et al. 2005; Aymerich et al. 2006; Urso et al. 2006). Among GCC+ isolates, *Staphylococcus xylosus, Staphylococcus equorum*, *Staphylococcus succinus*, and *Staphylococcus saprophyticus* are mentioned, often with *S. xylosus* predominant (Mauriello et al. 2004; Fontana et al 2005; Corbière Morot-Bizot et al. 2006; Drosinos et al. 2007).

Improvement of quality and safety of sausage and other fermented meat products can be achieved by the introduction of selective decontaminating procedures targeted toward spoilage and pathogenic bacteria but preserving technological microbiota (Ammor et al. 2004), and by the addition of microbial starter cultures selected from meat-processing environment in order to drive the fermentation process.

Meat starter cultures are "preparations which contain living or resting microorganisms that develop the desired metabolic activity in the meat" (Hammes 1996). Inoculation of the sausage batter with a starter culture composed of selected LAB (i.e., homofermentative lactobacilli and/or pediococci) and GCC+ (i.e., nonpathogenic, coagulase-negative staphylococci and/or kocuriae) improves the quality and safety of the final product and standardizes the production process (Campbell-Platt and Cook 1995; Hugas and Monfort 1997; Lücke 1998, 2000; Hüfner and Hertel 2008; Talon et al. 2008).

The aim of this chapter is to provide the most recent information on bacterial inoculants and their potential functionalities, explaining how they can improve the quality of fermented meat products.

Starter Cultures for Fermented Meats

History

Research on the use of starter cultures in meat products began in the United States in the 1940s, inoculating the batter with lactobacilli, with the aim being to govern and accelerate fermentation. In the late fifties, the Finn Niinivaara (1955) helped to launch this idea in Europe, developing mixed cultures of *Micrococcus* sp. and *Pediococcus cerevisiae*. After this experience, a first generation of meat starter bacterial cultures, generally based on microorganisms derived from cultures for vegetable fermentation, was developed. These bacterial cultures, mainly selected for their acidification properties, were usually composed of *L. plantarum* and members of the genus *Pediococcus*. Successively, a new generation of starter cultures composed of strains isolated from meat, such as *L. sakei* and coagulase negative staphylococci (CNS), was developed that harbored phenotypic traits of technological relevance (Buckenhüskes 1994). This second generation is now widely used in the industrial processes of fermented meat production.

In the last years, more efforts have been dedicated to the study of technological properties of LAB and staphylococci isolated from traditional fermented sausages to develop functional starter cultures, with increased diversity, stability, and industrial performance (García-Varona et al. 2000; Mauriello et al. 2004; Casaburi et al. 2005; Drosinos et al. 2007). Using comparative genomics, microarray analysis, transcriptomics, proteomics, and metabolomics, the natural diversity of wild strains that occur in traditional artisan foods is being explored. These approaches permit rapid high-throughput screening of promising wild strains, with interesting functional properties that lack negative characteristics to develop starter cultures based on indigenous technological bacteria of traditional sausages, since these strains are well adapted to the environment (Villani et al. 2007; Talon et al. 2008).

Bacterial Starter Cultures for Meat Fermentations

Lactic acid bacteria play a major role in the microbial consortium of fermented and cured meat: they affect both the technological properties and the microbial stability of the final product through the production of lactic and acetic acids, and the consequent pH decrease. At pH values of 4.6–5.9, muscle proteins coagulate and lose their water-holding capacity. This results in an improvement in the final product's sliceability, firmness, and cohesiveness. Ripening is also favored at this acidic environment, as well as color development. Moreover, the accumulation of lactic and acetic acids inhibits growth of spoilage and pathogenic microorganisms. Gram-positive catalase-positive cocci also have a relevant role in the manufacture of fermented sausages. They enhance color stability, prevent rancidity, reduce spoilage, decrease processing time, and contribute to flavor development. Yeasts and molds are used mainly for flavor development, the first through carbohydrate fermentation and the latter by lactate oxidation, proteolysis, degradation of amino acids, and lipolysis (Hugas and Monfort 1997; Lücke 2000).

Actually, most of the commercially available meat starter cultures contain mixtures of LAB (*Lactobacillus* and *Pediococcus*) and GCC+ (*Staphylococcus* and *Kocuria*). These bacterial groups are responsible for basic microbial reactions that occur simultaneously during fermentation. Table 10.1 lists

Table 10.1. Composition of some commercial starter cultures used for meat fermentation

Species	Functional and technological properties for meat fermentation	Quality characteristics
Lactobacillus curvatus, Staphylococcus carnosus	Fast acidification, and positive mild aroma developments as well as a stable color in the product. The final pH may be adjusted with the amount of fermentable sugars added to the meat mix.	Preservation Firmness (consistency) Aroma
Pediococcus acidilactici, Pediococcus pentosaceus,	Create a combination of normal acidification, a positive aroma development, and a good, stable red color in the product. The final pH may be adjusted with the amount of fermentable sugars added to the meat mix.	Preservation Firmness (consistency) Aroma
Staphylococcus xylosus, Pediococcus pentosaceus	Acidification process initiates quickly and results in a medium pH-decline. *S. xylosus* gives a strong and stable color and an aromatic flavor.	Preservation Firmness (consistency)
Pediococcus acidilactici, Lactobacillus curvatus and *Staphylococcus xylosus*	Fast fermentation, distinct and very good taste, good color formation and stability. Due to bacteriocin production, both *L. curvatus* and *P. acidilactici* contribute to suppressing growth of *Listeria monocytogenes*.	Preservation (pH and bacteriocin) Firmness (consistency) Aroma
Lactobacillus sakei, Staphylococcus carnosus	Mild acidification, and positive mild aroma developments as well as a stable color in the product.	Preservation Firmness (consistency) Aroma
Pediococcus pentosaceus, Staphylococcus carnosus	Mild acidification, and positive mild aroma developments as well as a stable color in the product*	Preservation Firmness (consistency)
Lactobacillus pentosus, Staphylococcus carnosus	Aromatic cultures with intermediate acidification	Color Aroma Preservation
Lactobacillus sakei, Staphylococcus xylosus and *Staphylococcus carnosus*	Proteolysis Amino acid catabolism Lipolysis Antioxidant properties: catalase and SOD Nitrate reduction	Color Aroma Preservation
Staphylococcus equorum	Flavor development Nitrate reduction	Color Aroma Preservation
Kocuria varians	Nitrate reduction	Color Preservation

*Bacteriocin production is discussed in Chapter 14.

the compostition of some commercial starter cultures.

Lactic Acid Bacteria

The genus *Lactobacillus* is of great importance in meat fermentation, and for this reason, species of this genus are frequently used as starter cultures in sausage and cured meat production. The genus *Lactobacillus* includes more than 150 different species, with a large variety of phenotypic, biochemical, and physiological traits (Axelsson 2004). The diversity and complexity of *Lactobacillus* genus is reflected by the presence of three phylogenetic groups: the *L. casei* subgroup, containing the facultative heterofermentative lactobacilli; the *Leuconostoc* group, which encompass the obligate heterofermentative; and the *L. acidophilus* group, composed of obligate homofermentative lactobacilli.

Only a limited number of *Lactobacillus* species is commonly isolated from meat fermentations and used as starter cultures. Among them, *L. sakei*, *L. curvatus* and *L. plantarum*, belonging to the sub-group of facultative heterofermentative lactobacilli, are generally used for this purpose. The main energetic metabolism of these bacteria is the dissimilation of sugar to organic acid by means of glycolysis and phosphoketolase pathways. When hexoses are the energy source, lactic acid is the major fermentation end product. Several studies clearly demonstrated that *L. sakei* is the predominant species in fermented meat products, and its use as a starter culture for sausage production is widespread (Leroy et al. 2006).

The first complete genome sequence of bacteria from meat fermentation was that of the sausage isolate *L. sakei* 23K (Berthier et al. 1996). Its 1.88-Mb chromosome, which encodes 1,883 predicted genes, harbors the genetic determinates for a specialized metabolic repertoire that reflects the adaptation to meat fermentation (Chaillou et al. 2005). The intra-specific diversity of *L. sakei* species has been investigated (Chaillou et al. 2008) by analyzing the genomic variations. This study revealed that *L. sakei* strains show extensive differences in chromosomal size, which range from 1.8 to 2.3 Mb. Cluster analysis revealed that there are ten different strains clusters, comprising two main groups of strains: *L. sakei* subsp. *carnosus*, the more diverse, comprised of seven clusters; and *L. sakei* subsp. *sakei*, comprised of three clusters.

L. sakei has evolved to adapt itself to the meat environment, harboring the genetic function that gives it the ability to grow and survive there. *L. sakei* seems very well suited to derive energy from other compounds that are more abundant in meat. Its adaptation to meat, an environment rich in amino acids because of the activity of endogenous proteases, has caused it to lose biosynthetic pathways for amino acid synthesis. *L. sakei* is therefore auxotrophic for all amino acids except aspartic and glutamic acid (Champomier-Verges et al. 2002). Amino acid metabolism can provide an alternative energy source for *L. sakei* when glucose is exhausted, and this affects the sensorial properties of the sausage, as discussed later. The genome shows a particularly well-developed potential for amino acid catabolism, and in addition, *L. sakei* has the ability to use purine nucleosides for energy production (a unique property among lactic acid bacteria).

Although *L. plantarum* has been identified as part of the meat microbiota and is used as starter cultures for meat fermentation, this species lacks the specific adaptation to meat environment found in *L. sakei*. *L. plantarum* is a highly versatile bacterium, frequently encountered in a variety of different environments, such as vegetable and dairy fermentation and the gastrointestinal tract of warm-blooded animals. The metabolic and environmental flexibility of this organism is reflected by the size of its genome, 3.3 Mb (Kleerebezem et al. 2003), which is the

largest of the *Lactobacillus* genus. Although, *L. curvatus* is frequently isolated from meat fermentations and has a role in the control of undesirable bacteria due to the production of antimicrobial peptides (see Chapter 14), less information on its physiology and genetics is available.

Pediococci, although they do not compose a relevant part of the microbial community of European fermented sausages, occasionally occur in small amounts (Papamanoli et al. 2003). They are more common in fermented sausages from the United States, where they are intentionally added as starter cultures to accelerate acidification of the meat batter. Pediococci are Gram-positive, coccus-shaped lactic acid bacteria, showing the distinctive characteristic of tetrad formation via cell division in two perpendicular directions on a single plane. Pediococci is a typical example of a rapid fermentative organism, with a higher optimum growth temperature requirement, and of homofermentative lactate production during sausage fermentation (Axelsson 2004). Phylogenetically *Pediococcus* species belong to the *L. casein-Pediococcus* sub-cluster of the *Lactobacillus* cluster. The genus consists currently of nine species, but only *P. pentosaceus* is generally used as a starter culture for meat fermentation. The species *P. cerevisiae*, frequently mentioned as a starter culture, has now been reclassified as *P. pentosaceus*. The genome-sequencing project of *P. pentosaceus* ATCC 25745 is complete (http://genome.jgi-psf.org/draft_microbes/pedpe/pedpe.info.html).

Gram-Positive Catalase-Positive Cocci (GCC+)

Micrococcaceae were frequently mentioned as components of meat starter cultures, but this term generally referred to members of the *Staphylococcus* genus (which belongs to the family *Staphylococcaceae)*. *Staphylococcus* were originally grouped with other Gram-positive cocci, such as *Micrococcus* genus, because these two genera often cohabit the same habitats. However, molecular taxonomy has revealed that these genera are phylogenetically separate and distinct. The genus *Staphylococcus* belongs to the *Clostridium* subdivision of Gram-positive bacteria, while *Micrococcus* is part of the *Actinomycetales*., *Kocuria varians* (formerly classified as *Micrococcus varians*), is a member of the *Micrococcaceae* family, and is used in meat starter cultures for its nitrate reductase ability.

The genus *Staphylococcus* comprises 41 validly described species and subspecies of Gram-positive, catalase-positive cocci (Ghebremedhin et al. 2008; Bannerman 2003; Kwok and Chow 2003; Spergser et al. 2003), 10 of which contain subdivisions with subspecies designations (Place et al. 2003; Spergser et al. 2003; Garrity et al. 2004). The staphylococci present a spherical shape, and the cells are often grouped to form clusters. These microorganisms are widespread in nature; their major habitats are skin, skin glands, and the mucous membranes of mammals and birds. Some species, mainly coagulase-negative staphylococci (CNS) such as *Staphylococcus xylosus*, *S. carnosus*, *S. equorum*, and *S. saprophyticus*, are frequently isolated from dry fermented sausages, but other species occur, too. Staphylococci are facultative anaerobes capable of metabolizing a number of different sugars. Under anaerobic conditions, the major end product is lactic acid, but acetate, piruvate, and acetoin are also formed.

Since *S. xylosus* and *S. carnosus* are highly competitive in meat fermentation, present important technological properties, and generally lack virulence determinants, these are the most common CNS species used as starter cultures. These organisms show the ability to survive under environmental stress, such as high salt and low temperatures encountered during meat fermentation. CNS primarily contribute to the

development and stability of the desired red color of fermented sausages by means of their nitrate reductase activity (Miralles et al. 1996). In addition, they contribute to the development of other organoleptic properties such as texture and flavor (Hammes and Hertel 1998). These functions are accomplished by specific enzymes involved in the metabolism of proteins and lipids. Previous studies have demonstrated that the aroma of fermented meat products can be modulated by the presence of different *Staphylococcus* spp. (Berdagué et al. 1993; Stahnke 1995; Sondergaard and Stahnke 2002).

A deeper view of the technological properties of *S. carnosus* derives from the analysis of its genome sequence (Rosenstein et al. 2009). *S. carnosus* TM300 has a genome size of 2.56 Mb, similar to that of pathogenic members of the *Staphylococcus* genus, such as *S. aureus* (2.71–2.91 Mb) and *S. epidermidis* (2.49–2.64). Although this species has a set of conserved genes corresponding to 46%–50% of the entire chromosome, in common with *S. aureus*, *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*, the lack of known staphylococcal virulence factors in *S. carnosus* was confirmed by genome sequence. Thus, gene coding for *alpha*-hemolysin, *gamma*-hemolysin, exfoliative toxins, and superantigens, such as toxic shock syndrome toxin 1 and enterotoxins, was not found in *S. carnosus* TM300 genome. A complete set of genes involved in meat adaptation and coding for technological relevant properties is harbored in the genome of *S. carnosus*. Genome-based analysis of the metabolic pathways for energy generation revealed that this species possesses the genetic potential for the transport into the cell and the metabolism of several sugars occurring in meat or added in the batter, such as glucose, lactose, and ribose. All the enzymes of the glycolytic pathway, the lactate dehydrogenase and the tricarboxilic acid cycle, and all components of the respiratory chain are coded by the *S. carnosus* genome, allowing the shift from aerobic to anaerobic metabolism as a function of environmental conditions.

The genome sequencing of *S. xylosus* is ongoing, and information can be obtained at www.cns.fr/externe/English/Projets/Projet_NN/NN.html. Knowledge of the whole chromosome sequence of *S. xylosus*, whose size has been estimated to be 2.86 Mb (Dordet-Frisoni et al. 2007), will provide for a better understanding of the physiology of this species. A proteomics approach to study cell-envelope proteins of *S. xylosus* has been developed (Planchon et al. 2006, 2007), in which a significant set of cell-enveloped proteins can be recovered. When such information is integrated with future analyses of the transcripts, a more integrated and comprehensive knowledge of the mechanism by which meat starter bacteria contribute to the fermentation of meat can be obtained, as can how these bacteria interact with one another.

Starter Cultures: Technological Advantage in the Meat Environment

Competitiveness

To make the ideal starter culture for any particular technology and recipe, it is necessary to understand the function we seek and to have tools to monitor the efficacy of the culture (Hansen 2002). One of the fundamental properties of bacterial starter cultures is the ability to compete with the adventitious microbiota of meat, to colonize this environment, and to dominate the microbial community of the fermented products. The starter culture must compete with the natural microbiota of the raw material and undertake the metabolic activities expected of being conditioned by its growth rate and survival in the conditions prevailing in the sausage (i.e., an anaerobic atmosphere, rather high salt concentrations, low temperatures, and low pH).

Two of the most common preservative conditions employed in meat processing are low temperatures and high salt concentrations. *L. sakei* is remarkably well equipped to cope with these conditions. It contains several transporters for osmoprotective substances and has more cold stress proteins than other lactobacilli. *L. sakei* has psychrotrophic and osmotolerant properties, and is able to grow at low temperatures and in the presence of up to 10% sodium chloride (NaCl). These physiological features are associated with the presence in its genome of a higher number of genes coding for stress-response proteins, such as cold shock and osmotolerance proteins, than found in other lactobacilli. *L. sakei* lacks proteins involved in adhesion to intestinal mucous, but its genome codes for numerous proteins that may be involved in adhesion to the meat surface (e.g., to collagen), aggregation, and biofilm formation. Thus, the bacterium seems well equipped to adhere to and spread on a meat surface (Eijsink and Axelsson 2005).

Sanz and Toldrá (2002) reported an arginine-specific aminopeptidase activity in *L. sakei* that is important for the release of the free amino acid, since it could be further channeled into the arginine deiminase pathway. The genes encoding the proteins required for arginine catabolism in *L. sakei* are organized in a cluster (Zúríga et al. 2002), and their transcription is repressed by glucose and induced by arginine. Arginine, in particular, is an essential amino acid for *L. sakei* and specifically promotes its growth in meat; it is used as an energy source in the absence of glucose (Champomier-Verges et al. 1999). The concentration of free arginine in raw meat is low, although it is relatively abundant in muscle myofibrillar proteins. Moreover, the genome analysis has shown that *L. sakei* harbors a second putative arginine deaminase pathway, containing two peptydil-arginine deaminases, enzymes that can contribute to the metabolism of arginine (Chaillou et al. 2005), thus increasing the competitiveness of *L. sakei* in a meat environment.

The competiveness during fermentation is strictly related to the ability of the cells to adapt to the environmental conditions of the meat batter and to the ecological conditions present during fermentation. In a study on *L. sakei* gene expression, environmental conditions of sausage were found to induce 15 genes (Hüfner et al. 2007). Consistent with the expected metabolic adaptation, these genes code for proteins involved in the amino acids and carbohydrate transport, lipid metabolism, and stress response. The inactivation of the heat shock regulator gene *ctsR* resulted in an improved growth of *L. sakei* in fermented sausages.

The ability of CNS to colonize cured and fermented meats has been well described (Leroy et al. 2006). Thus, these organisms, which are present in the adventitious microbiota of meat or are added as starter cultures to the batter, become a dominant population during fermentation. Physiological properties, such as the ability to grow at low temperatures and low water activity, contribute to the competiveness. Information derived from the *S. carnous* genome provides a scientific basis for adaptation to low water activity environments, such as cured and fermented meat. Nine pathways involved in osmoprotection, which contribute to the accumulation of biocompatible solutes in the cytoplasm, are present in *S. carnous* TM300. These include four proline transport systems; three glycine betaine transporters; one multicomponent transporter for choline, glycine betaine, and carnitine; and one system for the choline uptake (Rosenstein et al. 2009).

Acid Production

Sugars (glucose and occasionally lactose or sucrose) are usually included in the industrial manufacture of fermented meat products, though in Spain, chorizo is traditionally manufactured with little or no added sugar.

During fermentation and ripening, LAB convert glucose (their primary energy source) to lactic acid, which is the main component responsible for the pH decrease. This acidification has a preservative effect, due to inhibition of pathogenic and spoilage bacteria with little resistance to low pH, and it contributes to the development of the typical organoleptic characteristics of the fermented sausages (Bover-Cid et al. 2001). Although it is well established that fermentable carbohydrates have an influence on flavor, texture, and yield of fermented sausages, carbohydrates for use in dry sausages formulations are generally chosen to ensure an adequate initial drop in meat pH (Bacus 1984; Lücke 1985) for preservation reasons, and less importance is given to the product texture. The level of acidification and the selection of the starter culture to be used depend on the desired sensorial properties of the product. In northern European sausage technologies, more acid products are preferred, obtained by adding *Lactobacillus* starter cultures and more carbohydrates to the sausage matrix (0.6%–0.8%). On the other hand, less acidic products are obtained using a lower concentration of glucose and also by using *Staphylococcus* starter cultures, as occurs in typical southern European fermented sausages. In these last products, which are characterized by a longer ripening period (up to 60 days), an increase of pH occurs in the later stages of fermentation, related to ammonia release from ATP and amino acid metabolisms.

Acidification could also be the result of alternative pathways. In *L. sakei*, the presence of genes involved in the energetic catabolism of nucleoside, such as adenosine and inosine, is an example of the adaptation of this organism to the meat environment. Glucose, the favorite carbon source of *L. sakei*, is rapidly consumed in meat, while adenosine and inosine are abundant, reaching twice the concentration of glucose. In addition (as shown in Fig. 10.1), *L. sakei* harbors genes coding for adenosine deaminase, inosine hydrolase, and nucleoside phosphorylase, all of which enable the release of a ribose moiety from nucleoside (adenosine and inosine) and its subsequent metabolism (Chaillou et al. 2005). Moreover, the presence of methylglyoxal synthase, a novel genetic trait in lactic acid bacteria, has been proposed as a pathway to counteract frequent glucose starvation and modulate the metabolism of alternative carbon sources (Chaillou et al. 2005).

The effect of environmental challenges on the growth and acidification kinetics of *L. sakei* in sausages has been recently studied by Hüfner and Hertel (2008). In this study, it was demonstrated that *L. sakei* improves its acidification performances if cells are exposed to sub-lethal stresses, such as cold and osmotic shocks. This adaptation to stress improves the performance of *L. sakei* during sausage fermentation.

Catalase Activity

The metabolism of most lactic acid bacteria, such as the adventitious lactobacilli that contaminate raw meat, could lead to the formation of hydrogen peroxide, a compound that interferes with the sensorial properties of meat products, as it is involved in discoloration of nitroso-heme pigment and lipid oxidation. Bacterial strains used in meat cultures can produce catalase, antioxidant enzymes that cause disproportionate levels of hydrogen peroxide compared with oxygen and water, preventing the risk of reduced quality in the fermented meat. Thus, catalase production is considered a relevant technological property of starter cultures for fermented meat products (Leroy et al. 2006). Production of this antioxidant enzyme is a common trait in aerobic bacteria, such as CNS. The characterization of catalase and superoxide dismutase in *S. carnosus* and *S. xylosus* has been reported. The catalase gene *kat*A of *S. xylosus* has been studied in detail (Barrière et al. 2001a, b, 2002). Transcriptional activity of

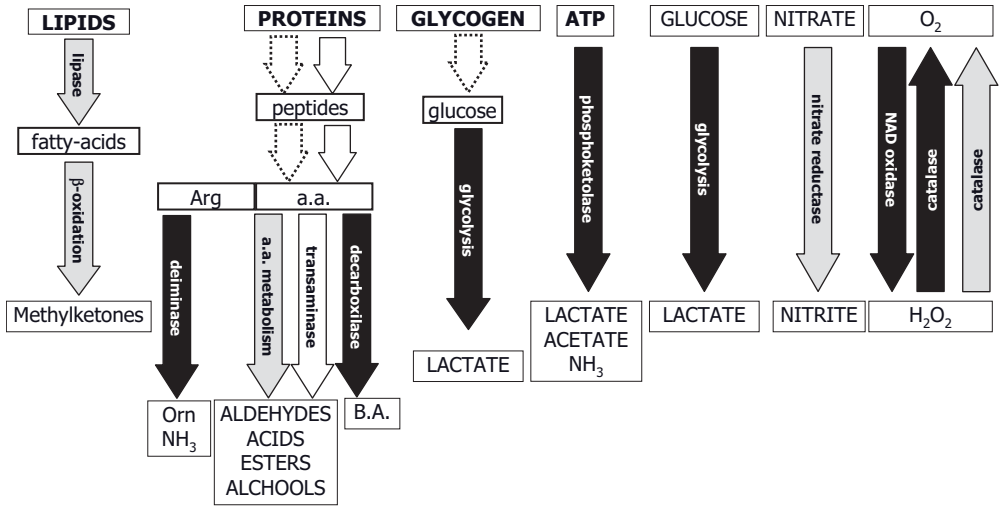

Figure 10.1. Meat starter culture bacteria: major metabolic pathways in meat fermentation. Main enzymatic activities of coagulase negative staphylococci and lactobacilli are indicated by light grey arrows and dark grey arrows, respectively. The metabolic activities ascribed to both bacterial groups are indicated by white arrows. Dotted-line arrows indicate action of endogenous meat enzymes. Abbrevations: a.a., amino acids; Arg, arginine; B.A., Biogenic Amines; Orn, ornithine. Sugars added to the batter are rapidly metabolized to lactate by starter cultures of lactic acid bacteria. Glycogen, proteins, and lipids catabolism are also used for microbial growth during fermentation. Ribose is released by ATP hydrolysis, and the subsequent metabolism of ribose-derived molecules is used for energy production by *L. sakei*. When sugar concentration declines, free amino acids (a.a) are utilized for microbial growth. Via the arginine deiminase (ADI) pathway, arginine is converted to ornithine and supports the growth of lactobacilli in the latter stage of meat fermentation. Staphylococci modulate the aroma through the conversion of amino acids (particularly the branched-chain amino acids leucine, isoleucine, and valine) into methyl-branched aldehyde, methyl-branched acids and sulphites, diacetyl, and ethyl ester. The methyl ketones (2- pentanone and 2-heptanone) derive from intermediates of an incomplete ß-oxidation pathway in staphylococci.

this gene is activated and induced by oxygen and hydrogen peroxide upon entry into the stationary phase. Moreover, a second gene coding for heme-dependent catalase has been detected in *S. xylosus*. The well-described antioxidant property of *S. carnous* TM300, involved in the protection of meat products from hydrogen peroxide damage, depends on a set of genes, one superoxide dismutase, two catalases, and various peroxidases, involved in the protection against oxygen reactive species (Rosenstein et al. 2009).

Although lactic acid bacteria have long been considered as catalase-negative microorganisms, two groups of catalase activity have been reported in the last decade in genera *Lactobacillus*, *Pediococcus*, and *Leuconostoc*. The first group is defined as heme catalase and the second group nonheme Mn-containing catalase. The presence of a heme-dependent catalase has been demonstrated in *L. plantarum* (Igarashi et al. 1996) and *L. sakei* (Noonpakdeea et al. 1996); it can be active in meat products because these substrates contain abundant heme sources (Hertel et al. 1998). Moreover, analysis of the genome of *L. sakei* revealed that this meat organism harbors systems for protection against reactive oxygen species, such as Mn-dependent SOD and heme-dependent catalase (Chaillou et al. 2005). The *L. sakei* genome contains genes encoding a heme-dependent catalase, a superoxide dismutase, and a NADH oxidase to cope with reactive oxygen species, and there are several systems to cope with changes in the redox potential.

A summary of the main metabolic pathways used by the meat starter bacteria (*Lactobacillus* and *Staphylococcus*) is given in Figure 10.1.

Nitrate Reduction

Nitrate is added to fermented sausages for its capacity to obtain and fix the typical color of cured products, rather than for its antimicrobial properties. To be effective, the added nitrate must be reduced to nitrite. Besides contributing to flavor, *Staphylocuccus* and *Kocuria* also have a role because of their nitrate reductase and antioxidant activities (Talón et al. 1999, 2002). These microorganisms reduce nitrate to nitrite, which is important for the formation of nitrosylmyoglobin, the compound responsible for the characteristic red color of fermented meats. The nitrate reductase activity is widespread in CNS; it has been detected in *S. xylosus*, *S. carnosus*, *S. epidermidis*, *S. equorum*, *S. lentus*, and *S. simulans* (Talón et al. 1999; Mauriello et al. 2004). In *S. carnosus*, the molecular genetic determinants for nitrogen regulation, the *nre*ABC genes, were identified and shown to link the nitrate reductase operon (*nar*GHJI) and the putative nitrate transporter gene *nar*T. The data provide evidence for a global regulatory system, with oxygen as the effectors molecule (Fedtke et al. 2002). The high dissimilatory nitrate respiration, typical of *S. carnosus* and involved in nitrate reduction in meat products, was found to be present in the genome of *S. carnosus* TM300 (Rosenstein et al. 2009).

Flavor Formation

The flavor and aroma of fermented meats is a combination of several elements. Lactic acid bacteria produce lactic acid and small amounts of acetic acid, ethanol, and acetoin; however, to ensure the sensory quality of fermented sausages, the contribution of the proteolytic and lipolytic activities of staphylococci is fundamental.

The pattern of the proteolysis in fermented sausages is influenced by several variables, such as product formulation, processing condition, and starter culture (Hughes et al. 2002). The volatiles so far recognized as being produced by staphylococci are primarily amino acid catabolites, piruvate metabolites, and methylketones from incomplete β-oxidation of fatty acids (Stahnke et al. 2002). In particular, *S. xylosus* and *S. carnosus* modulate the aroma through the conversion of amino acids (particularly the branched-chain amino acids BCAA: leucine, isoleucine, and valine). The BCAA can be degraded into methyl-branched aldehydes, alcohols, and acids by *S. xylosus* and *S. carnosus* (Vergnais et al. 1998; Larrouture et al. 2000; Beck et al. 2002). Furthermore, addition of *S. carnosus* starter culture has been shown to decrease the maturation time of Italian dried sausages by more than two weeks (Stahnke et al. 2002). Olesen et al (2004) reported that curing conditions had a considerable influence on the development of volatile compounds in sausages. In addition, major differences were observed in the development of volatile compounds, depending on whether *S. xylosus* or *S. carnosus* were used as starter culture.

Even though microbial proteolytic activity is generally low in the conditions found in fermented sausages (Kenneally et al. 1999), a minor, strain-dependent activity may still partly contribute to initial protein breakdown (Molly et al. 1997; Fadda et al. 1999a, b, 2002; Sanz et al. 1999). Several studies lead the hypothesis that both endogenous and bacterial peptidases are required for complete hydrolysis of oligopeptides, and the activity of these enzymes could be strongly involved in the quality of the final product (Rodríguez et al. 1998; Fadda et al. 1999a, b; Mauriello et al. 2002, 2004; Casaburi et al. 2005; Drosinos et al. 2007). It has been described *in vitro* that several *Lactobacillus* spp. exhibit proteolytic activity on porcine muscle myofibrillar and sarcoplasmic proteins. Fadda et al. (2001a) reported the contribution of

curing conditions to the generation of hydrophilic peptides and free amino acids by the proteolytic activity of *L. curvatus* CRL 705. Moreover, it has been demonstrated that *L. sakei* plays an important role in amino acid generation (Fadda et al. 1999a, b; Sanz et al. 1999).

Lipolysis, together with proteolysis, is believed to play a central role in aroma formation. This phenomena is only the first step in the process and is followed by further oxidative degradation of fatty acids into alkanes, alkenes, alcohols, aldehydes, and ketones (Viallon et al. 1996; Chizzolini et al. 1998), which enhances the development of the flavor. In fact, medium- and long-chain fatty acids act as precursors of aroma compounds, whereas the short-chain fatty acids (Co6) lead to strong cheesy odors (Ansorena et al. 2001). Although some authors (Molly et al. 1997; Kenneally et al. 1998; Galgano et al. 2003) have concluded that tissue lipases are primarily responsible for lipolysis during fermentation, numerous studies over the last decade described lipolytic bacteria, especially staphylococci (Hugas and Monfort 1997; Montel et al. 1998; Mauriello et al. 2004). Hugas and Monfort (1997) highlighted the need to use selected strains of Gram-positive, catalase-positive cocci to ensure sensory quality of fermented sausages. Moreover, Stahnke et al. (2002), Beck et al. (2004), and Olesen et al. (2004) described the capability of *Staphylococcus xylosus* and *Staphylococcus carnosus* strains to modulate the aroma through the conversion of amino acids and free fatty acids (FFA). Strains of *S. xylosus* have been recommended for the production of the very aromatic sausages of southern Europe (Samelis et al. 1998).

Bacteriocin and Biopreservation

In recent years, there has been a considerable increase in studies of the natural antimicrobial compounds on and in food produced by LAB, referred to as bioprotective cultures. Bioprotective cultures may act as starter cultures in food fermentation processes, such as dry sausage manufacturing, or they may protect foods without any detrimental organoleptic changes.

The ability to produce different antimicrobial compounds, such as bacteriocins and/or low-molecular mass antimicrobial compounds, may be one of the critical characteristics for effective competitive exclusion. As mentioned above, one of the main roles of meat LAB starter cultures is the rapid production of organics acids; this inhibits the growth of unwanted biota and enhances product safety and shelf life. Likewise, several authors have reported the role of *Staphylococcus* in proteolysis, lipolysis, and formation of flavor in sausages (Berdagué et al. 1993; Montel et al. 1998, 1996; Engelvin et al. 2000; Stahnke 2002; Olesen et al. 2004; Tjener et al. 2004). Some strains are able to produce antimicrobial substances (Martín et al. 2007).

The production of bacteriocins, one of the most promising technological features of starter cultures, is discussed in Chapter 14.

Probiotics

Foods that have health benefits beyond their nutritional content (functional foods), and particularly foods containing probiotics, are products that are growing in popularity. Probiotics are available as dietary supplements or they may be incorporated directly into foods. They are live microorganisms that when administered in adequate amounts, confer a health benefit to the host (FAO 2006); they are added to a variety of foods. Recently, attention has been directed to the use of fermented sausages as a food carrier because these products could contain high numbers of viable lactic acid bacteria. To use probiotics as starter cultures for fermented sausages, in addition to the demonstrated probiotic features (FAO 2006), other properties are demanded.

Although dairy products are the most commonly used food vehicles for delivery of probiotics, the future of dry-fermented sausages in this field has been termed "promising" (Incze 1998). The probiotic culture should be well adapted to the conditions of the fermented sausage in order to dominate in the final product, competing with other bacterial populations from meat and from the starter culture. In addition, the culture should not develop off-flavors in the final product.

The potential for dry-fermented sausages to serve as a vehicle for probiotic organisms has been comprehensively reviewed by Työppönen et al. (2003). Most of the studies discussed in this review relied on the fermentative abilities of the probiotic organisms used, so the selection of probiotics was limited to organisms that were capable of fermenting carbohydrates in meat.

Various studies have shown that probiotic organisms have poor survival in fermented foods such as yoghurt, fermented milks, and dry-fermented sausages (Kailasapathy and Rybka 1997; Lücke 2000; Shah 2000; Shah and Ravula 2000; Erkkilä et al. 2001). Dry-fermented sausages with their low a_w and pH, plus curing salts and competing organisms, would seem to present a challenging environment for the survival of probiotics during processing. Kearney et al. (1990) was the first to report the use of microencapsulation in alginate to protect starter cultures during meat fermentation. Recently, Muthukumarasamy and Holley (2006) used microencapsulation technology as a means to protect a recognized probiotic organism (*L. reuteri*) from the harsh environment during sausage processing. Based on their results, the authors suggest that microencapsulation may be an option for formulation of fermented meat products with viable health-promoting bacteria. Another approach for selecting bioprotective and probiotic cultures for use in dry-fermented sausages involved the isolation of LAB, which possess acid and bile resistance, from finished products (Papamanoli et al. 2003; Pennacchia et al. 2004). This approach requires an extensive study of the isolates for other beneficial properties, such as intestinal colonization potential and inhibitory activity against pathogenic bacteria.

Commercial probiotic cultures, such as strains *L. rhamnosus* GG, *L. rhamnosus* LC-705, *L. rhamnosus* E-97800, and *L. plantarum* E-98098, have been tested as functional starter culture strains in northern European sausage fermentation without negatively affecting the technological or sensory properties, with the exception of *L. rhamnosus* LC-705 (Erkkilä et al. 2001). Klingberg et al. (2005) identified *L. plantarum* and *L. pentosus* strains, originating from the dominant NSLAB of fermented meat products, as promising candidates for probiotic meat starter cultures suitable for the manufacture of the Scandinavian-type fermented sausage.

It is worthwhile to mention that in the European Union there is specific regulation (EC No 1924/2006) aimed at ensuring that any health claim made on a food label is clear, accurate, and scientifically substantiated.

Safety of Selected Bacterial Starter Cultures

Members of the genus *Lactobacillus* and *Pediococcus* are generally considered nonpathogenic for the consumer. The safety of these two bacterial genera has recently been assessed by EFSA in the risk-assessment approach named Qualified Presumption of Safety (EFSA 2008). However, risk factors could be the production of biogenic amines or the presence of transmissible determinants for the antibiotic resistance. The *Staphylococcus* genus also encompasses several species responsible for infections or intoxications. For this reason, the production of enterotoxins and the presence of acquired

resistance to antibiotic are major concerns in CNS.

Biogenic Amines

The accumulation of biogenic amines (BA) in foods requires the presence of amino acid precursors, microorganisms with amino acid decarboxylase activity, and favorable conditions (temperature and pH) for growth and decarboxylation. The large quantities of protein present and the proteolytic activity found during the ripening of meat products provide the precursors for later decarboxylase reactions performed by both starter cultures and wild microbiota (Suzzi and Gardini 2003; Komprda et al. 2004). The presence in food of biogenic amines (BA), such as cadaverine, putrescine, spermidine, histamine, phenethylamine, agmatine, and tyramine, is a health concern because their biological effect can lead to toxicological symptoms, such as pseudo-allergic reactions, histaminic intoxication, and interaction with drugs (Shalaby 1996). Excessive consumption of these amines could cause nervous, gastric, intestinal, and blood pressure problems (Suzzi and Gardini 2003). Nowadays, increasing attention is given to BA because of the growing number of consumers who are sensitive to them; in such people, the action of amine oxidases, the enzymes involved in the detoxification of these substances, is deficient (Suzzi and Gardini 2003). High levels of BA, especially tyramine but also histamine and the diamines putrescine and cadaverine, have been described in fermented sausages (Hernández-Jover et al. 1997a, b; Bover-Cid et al. 2000a, b).

Many LAB from meat and meat products can decarboxylate amino acids (Bover-Cid and Holzapfel 1999). Rosenstein et al. (2009) reported that *S. carnosus* encodes an ornithine decarboxylase (Sca0122) that could account for the synthesis of putrescine from ornithine or cadaverine from lysine. Many studies have reported a significant correlation between pH and BA contents, the lowest pH generally being characterized by highest amine levels (Vandekerckove 1977; Eitenmiller et al. 1978; Halàsz et al. 1994; Bover-Cid et al. 1999; Parente et al. 2001), according to the hypothesis that biogenic amine production could be a protective mechanism for microorganisms against acidic environmental conditions.

The final BA contents in fermented sausages depend on the microbial composition of meat used as raw material, but also on the type and activity of the starter culture inoculated. Most strains of *L. curvatus*, one of the main species used as a starter in sausage production, are associated with high BA production (Bover-Cid and Holzapfel 1999; Pereira et al. 2001).

The use of starter cultures with negative decarboxylase activity was shown to prevent the growth of biogenic amine producers and lead to end products nearly free of BA, as long as the raw material was of sufficient quality. Several papers have reported on the ability of selected starter culture (*L. sakei* CTC494) to greatly reduce BA accumulation in fermented sausages (Bover-Cid et al. 2001; González-Fernández et al. 2003). This negative-decarboxylate strain can decrease the pH quickly during the fermentation step and be predominant throughout the process, thus preventing the growth of bacteria that can produce BA.

The introduction of starter strains that possess amine oxidase activity might be a way to further decrease the amount of BA produced during meat fermentation (Martuscelli et al. 2000; Fadda et al. 2001b; Gardini et al. 2003; Suzzi and Gardini 2003).

Antibiotic Resistance

The safety of bacterial strains intentionally added to food, such as starter cultures used for meat products, is becoming an issue. Although meat starter cultures have a long

history of apparent safe use, safety concerns can be associated with lactic acid bacteria and, more frequently, with CNS. A risk factor potentially associated with all bacterial groups used as starter cultures for sausage is the presence of acquired genes for antimicrobial resistance.

The food chain has been recognized as one of the main routes for the transmission of antibiotic-resistant bacteria between animal and human populations (Witte 2000). The European Food Safety Authority has recently concluded that bacteria deliberately introduced in the food chains, such as the starter cultures, might pose a risk to human and animal health because of carrying acquired resistance genes (EFSA 2007). Fermented meats that are not heat treated before consumption provide a vehicle for such bacteria and can act as a direct link between the indigenous microbiota of animals and the human gastrointestinal tract.

Lactobacilli are generally recognized as safe and are not responsible for human infections in healthy people (Z'Graggen et al. 2005). However, they might act as reservoirs of transmissible antibiotic-resistance genes that under certain conditions could be transferred to food or gut microbiota (Jacobsen et al. 2007). In addition, the emergence of antibiotic-resistant food-borne pathogens originating from meat products (Doyle and Erickson 2006) raises the question of the possibility of gene transfer between industrial bacterial species and food-borne pathogens.

Several studies have reported antibiotic resistance in LAB from meats and meat products; a few strains involved in sausage fermentation such as *L. sakei*, *L. curvatus*, and *L. plantarum* have been found to show such resistance (Holley and Blaszyk 1997; Teuber and Perreten 2000; Gevers et al. 2003). Some genetic determinants, such as chloramphenicol acetyltransferase (*cat*-TC), erythromycin (*erm*B), and tetracycline (*tet*L, *tet*M, and *tet*S resistance genes), have been identified, suggesting that horizontal gene transfer may have occurred (Ahn et al. 1992; Tannock et al. 1994; Lin et al. 1996; Gevers et al. 2003; Ammor et al. 2008)

Antimicrobial resistance in CNS has been studied in detail due to its clinical relevance. These bacteria display a high prevalence of antibiotic resistance (Agvald-Ohman et al. 2004) and can constitute reservoirs of antibiotic-resistance genes that can be transferred to other staphylococci (Wielders et al. 2001). Antibiotic-resistant strains were found in food (Gardini et al. 2003; Martin et al. 2006), and genes for antimicrobial resistance to tetracycline, *tet*(M) and *tet*(K); erythromycin, *ermB* and *ermC*; and two β-lactams (*blaZ* and mecA) have been detected in CNS isolated from fermented meat. Moreover, *S. xylosus* strains, isolated from poultry infections, were found to be resistant to streptogramins, harboring the *vatB* and the *vgaB* genes.

An additional concern is that, even in the absence of selective pressure, mobile genetic elements carrying antibiotic resistance can be transferred at high frequency through the microbial community during sausage fermentation (Vogel et al. 1992; Cocconcelli et al. 2003).

For these reasons, the absence of acquired resistance to an antibiotic of clinical relevance should be a parameter to be used in the selection of starter cultures for food.

Toxigenic Potential

Some members of the CNS group, primarily *S. epidermidis*, are common nosocomial pathogens, and the presence of regulatory elements, involved in the control of virulence factor synthesis, has been recently identified. Remarkably, strains of *S. xylosus* were isolated from patients who had an underlying disease. The same species has been reported to be involved in infections of poultry (Aarestrup et al. 2000).

Although CNS of food origin has not been found to produce nosocomial infections,

some strains that produced enterotoxins have been described. Vernozy-Rozand et al. (1996) reported enterotoxin E as the most frequent enterotoxin found in *S. equorum* and *S. xylosus*, though Martin et al. (2006) reported that the occurrence of staphylococcal enterotoxin genes in CNS from slightly fermented sausages was rare, detecting only *ent*C in *S. epidermidis*. The coagulase-positive species, *S. intermedius* and *S. aureus*, have been more frequently related to staphylococcal intoxication to date. Moreover, *S. intermedius* are opportunistic pathogens in implant infections, due to their ability to form biofilm on prosthetic materials.

The *S. xylosus* and *S, carnosus* strains currently used as starter cultures or isolated from fermented meat products generally lack toxin genes. The absence of genes coding for staphylococcal enterotoxins or enterotoxin-like superantigens is a qualification required for CNS strains selected for use as starter cultures.

Conclusion

Selected starter cultures are powerful tools to drive the fermentation of meat products, allowing meats to reach the desired targets of quality and safety. Their use in meat fermentation results in an acceleration of fermentation time, an improvement of safety (by reducing undesirable microorganisms), and a better final quality of the products. The choice of a selected starter culture should be seen in the context of its application, since functionality will depend on the type of sausage, the technology applied, the ripening time, and the ingredients and raw materials used.

Moreover, the new information derived from genetic analyses, such as genomic and transcriptomic, provide a new instrument for a scientifically sound selection of new tailor-made starter cultures harboring the desired physiological traits. The microbiota of traditional sausages will provide the source for the selection of strains well adapted to the environment and able to compete with contaminant bacteria.

In the process of selection and choice of a starter culture, the safety aspects such as antibiotic resistance, virulence genes, and undesirable metabolite formation should not be overlooked.

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Chapter 11

Drying

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Introduction

Drying is probably the most ancient way of preservation, along with heating (cooking, frying, etc.) and chilling or freezing. While chilling and freezing were applicable only seasonally or in arctic regions, drying as such or in combination with smoking could be practiced all over the world. This method was of vital importance to early humans, when fishing and hunting were not always successful and/or when the meat and fat of a large animal was too much to be eaten within a short period of time. Unsalted or salted dried meat also had great importance during wars and maritime navigation.

As a consequence of the development in general, meat processing (drying) began to be done at a larger scale, and simple ways of "conditioning" were worked out and gradually improved. Good dried meat products were also made before air conditioning. Naturally, the process needed "conditioning" at that time, too, but this process had other names. The drying rooms had big doors and windows, and the air conditions were regulated by opening and closing the doors and windows according to the daily outside temperature and humidity. The water loss of the product was examined by touch, and then eventually, on the basis of color, shape, and flexibility of slices. This procedure needed a lot of experience, and it was still not always precise and well-founded enough. That is why there was a strong demand for the classical salami-drying specialist ("salami-masters"), who had high prestige and earnings.

Basis of Drying

During drying, water is removed as vapor. Thus:

- the activity of microorganisms decreases because the portion of water they can utilize decreases, and this in turn means the shelf life of the product increases
- the mass and volume of product decreases
- the texture will be harder
- aroma compounds develop during longer processes, mainly in nonheated products

By means of these changes, meat products can be manufactured, stressing and improving the favorable texture of raw meat and developing products with extraordinary attributes.

The Effect of Preservation

The pressure of water vapor will be constant in the closed space around the material. This pressure is lower or equal to vapor pressure above pure water at the same temperature. The numerical expression of this is the water activity (a_w):

$$a_w = \text{water pressure above the material} / \text{water pressure above the pure water}$$

Microorganisms cannot grow below their specific a_w (Table 11.1.) (IFT/FDA 2003; Leistner and Rödel 1976; Mossel 1971).

Table 11.1. The lower a_w range of development of microorganisms

Bacteria	Yeasts	Molds	a_w
E. coli			0.99
Str. fecalis			0.98
Vib. metschnikovii			0.97
Pse. fluorescens			0.97
Clo. botulinum			0.97
Campylobacter ssp.			0.97
Shighella			0.97
Yersinia enterocolitica			0.97
Clo. perfringens			0.96
Bac. cereus			0.96
Bac. subtilis			0.95
Sal. newport			0.95
Ent. aerogenes			0.94
Microbacterium			0.94
Vib. parahaemolyticus			0.94
Lac. viridescens	*Schizosaccharomyces*	*Rhisopus*	0.93
		Mucor	0.93
	Rodotorula		0.92
Mic. roseus	*Pichia*		0.91
Anaer. Staphylococcus			0.91
Lactobacillus	*Saccharomyces*		0.90
Pediococcus	*Hansenula*		0.90
	Candida	*Asp. niger*	0.88
		Debaryomyces	0.88
	Torulopsis	*Cladosporium*	0.87
Staphylococcus aureus	*Torulaspora*	*Paecilomyces*	0.86
Listeria monocyt.			0.83
		Penicillium	0.80
		Asp. ochraceus	0.80
Halophilic bacteria			0.75
		Asp. glaucus	0.72
		Chrysosporium fastidum	0.70
Zygosaccharomyces rouxii		*Monascus bisporus*	0.60

The a_w of the material has to be decreased to a certain level in order to inhibit the growth of contaminating microorganisms. During drying, this decrease will be achieved by lowering the moisture content. It is necessary to know the relation between the moisture content and a_w. This relation is rather complicated and changes from material to material. Two groups of components are able to diminish the a_w value:

- the water soluble compounds
- materials able to swell in water (In meat, structural proteins are such components.)

In meat products, the first group is important, and in meat meal, it is the second one.

It needs to be stressed that the other components of meat—for example, fat—are ineffective in terms of a_w, and therefore, a_w does not depend on the material's moisture content but on the ratio of water to the effective components. The raw fat has an a_w close to 1 independent of its low moisture content.

The soluble components decrease a_w value by means of their "diluting" effect. The basic relation for a_w is:

a_w = number of water particles/(number of water particles + number of particles in solution) (Nernst law)

Number of particles = mass of component in the solution/mass of particles

The mass of a particle is the molecular mass in the case of molecules, and ion mass in the case of ions. If the solubility is limited in a saturated solution, no more soluble component goes into solution, and consequently the a_w does not change either.

Some soluble particles, particularly ions, further decrease the water activity because they bind water particles.

During preservation, the task is to diminish the a_w to the desired value. This value depends on the storage temperature of the product. Dried meat products need no chilling; they can be stored at room temperature. Traditional dried meat products have a pH of about 6.0; they need an a_w below 0.9. The lactic fermented ones have a pH about 5.0, and in this latter case, an a_w below 0.95 is needed for ensuring a safe product (Fig. 11.1) (Incze 2004).

The swelling components bind a little part of water independent of the a_w; another part of water is bound increasingly parallel with a_w-increase. The amount of bound water depends on the state of swelling material (denaturation).

The contents of water, soluble materials, and swelling materials together determine the a_w; the other components have no influence.

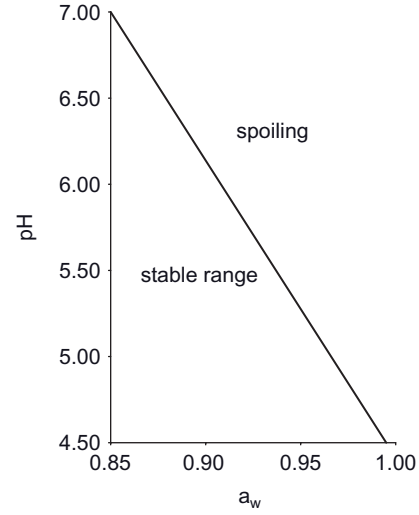

Figure 11.1. The stable a_w-range as a function of pH.

Fat-free raw meat has a content of water of about 80%, with swelling protein content of about 19% and soluble components at about 1%. Figure 11.2 shows the domain of a_w water content connection calculated from various experiments (Hamm 1972; Lewicki 2004; Ruiz-Ramirez et al. 2005a). The figure

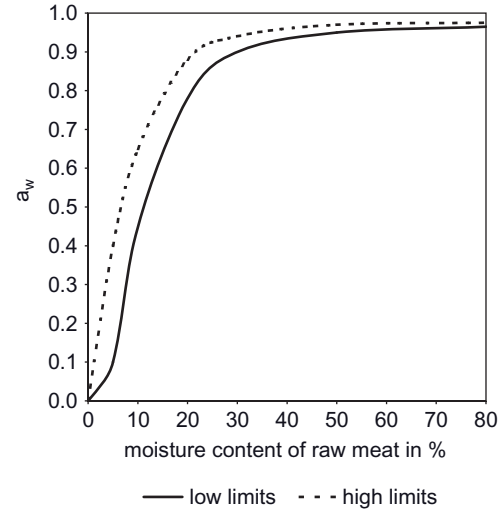

Figure 11.2. Relationship between a_w and moisture content of raw meats.

also shows that the water content of raw meat must be decreased to 40% to get an a_w of 0.90.

The meat loses 60% of its original mass during this process. This loss and the amount of used energy is too high. In addition, the product has a skin-like texture; the product can become edible only by a complicated swelling process.

That is why raw meat as such is not dried without some supplementary treatment. Meat powder and meat granules are produced from cooked meat to use in soup powders, soup insets, and feeds.

Less drying is necessary when soluble materials are introduced into the meat. Two materials, NaCl and sugar, can be used because of health and organoleptic reasons. Lower amounts of NaCl than sugar is necessary for a sufficient reduction of a_w, because of the lower particle mass of NaCl. NaCl is used in meat products and in some sour vegetables, while sugar is added to fruits and candies because of accustomed taste.

The Na+ and Cl− ions bind about two molecules of water in solution; therefore, the NaCl decreases the a_w more than would be expected according to Nernst law (Fig. 11.3).

The saturated solution of NaCl contains 26.5% NaCl (a_w = 0.75). The NaCl content is much lower in meat products; NaCl does not separate in solid form (Krispien et al. 1979). Other meat components (phosphates, amino acids) can be crystalized by strong drying (Zukál 1959).

The fat-free part of raw meat must contain 6.6% NaCl to reach a_w = 0.95. Such product is inedibly salty. The salty taste can be decreased by adding fat to the mix. This method is used when producing meat creams, but these products are not dried. Another method to reduce the salty taste of the end product is to start with lower salt content and then dry to the necessary extent. In this case, the salty taste eases because of the higher content of proteins and fat. This method is used with dried meat products; therefore, the raw material of these products is salted or cured meat. Another benefit of the salt treatment is that salt swells the meat, and so the chewiness and sliceability of the product is improved. These are important attributes of dried meat products.

During the planning of a_w reduction, the following factors need to be taken into account:

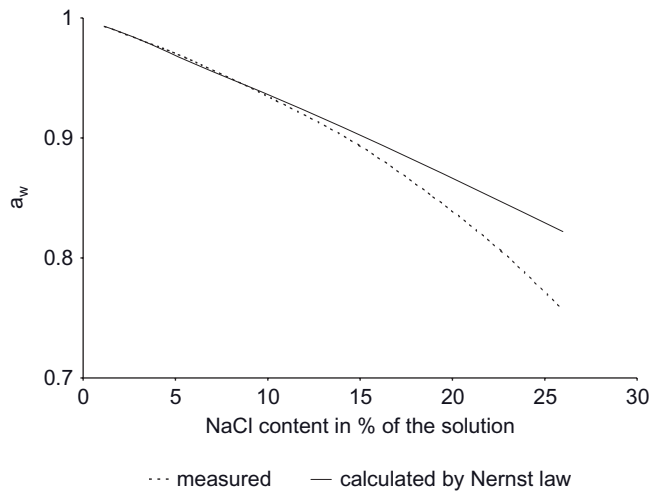

Figure 11.3. The a_w of NaCl solutions.

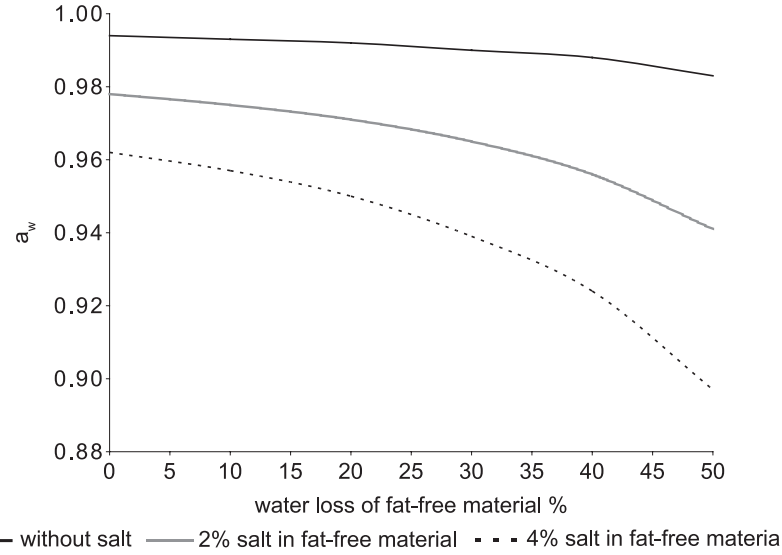

Figure 11.4. Change of a_w as a function of water loss and initial salt content of meat products.

- the characteristic composition of the end product (water, meat-protein, salt, other soluble materials, insoluble materials)
- a_w to be reached.

Meat's a_w—which is necessary for stability—depends on pH. The higher the pH, the lower the a_w must be (Fig. 11.1).

The loss of mass and the initial salt content can be calculated on the basis of the planned end characteristics (Fig. 11.4).

The next task is to determine the drying rate. To do this, we need to understand the drying process.

The Drying Process

During drying

- Water vapor evaporates from the surface of the product, and consequently, the composition of the surface layer changes.
- Materials move from one layer of the product to the other.
- The thickness (and eventually the shape too) of the layers changes to different degrees.
- The mechanical and organoleptical properties of the originally soft material improve.

Details of the Steps

The water vapor leaves the surface layer of the product if the a_w of the surface layer is higher than the relative humidity of the air around the product. In reversed cases, the surface layer binds water and becomes wet. The rate of drying depends on:

- the measurements of the drying surface
- the difference between the a_w and the humidity of the air (driving force)
- the characteristics of the outer layers with pores (e.g., casing, mold, surface layer of the product/drying resistance)

The drying surface is the geometrical surface multiplied by the ratio of the water-permeable elements (meat) to the surface. Ham with skin dries only on the meat side; the skin side is isolated by the fat. This barrier effect is utilized during ripening of some types of hams. If the meat surface is smeared

with fat, the ripening process that produces aroma proceeds with very slow drying.

The geometric surface of chopped products decreases during drying because the diameter of the rods decreases. The permeable part of the surface decreases to a higher degree than the geometrical surface because the impermeable fat particles flatten out, occupying a greater part of the surface. The water vapor leaving the surface increases the moisture content of the ambient air. The relative vapor content will be higher because the evaporation cools down the surface and the environment. The air surrounding the product has to become drier and warmer to maintain the driving force.

The high rate of air circulation decreases the air-side drying resistance too. The product-sided resistance depends on the porosity of the casing, the mold, and the fat film on the surface. This resistance steadily increases during the process, lowering the drying rate.

The salt content of the surface layer grows with the water loss and decreases with the diffusion of salt into the inner layer. The driving force of diffusion is the difference in the salt content compared with the water content. The diffusion of salt seems to be quicker because the salt content along the radius of product is more equilibrated than the water content. So the salt content compared with the water mass is lower in the outer layers (Fig. 11.5) (Kárpáti 1960; Gou et al. 2004).

In the initial phase of drying, the moisture content of outer layers decreases, while the inner layers lose water in the later periods. The equilibration of water content becomes slower and slower, which in turn slows down drying. The differences in water content of the layers barely changes after the end of drying (Fig. 11.6) (Imre 1974).

The mechanical properties of dried meat products, such as chewiness, easy slicing, appropriate firmness, and sufficient flexibility, are very important. During drying

- the initial plasticity comes to an end.
- the mentioned attributes increase.
- chewiness and sliceability improve.

The firmness is highest in the surface layer as a consequence of the higher content of dry material (Fig. 11.7) (Kovács 1961; Ruiz-Ramirez et al. 2005b). Too low water content in overdried products causes denaturation of proteins and a loss of swelling capacity. The result is a product that is too chewy.

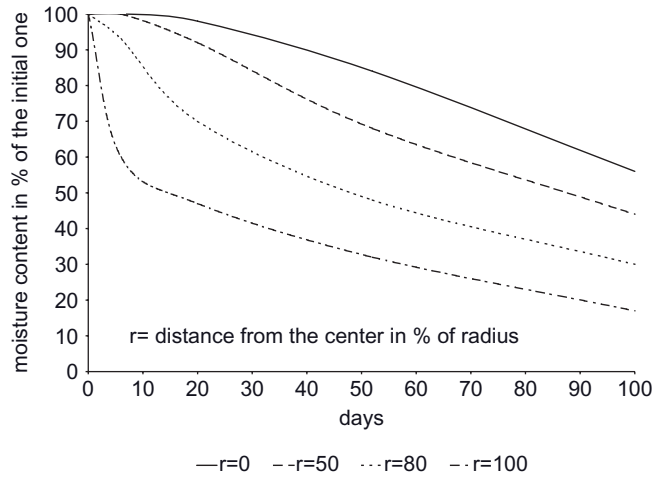

Figure 11.5. Pattern of moisture content in the layers of salami during drying.

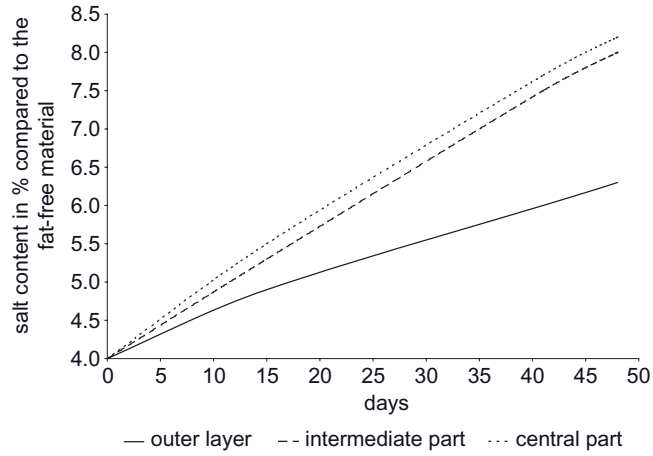

Figure 11.6. The salt content in the layers of salami during drying.

The loss of volume causes tensile strength in the outer layer; therefore, at first, this layer stretches plastically, and later, elastically. Too rapid drying makes denaturation in the external layer, so this layer becomes irreversibly hard (case hardening). The layer should shrink during further drying, but it is unable to do so. The external layer will be separated from the inner layers, causing hollows and breakages. Therefore, air gets into the deeper part of the product.

The oxygen itself and the initiation of aerobic microbe activity makes the inner part rancid and green, with a bad odor. That is why the meat products must be dried slowly in order to avoid case hardening. The air holes remaining in the sausage batter during stuffing mechanically weaken the product. Such products become hollow more easily.

The chopped products always have fat particles. Case hardening, air holes, and form

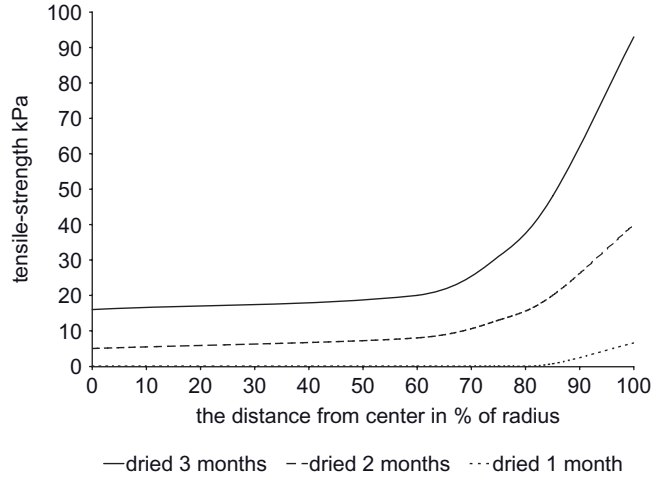

Figure 11.7. The change of tensile strength of the layers of salami during drying.

defects occur too often without this component. However, fat softens the product, so more drying is necessary for the sufficient hardness. The fat particles need to stick to meat particles; otherwise, the product becomes crumbly. Good adhesion requires a surface of fat tissue particles without fat film and a surface of meat covered with solved proteins, gluing the surfaces together.

Smeared fat slows down the drying rate by covering the outer surface and by blocking inner channels that could otherwise serve for moisture migration.

As a result of the enzymatic decomposition of proteins, the meat softens during the drying-ripening (Toldrá 2006). The soft texture can be controlled by mild thermal treatment (under 50°C) at the end of the drying (Morales et al. 2008).

Preparation

The raw material of dried products has to be selected following stricter hygienic directives than that of cooked products because of the initial, critical period of drying and because of the rational decisions to be reached for achieving the proper a_w.

Meat pieces will be salted without water (dry salting). In the preparation of the raw mass of chopped products, the fat must be chopped frozen, with a sharp knife, to avoid the fat film. During stuffing, smearing and air holes have to be avoided. Air holes cannot be left over in the mass (vacuum chopping, filling).

Drying

Meat products are dried in ventilated rooms, hung upon suitable (eventually mobile) frames. The size of the room and the number of levels depends on the capacity, the timing of production, and the choice of products. The frames are mostly mobile, so that they can be cleaned and loaded more easily.

Air Circulation

Canals for both blowing air and sucking it are built into drying rooms. In one-level rooms, the blowers are along the sides at the bottom and blow the air horizontally to the middle of the room; or the blowers are high on the walls and blow vertically down along the walls (Fig. 11.8). Rooms with more levels have horizontal blowers placed at the bottom of all levels, while sucking the air back takes place higher, mostly in the middle of the room.

Control of Air Conditions

The only regulating factor in the drying of meat products is, in fact, the air. The amount of water vapor, the temperature, and the rate of circulation must be controlled. The temperature is the most effective regulator; traditional meat products, especially at the beginning of drying, need low temperature (about 10°C) to prevent microbial activity. The product dries more slowly because control is more difficult at this temperature; however, the danger of case hardening and other mechanical faults is also lower. The range of the relative humidity expands from 70% to 95%, according to the product's drying program.

When working with the starter culture for manufactured meat products, initial (incubation) temperature is in general 20–24°C. (See Chapter 21 on mold ripened sausages.)

The regulation of air parameters is performed in the device connected to the drying room. The air sucked from the room is mixed with outside air from time to time. The superfluous humidity is eliminated by condensation or by absorption. With condensation, the temperature of the air is lowered below the dewpoint temperature, and the water partially condenses. The dry air is warmed and eventually moistened. To absorb water, two methods are used. One of the methods is by

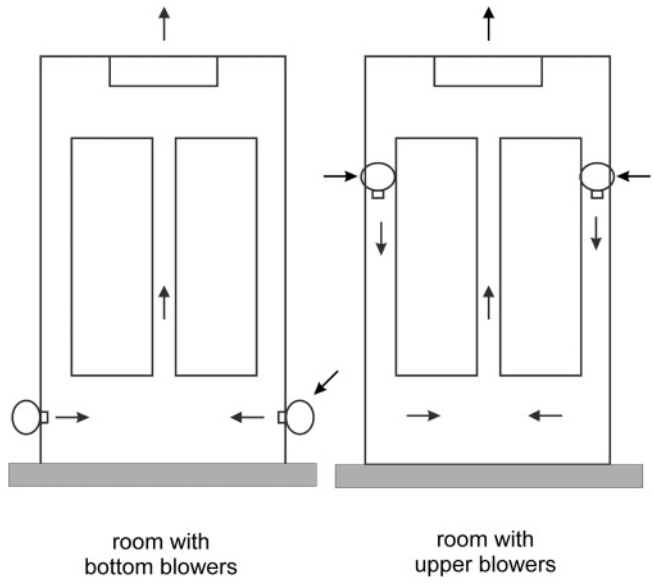

Figure 11.8. Front view (cross section) of a single level drying room.

spraying an absorbing fluid into the air. The other method sends air through layers of water-absorbing granules (e.g., silica gel). These systems leave out the cooling and warming of the air to achieve moisture condensation. The regulation of air humidity is easier, too. However the absorbing medium must be changed or regenerated. The total energy demand is lower as well (Fig. 11.9).

The dried air is recirculated into the drying room (Imre 1974). Temperature and relative humidity of this air is adjusted to the drying program. This program determines the rate of air circulation, too. The circulation is either

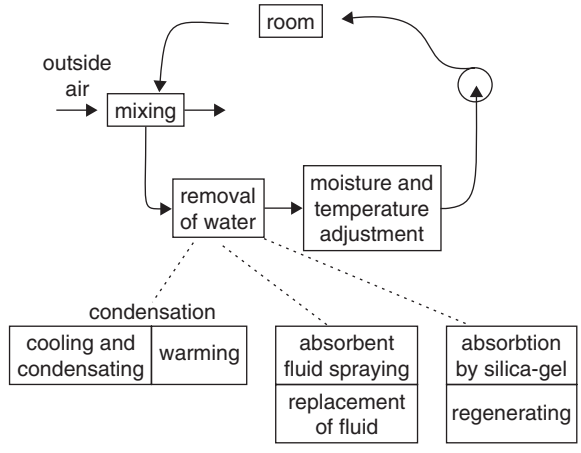

Figure 11.9. The arrangement of the air control unit.

continuous, with relatively low velocity, or periodic. With the periodic system, the room is rinsed through with relatively dry, high-velocity air. Then air circulation stops, and the product saturates the air; then the rinse comes again. In this system, the product regulates its own drying, and case hardening can be avoided.

Smoking needs lower humidity. The smoke hinders the growth of microbes and rancidity on the surface, makes the evaporation easier by tanning the outer sausage layer, and gives a specific, pleasant flavor.

Mold growth needs high humidity. The cover of mold balances the drying rate, hinders the rancidity, and helps to develop aroma by its enzyme-activity. Smoking and mold growth make the drying slower and need special drying programs (Andres et al. 2007; Zukál 1973).

Observation of Drying

The temperature, humidity, and velocity of the blown and sucked air are easy to measure and register at the input and output of the air channels. However, these data give an overall view inside the room. This distribution must be periodically examined at various places in the room.

The water loss can be measured simply by the (eventually continuous) weighing of some of the product units in the room. This can be solved by weighing devices mounted on the drying frames.

Special Methods for Drying

In order to avoid case hardening, a phenomenon occurring mainly in the first part of ripening-drying, a method was worked out where after stuffing, sausages were put in tanks with saline. Sausages with lower salt content lose water into the higher-concentration salt solution; in other words, the sausages dry. When the salt content of the saline and the sausage are adjusted properly, an equilibrium can be reached, and sausages with lower moisture content are then dried further the traditional way.

In the Quick-Dry-Slice (QDS) technology (Comaposada et al. 2008), sausages are fermented, and, after fermentation, they are frozen and sliced; in this form, they are dried by convection and vacuum drying. This method of drying lasts 30 minutes only, and the products are similar in quality to the sausages produced traditionally, according to the authors.

One might call it “indirect drying” when part of the meat is freeze dried, which lowers initial moisture and shortens total drying time.

At present, none of these technologies can be considered to be widespread. This is due to several reasons, such as high-energy demand, the need for further investments, the need for more space, problems with reaching high quality, or even in some cases, no real-time savings.

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Chapter 12

Smoking

Zdzisław E. Sikorski and Edward Kołakowski

Introduction

Smoking, drying, and salting of meats belong to the oldest methods of food preservation. Ages ago, meat hung above a fire was preserved by the combined action of drying and smoking, which was often preceded by pickling in brine. Smoking extended the shelf life and changed the sensory properties of the meats. The procedures of smoking have been gradually improved to suit the requirements of people in different regions of the world in respect to shelf life and sensory properties. The role of the preservative effect of smoking diminished in many countries, while flavoring and safety are of paramount importance for the processor and consumer. Nowadays, various smoking procedures are applied throughout the world in rural households for treating the meat and sausages for domestic use, as well as in large processing plants for the market. It is estimated that as much as 40%–60% of the total amount of meats and meat products are smoked.

The meats hung in a kiln are exposed to smoke and heat for a time sufficient to cause the desirable sensory and preservative effect. The smoke generally comes from smouldering wood chips or sawdust, either directly below the hanging meat or in an external generator. Its density, relative humidity, and flow rate are controlled in a traditional smoking oven by natural draft and depend on the construction of the kiln, the weather conditions, and the actions of the operator. In modern, automatic smokehouses, the draft is forced by mechanical equipment and shaped according to a computer program adjusted to the kind of smoked goods. The temperature of the smoke affects the sensory properties and the preservative effect, and controls the rate of the process. Cold smoking takes place in the range of 12–25°C and warm smoking at 23–45°C. In hot smoking, since thermal denaturation of the meat proteins is required, the smoke temperature during various stages of the process ranges from about 50° to 90°C.

In the past few decades, various aspects of the process of meat smoking have progressed. In traditional smoking, the most significant developments include:

- control of the composition of the smoke by applying rational procedures of smoke generation;
- use of engineering principles regarding heat and mass transfer to shorten the processing time and control the weight loss of the product;
- optimization of the process parameters to assure the required sensory properties and safety of the smoked goods
- modernization of smokehouses, which affects smoke generation as well as handling of the smoked material and control of the process; and
- treatment of the spent smoke to avoid pollution of the environment.

In nontraditional smoking, various new liquid smoke preparations have come into

use, along with procedures for their application.

Curing Smoke

Generation and Properties of Wood Smoke

Curing smoke develops as a result of the partial burning of wood with a limited oxygen supply. Generally, hardwood is used, mainly oak and beech. However, for imparting specific color or flavor to some products, wood from other trees that are rich in resins, including coniferous, as well as heather, may be used. In some areas, other carbohydrate-rich material (e.g., bagasse [sugarcane], beet refuse from sugar making, or coconut husks) is used.

The smoke consists of gaseous products of thermal degradation and subsequent partial oxidation of the wooden material and of the dispersed soot and compounds present in fluid or particle form. The temperature of thermal degradation in the wood constituents—hemicelluloses, cellulose, and lignin—ranges from 180° to 300°C, 260° to 350°C, and 300° to 500°C, respectively. The temperature in the glowing zone may even reach up to 900°C.

The numerous components of the smoke differ in chemical and physical properties. The gases and low-boiling compounds constitute the gaseous phase, while the higher boiling ones are dispersed in the form of fluid droplets or solid particles. The mass of the dispersed phase makes up about 90% of the total mass of the smoke. The proportion of different smoke constituents in both phases depends not only on their chemical composition, but also on the conditions of smoke generation and the temperature and turbulence in the duct leading from the generator to the smokehouse. Cooling increases the weight proportion of the higher boiling compounds, while heating raises the concentration of vapors. Brownian motion leads to coalescence of some particles. Due to the action of the gravitation and centrifugal forces, as well as the temperature gradient, some components are deposited on the smoked goods, in the smoke ducts, and on the walls of the smokehouse. This leads to changes in their concentration in the smoke. The surface electrical charge of the particles also contributes to the physicochemical state of the aerosol. The dispersed components absorb and disperse light; thus their concentration affects the optical density of the smoke. The optical density is proportional to the number of particles in a unit volume of the smoke. In constant conditions, when the dimensions of the particles do not change, the optical density is correlated to the mass concentration of the dispersed components in the smoke. Thus photo optical measurements can be used for the determination of smoke density, which reflects the contents of all components.

Chemical Composition of Smoke

Introduction

The chemical composition of smoke and smoke condensates produced from various kinds of wood was comprehensively reviewed over two decades ago (Tóth and Potthast 1984). In numerous later publications, the effect of the conditions of smoke production prevailing in different generators has also been investigated.

Wood smoke contains air, water vapor, CO_2, CO, and at least several hundred organic compounds in different concentrations. About 400 of them have been unequivocally identified by chromatographic and spectral analytical methods. The composition of the smoke depends on the kind of wood used for smouldering (i.e., mainly on its dryness and the contents of hemicelluloses, cellulose, lignin, and resins), as well as on the temperature and access of air to the zone of oxidation of the volatile products. The content of water

vapor in the smoke is related to the humidity of the wood and air. The relative humidity of the smoke varies within a broad range and can be controlled by the operator. Although numerous investigations have been carried out on the effect of the parameters of generation on the composition of the curing smoke, it is still not possible to predict precisely the contents of various compounds in the smoke. However, it is known which factors affect the generation of phenols, aldehydes, ketones, alcohols, acids, esters, and hydrocarbons. The concentrations of these fractions in the curing smoke, in mg/m^3 of the aerosol or in mg/100 g of wood, differ considerably as reported by different investigators, since the conditions of smoke production in various experiments were different. The yield and gross chemical composition of smoke depends more on the temperature and oxygen access than on the humidity and kind of wood.

The Main Groups of Compounds

The phenolic fraction of wood smoke consists of about 250 components, with 85 of them identified. Phenols are formed primarily due to pyrolysis and oxidation of lignin, at comparatively low temperature (200–400°C), and cellulose at 700°C. The total contents of phenols depend on the kind of wood, temperature, and the density of the analyzed smoke. According to different data, it may be from 10 to 200 mg/m^3; the yield of phenols from 100 g of wood ranges from 50 to 5000 mg. This fraction includes compounds containing one, two, or three hydroxyl groups bound to the benzene ring, besides alkyl or ether derivatives, as well as those containing additional alcohol, aldehyde, acid, and ester groups. Therefore, they differ in water solubility, boiling point, sensitivity to oxidation, chemical reactivity, sensory properties, and antibacterial activity. Among the identified phenols, those present in the highest concentrations are syringol, guaiacol, pyrocatechol, phenol, and their various alkyl derivatives. The highest yield of phenols, especially of guaiacol and syringol and their derivatives, compounds that are essential for the sensory and preservative action of smoking, is at 400–600°C.

Aldehydes and ketones of smoke form a group of about 110 compounds, which includes also a number of aldehydealcohols, ketoalcohols, and ketoaldehydes. In the smoke from alder and fir wood, 28 and 34 carbonyl compounds, respectively, were identified (Borys 1978). Aliphatic and cyclic carbonyl compounds, as well as furan derivatives, are the products of pyrolytic degradation of cellulose and hemicelluloses, while aromatic carbonyls are formed from lignin. The total content of carbonyl compounds ranges from about 25 to 110 mg/m^3; thus it is similar to that of phenols. The carbonyls present in the highest concentrations are acetaldehyde, formaldehyde, and acetone; also, several O-heterocyclic carbonyls have been identified in wood smoke (e.g., furfuraldehyde and 5-hydroxymethyl-2-furaldehyde).

Wood smoke contains several aliphatic and aromatic alcohols, including methanol, ethanol, allylalcohol, n-amylalcohol, benzyl alcohol, and phenylethylalcohol. Methanol may be the substrate for the generation of formaldehyde and formic acid.

The group of carboxylic acids in wood smoke consists of about 30 various compounds. In the aliphatic fraction, the following acids were identified: acetic, propionic, isobutyric, butyric, crotonic, isocrotonic, valeric, isovaleric, heptanoic, caprylic, and nonanoic (Kłossowska 1979). Among the dicarboxylic acids are oxalic, malonic, fumaric, maleic, and succinic acids. Wood smoke also contains several ketocarboxylic acids. In the esters group, the methyl esters of formic, acetic, butyric, and acrylic acids were identified, as well as the benzoic acid ethyl ester.

One of the important groups of smoke constituents contains aliphatic and aromatic

hydrocarbons. In the fraction of about 20 aliphatic hydrocarbons, the compound present in the highest concentration is methane, known also as the product of dry distillation of wood. Much larger is the group of polycyclic aromatic hydrocarbons (PAH) identified in numerous investigations (Obiedziński and Borys 1977). Some smoked products contain up to 100 different PAH and their alkylated derivatives, of molecular weights from 116 (indene) to 302 (dibenzopyrenes). They are generated at temperatures above 420°C and, having high boiling temperature, are present mainly in the dispersed phase of smoke. Thus the contents of PAH can be decreased by reducing the wood smouldering temperature and filtering the smoke.

Smoke flavorings produced commercially for the food industry contain only traces of PAH. They are generally smoke extracts filtered and separated from the tars or distillates of pyroligneous liquids. Various flavorings of different brands are available as aqueous solutions or in free-flowing, dry form on salt, yeast, or other material.

Wood smoke also contains a number of other chemicals, including NO, NO_2, and NO_3, as well as various heterocyclic compounds, including the N-heterocyclic pyrrole, pyrazine, and carbazole.

Accumulation and Interactions of Smoke Components in Meats

Deposition on Smoked Surfaces

Due to Brownian motion, the smoke particles and droplets undergo coalescence and settle on the smoked products under the effect of gravitational and centrifugal forces. Their natural, electrostatic charge and absorption in the wet surface layer also contribute somewhat to deposition on the meat. Thus, the high humidity of the smoke increases the rate of smoking. On wet surfaces, the deposition of components present in the dispersing phase is more effective than that of the particles and droplets. A rise in the temperature of the smoke also accelerates sorption of these compounds by the meat being smoked.

The quantity of different smoke components accumulated in the meat depends on the temperature, humidity, agitation, and composition of the smoke; the properties of the components, particularly their volatility and solubility; the characteristics of the surface of the product; and the duration of smoking. Wet surfaces absorb about 20 times more phenols than dry ones. The published data on the total amount of smoke components absorbed by meat products are incomplete and vary within a broad range. The large range of values is caused both by the properties of the products and the parameters of smoking, as well as by differences in the analytical procedures applied by various investigators. The use of phenol as a standard in colorimetric determination of total phenols may lead to significant underestimation, since the amount of phenol in smoked meats is rather low. The content of phenols in different smoked sausages, according to published data, ranges from about 0.02 to 300 µg/g. In smoked pork belly strips and summer sausage, the total recovery of phenols was, according to Lustre and Issenberg (1970), about 280 µg/g and 60 µg/g, respectively. The composition of the absorbed fraction depends more on the conditions of smoking, especially the humidity of the surface of the meats, than on the concentration of individual phenols in the smoke. The quantity of formaldehyde in cold smoked goods may be as high as 20–40 µg/g. The amount of formaldehyde in different assortments of sausages may reach 2 to 50 µg/g; the surface layers of some products may contain about five times more than the inner layer.

The rate of deposition of smoke components can be greatly increased by applying high-voltage electrostatic precipitation, as in

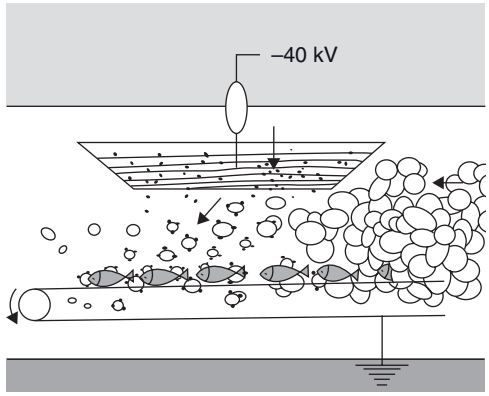

Figure 12.1. Artist's view of the principle of electrostatic smoking. (Courtesy of Paweł Kowalski.)

an electrostatic filter. If the meat is placed on a metal conveyor connected by a conducting wire to the ground, and a corona discharging wire electrode under high voltage is arranged nearby, the smoke particles in the space between the meat and the electrode get ionized and deposited under the effect of the electrostatic field (Fig. 12.1). At a proper distance between the product and the electrode, a thick, viscous layer of smoke components can be deposited in a few minutes. Usually, the installation works at about 20 to 60 kV at an electrode distance of 7 to 20 cm. In the conditions for electrostatic smoking, not only deposition of the dispersed, ionized smoke particles is accelerated, but also that of the components of the vapor phase. This happens because the rapid movement of the particles creates an "electrical wind," which increases the motion of the smoke toward the meat surface.

Diffusion and Interactions

The components deposited on the product and dissolved in the humid surface film gradually diffuse into the deeper tissues due to the concentration gradient. The rate of diffusion is controlled by the properties of meat and of the deposited compounds, as well as by the thermal conditions of smoking. In electrostatic smoking, the rapidly formed thick, sticky coat of smoke components is initially not fixed to the meat surface and can be easily removed (Fig. 12.1). However, during subsequent heating to the desired temperature, rapid penetration of the smoke components into the mass of the meat takes place. Most phenols accumulate on the skin, on the sausage casing, and under the surface at about a 6 mm deep layer, especially in the fatty tissue. However, in some products, even as much as 60% of the total mass of phenols can diffuse into deeper layers. Carbonyl compounds and acids are rather equally distributed throughout the mass of some smoked meat products.

The smoke compounds accumulated in the meat cannot be 100% recovered by extraction, since many of them interact chemically with the meat components. The proteins and lipids of meat contain various reactive groups, which in appropriate conditions, especially at high temperature, can react with phenols, carbonyl compounds, and acids of the smoke. The most reactive are the –SH, $–NH_2$, and $–NH–C(=NH)NH_2$ groups of amino acid residues in proteins and of nonprotein nitrogen compounds, as well as the polyenoic fatty acids and different reactive oxygen species and oxidation products like $^{\cdot}O_2^{-}$, $^{\cdot}OH$, $RO^{\cdot}$, $ROO^{\cdot}$, and ROOH. Smoke compounds may react with amino acids in meat products, leading to a significant decrease in the contents of amino acids in acid hydrolysates (Seuss 1986). Coniferaldehyde and sinapaldehyde present in the smoke condensates were not recovered from the smoked meats in the experiments of Lustre and Issenberg (1970), possibly due to their interactions with the meat proteins. A significant decrease in the contents of smoke carbonyl compounds caused by reactions with gelatine was shown by Ziemba (1969). High loss of available lysine in sausage casings due to smoking was found by Ruiter

(1979). In laboratory experiments, the smoke phenols have been shown to reduce significantly the concentration of thiol and amino groups in solutions of amino acids, peptides, and proteins and in meat. Such interactions may decrease the lysine availability somewhat. However, since the concentration of smoke components is the highest on the surface and in the thin outer parts of the smoked meat products, no significant decrease in the nutritional value of the meat proteins should be expected. Furthermore, the phenolic constituents of smoke absorbed by the sausage can be oxidized. The contents of guaiacol and phenol in smoked sausages stored 1 month may decrease by about 35%.

The Sensory Effects

Introduction

The desirable, sensory properties of smoked products result from the concerted action of salting or curing, seasoning, pre-drying, smoking, and heating, and in some cases also dyeing. The smoke compounds induce smoky color and flavor themselves and by interacting with the meat components, which results in the creation of other sensory-active substances. Interactions with the nitrogenous meat constituents may lead to some texture changes. The desirable intensity of sensory changes induced by smoking depends on the kind of meat products; some assortments are expected to acquire only a slight smoky note, while for others, mainly regional products, very heavy smoking must be applied to suit the typical consumer preferences.

The Color

The color developed on the surface of the products is due to the presence of colored smoke components and to the interactions of reactive compounds with those in the meat or sausage casings. The direct coloring role may be easily shown by exposing a plate of tin sheet to smoke in the high-voltage field of an electrostatic apparatus; in two or three minutes, the sheet turns yellow or brown. According to Ziemba (1969) and Ruiter (1979), a significant contribution to the formation of the color of smoked goods comes from the reactions of carbonyl compounds, mainly glycolaldehyde and methylglyoxal present predominantly in the vapor phase of the smoke, with the amino groups of proteins and nonprotein nitrogen compounds. The smoke phenols form stable colors in reactions with proteins at weak alkaline conditions.

The intensity J of the color of the smoked products is primarily related to the optical density of the smoke E_0 and the time of smoking τ:

$$J = kE_0\tau$$

The value of k increases with the rise in smoke temperature and velocity. High temperature favors the development of dark color, since it increases the concentration of the components of the dispersing phase of smoke and the rate of the carbonyl-amino reactions and polymerization of various components. The higher the temperature and water activity of the surface of small-caliber Brühwurst sausages, within limits set by other technological requirements, the darker is the color of the sausages. The kind of wood used for smoke generation is also important. Smoking with beech, maple, ash, sycamore, or lime-tree smoke leads to gold-yellow color; yellow-brownish tint comes from oak, nut, and alder smoke, and lemon-like from acacia smoke. Products treated with smoke from coniferous wood have dark coloration.

The Flavor and Taste

The smoke compounds are the dominant factor directly responsible for the smoky flavor. Smouldering wood smoke generated at 450–550°C is regarded as the most suitable for imparting the smoky flavor to smoked

meats. The products of thermal decomposition of cellulose and hemicelluloses are the result of carmelization and the source of fruity and floral scents, while the phenols generated by decomposition of lignin contribute the flavor associated with smoke, scorch, spices, vanillin, and clover. Various fractions of smoke condensates separated by chromatography reveal different flavors, including fruity, diacetyl-like, spicy, protein hydrolysate-like, or that of freshly baked bread. The desirable smoky flavor is associated with the presence of a mixture of syringol and 4-methylsyringol, although 4-allylsyringol, guaiacol, 4-methylguaiacol, and trans-isoeugenol also contribute to the typical sensory sensation. However, the multitude of variations of the smoky flavor is probably due to the contribution of the osmic effect of different carbonyl compounds and their reaction products, furans, esters, short-chain carboxylic acids, pyrazine and its derivatives, terpenes, and other unidentified constituents, as well as various products of interactions of smoke compounds and reactive meat constituents.

The smoky taste is a result of the sensory properties of smoke constituents, mainly numerous phenols and carbonyl compounds, as well as various products of the interactions with proteins and lipids. Some results of experiments point to the crucial role of the fraction of smoke condensates containing guaiacol and its four derivatives, eugenol, phenol, 3 cresols, 4-ethylphenol, 3 xylenols, tyglic acid, and 4 carbonyl compounds.

The Antimicrobial Activity of Smoke Components

The shelf life of smoked meats depends on the time and temperature of heating during the process, on decrease in water activity, and on the antibacterial and antioxidant activity of smoke components. Thus the preservative effect is related to the effectiveness of the heat pasteurization, loss of water during processing, concentration of salt, and composition and quantity of smoke deposited in the meat. Various products preserved by curing and heavy smoking may have a shelf life of even several months at room temperature, while mild treatment, as applied in manufacturing of some frankfurter-type sausages, yields products that can be kept only a few days under refrigeration. By smoking frankfurters 30 minutes at internal temperatures 60–76°C, the total number of aerobic bacteria may be reduced by about two log cycles; higher temperature and longer processing time is slightly more effective. Smoke components delay the growth of microflora in cold-stored frankfurters, whereby the effect increases with the smoking time. Natural smoking can retard the onset of greening of frankfurters caused by *Leuconostoc mesenteroides* during storage (Anifantaki et al. 2002).

Numerous smoke compounds (phenols, carboxylic acids, and formaldehyde) in concentrations similar to those in heavily smoked goods are effective antimicrobial agents. Their activity against various microorganisms at different stages of development is not equal. The phenols prolong the lag phase of bacterial growth proportionally to their concentration in the product. Therefore, the amount of the smoke components deposited on the meats during smoking has a significant influence on the preservative effect. Generally, hot smoking decreases the number of viable microorganisms in the products by one to two log cycles, whereby the effect increases with the rise in processing time and temperature. Among the most effective antimicrobial agents of wood smoke are: guaiacol and its methyl and propyl derivatives, creosol, pyrocatechol, methylpyrocatechol, 2,6-dimethoxyphenol, and pyrogallol and its methyl ether. Formaldehyde inhibits *Cl. botulinum* in concentrations of 40 μg/cm^3. Adding to raw minced beef 8% of liquid smoke, containing in 1 cm^3 about 1.4–4.0 mg of phenols and 20–70 mg of carbonyl com-

pounds, may reduce by two log cycles the number of viable cells of *E. coli* O157 H7 after 3 days at 4°C. This result, however, was shown at a very high concentration of the liquid smoke, 8%, while the recommended percentage is 1.5% (Estrada-Muñoz et al. 1998). Several strains of thermotolerant *Staphylococcus epidermis* do not survive commercial hot smoking on inoculated rainbow trout. In cold-smoked salmon, the growth of *Listeria monocytogenes* was found to be inhibited proportionally to the smoking time; 12 hours of smoking reduced the number of the population by three log cycles. However, well-adapted strains may persist in the smokehouse environment, so that *L. monocytogenes* can often be found in vacuum-packaged cold smoked salmon. The total concentration of smoke components present in lightly smoked vacuum-packed nonrefrigerated foods is not high enough to effectively prevent the formation of *Cl. botulinum* toxin.

Generally the vegetative forms of bacteria are most sensitive to smoke. Molds are considerably resistant. A large population of molds and yeasts may survive in frankfurters smoked 30 minutes at an internal temperature 67°C. Smoking has little effect on the yeast count in the early stages of manufacturing of fermented sausages; however, in stored samples the population of yeast is lower in smoked sausages than in unsmoked controls.

The Antioxidant Properties of Smoke Components

The antioxidant effect of smoking was noticed previously by observing that the lipids in smoked meats and fish were resistant to oxidation (Watts and Faulkner 1954). Among the smoke components that have the highest antioxidant activity are phenols; some of them are more effective than butylated hydroxyanisole (E 320) and butylated hydroxytoluene (E 321) when applied in similar concentrations. To the most active smoke phenols belong: pyrogallol, 3-methylpyrocatechol, 4-methylpyrocatechol, pyrocatechol, butylhydroxytoluol, resorcinol, hydroquinone, α-naphthol, 4-methylguaiacol, 4-vinylguaiacol, and 4-trans-propenylsyringol. The antioxidant properties of the phenolic fraction of wood smoke were already recognized about 50 years ago (Kurko 1963, 1966). Liquid smoke in a concentration of 1.5% was shown to effectively retard the lipid oxidation in precooked beef patties during 90 days of storage at −15°C (Estrada-Muñoz et al. 1998).

Possible Health Hazards Caused by Smoked Meats

The health hazards associated with the smoking of meat may be caused by carcinogenic components deposited from wood smoke: PAH, N-nitroso compounds, and possibly also heterocyclic aromatic amines.

Most of the PAH contained in wood smoke have a molecular weight below 216 Da, and they are regarded as noncarcinogenic. However, smoke also contains highly carcinogenic or mutagenic PAH (Table 12.1). Very mutagenic and carcinogenic is benzo(a) pyrene (BaP). It has a molecular weight of 252 Da and has been chosen as the indicator PAH, representing the other carcinogenic hydrocarbons in wood smoke and smoked products. By applying the principle of toxic equivalency factors (TEF), it is possible to estimate the total equivalent exposure (TEQ) to PAHs in various smoked meat products

Table 12.1. Polycyclic aromatic hydrocarbons regarded as potentially genotoxic and carcinogenic for man

Benz [a]anthracene	Dibenzo[al]pyrene
Benzo[b]fluoranthene	Dibenz[ah]anthracene
Benzo[j]fluoranthene	Indeno[1,2,3-cd]pyrene
Dibenzo[ae]pyrene	Benzo[k]fluoranthene
Benzo[ghi]perylene	Dibenzo[ai]pyrene
Dibenzo[ah]pyrene	Chrysene
Benzo[a]pyrene	5-Methylchrysene
Cyclopenta[cd]pyrene	

relative to BaP. The proposed TEF for BaP and dibenzo(a,h)anthracene is 1, for benzo(a) anthracene, benzo(a)fluoranthene, indeno (1,2,3-c,d)pyrene, and benzo(k)fluoranthene it is 0.1, for chrysene and fluoranthene 0.01. Among PAH isolated from smoked products are mainly compounds of m.w. smaller than 216. In different smoked meat products, their total mass may be from about 30 to 250 times larger, while that of the heavy PAH about 10 times larger than that of BaP. If the specific carcinogenicity of various heavy PAH contained in smoked foods is taken into consideration, the total carcinogenicity of all PAH is about 10 times higher than would result from the content of BaP alone (Scientific Committee on Food 2002). Although many PAH are regarded as not carcinogenic, some of them may function in living organisms as synergists, increasing the carcinogenicity of other PAH.

German regulations in force since 1973 require that the content of BaP in smoked meat products not exceed 1 ng/g. In about 75% of market samples of meat products in Germany, the contents of BaP were not found to be higher than the limiting value, but in about 1% of investigated samples, even as much as 40 ng/g were present. The actual European limit of BaP in smoked meats and smoked meat products is 5 ng/g wet weight (Commission Regulation (EC) No 208/2005). Hot smoked sausages and smoked beef spreads usually contain below 1 ng/g, but some black smoked products even have as much as 55 ng/g. According to data reviewed by Simko (2002), the content of BaP in 10 different kinds of smoked meat and meat products ranged from 0.03 to 1.2 ng/g and was 17.1 ng/g in dark smoked meat products. In 1 g of flame-grilled sausages, 18 to 42 ng BaP were found. This could be compared with the contents of BaP in barbecued pork and beef: 1.5–10.5 ng/g, and in charcoal-broiled steaks 5–8 ng/g. Traditionally smoked fish may contain from about 1 to about 60 ng of BaP/g of product, depending on the method of smoking, the quality of smoke, and the protection of the edible parts by the skin. The external parts of the fish exposed to the smoke, especially the skin of eel, may contain up to five times more BaP than the flesh. In the edible parts of fish smoked in a modern automatic kiln with external smoke generation, the contents of BaP are about 0.1 ng/g.

Some smoked foods may also be contaminated with nitropolycyclic aromatic hydrocarbons. In smoked sausages, 1-nitropyrene, 2-nitronaphtalene, and 2-nitrofluorene were found in concentrations of about 4.2, 8.4, and 19.6 ng/g, respectively, while in roasted coffee beans the concentrations were 2.4, 4.0, and 30.1 ng/g.

Cured meats and bacon, as well as smoked cured meat products, contain several N-nitroso compounds, most of which are carcinogenic in laboratory animals. The contents of N-nitrosoproline, N-nitrosohydroxyproline, and N-nitrosodimethylamine in smoked cured mutton after cooking reached up to 230, 500, and 2.2 ng/g, respectively (Dennis et al. 1984). The aldehydes of smoke can react with cysteamine and cysteine, yielding various thiazolidine precursors, which can be easily nitrosated. Formaldehyde reacting with cysteamine and cysteine yields thiazolidine and thiazolidine-4-carboxylic acid, respectively, which, upon nitrosation, turn into N-nitrosothiazolidine and N-nitrosothiazolidine-4-carboxylic acid. In traditionally smoked fried bacon, the content of N-nitrosothiazolidine may be about 5 ng/g (Ikins et al. 1986). In the presence of glycolaldehyde from smoke, 2-(hydroxymethyl)-N-nitrosothiazolidine and 2-(hydroxymethyl)-N-nitrosothiazolidine-4-carboxylic acid (HMNTCA) may be formed. In various cured smoked products, including smoked ham, sausages, salami, pepperoni, and smoked poultry products, the contents of HMNTCA ranges from about 10 to 260 ng/g (Sen et al. 1993). Generally, these compounds occur in higher concentrations in

meat products smoked in traditional smoking ovens than in meats processed in modern smokehouses. The total amount of various N-nitroso compounds in smoked fried bacon, some of which are still unidentified, has been reported to be 430–6800 ng/g.

Heterocyclic aromatic amines, known to be generated due to pyrolysis of amino acids and proteins and in nonenzymatic browning, may be found in very heavy smoked goods in amounts lower than 1 ng/g.

The Equipment for Smoking

Introduction

The implements used for smoking meat and fish have been gradually improved during ages of development from very primitive burrows or huts with a fireplace on the ground to modern installations controlled electronically. However, in some parts of the world, the most ancient procedures and equipment may still be in use. In contemporary industrial smoking, high attention is paid to the temperature of smoke generation, proper circulation of the smoke and drying air in order to achieve the required degree and uniformity of smoke deposition, water evaporation, and heating, as well as to control all process parameters affecting the quality of the products. In accordance with the requirements of hygiene and environmental protection, the industrial installations also include the necessary gear for efficient cleaning and for neutralization of the spent smoke. The main components of smoking equipment are the generators of smoke and the smokehouses.

Smoke Generators

Smoldering-Type Generators

In primitive, usually small-scale or artisan processing, the smoke is produced in a fireplace directly below the meat, which is hung above it on spits or laid on a mesh. A fire is made of wood logs, and the burning logs are covered with a layer of damp sawdust or wood chips to keep the flame down and cause smoldering of the woody material. Here the control of the quantity and quality of the smoke depends totally on the kind of sawdust, the experience of the operator, and the weather conditions. Increasing or decreasing the volume of air entering into the fireplace by widening or shutting the inlet openings can adjust the smoldering temperature only coarsely. The smoke produced in such conditions may be high in PAHs. This principle is applied also in industrially manufactured generators, in which there are several boxes connected to one smoke duct. Here the uniformity of smoke output can be better assured by proper, simultaneous controlling of the air inlets to the boxes.

In industrial smoking, generators are most often used, in which the sawdust or wood chips are automatically fed onto a plate or grate heated to a controlled temperature of about 350°C. The air needed for smoldering is blown from below the plate. The temperature in the layer of sawdust depends on the temperature of the heated grate and on the volume of supplied air. When small mesh sawdust is used, the flow of air may be hindered and the temperature tends to be below that of the plate. Such smoke is rich in CO. Adding wood chips to the pile increases the temperature and makes the smoke richer in phenols. However, in the presence of too large chips, the development of smoke may be disturbed by the occurrence of flame.

Friction-Type Generators

The principle of action of different constructions of these generators depends also on thermal degradation and partial oxidation of woody material. However, the temperature needed to initiate the process results from friction of a log pressed at about 1 kg/cm^2 against a rotating drum or disk (Rasmussen 1956). Access of air is possible due to perfo-

rations in the rotor. The temperature in the friction zone may be controlled by adjusting the pressure applied to the log and the speed of rotation of the rotor. It is usually 300–350°C. The smoke is blown into the smoke duct by a fan that may be fixed on the shaft of the rotor. Because of comparatively limited access of air to the friction zone and low temperature, the smoke contains fewer products of thermal degradation and oxidation of lignin than that from the earlier described generators. The assets of the friction-type machine are low consumption of wood and the fact that the smoke production can be started and stopped instantaneously. However, because it makes a lot of noise during operation and needs electrical power for driving the rotor, it is not very often used in the industry.

Other Types of Generators

Thermal degradation of wood can also be accomplished by overheated steam; this takes place in the steam smoke generators covered by numerous patents (Fessman 1971). The sawdust is fed by a worm feeder into a reactor formed by a tilted pipe with perforated walls. Overheated steam at temperatures of usually about 200°C is blown through the perforations into the sawdust-filled reactor. At this considerably lower temperature, lignin does not undergo thermal degradation, and the smoke is rich in carboxylic acids and carbonyl compounds but relatively poor in phenols and polycyclic aromatic compounds. In some of these generators, additional sections of the reactor serve to oxidize the volatile decomposition products by oxygen-enriched air.

Wood smoke can also be produced by blowing a stream of hot air or air/inert gas mixture countercurrently across a bed of sawdust that is being fed at a controllable rate into a fluidization chamber that has the form of a truncated cone (Nicol 1962). The temperature of the fluidized bed of sawdust or chips of low mesh size is kept below 400°C to prevent self-ignition. The composition of the smoke can be controlled by modifying the content of oxygen in the air mixture. Self-ignition of the fluidized sawdust bed may be prevented even at a temperature as high as 750°C if the concentration of oxygen in the gas mixture is reduced to 6% (Balejko and Miler 1988). The quality of the smoke produced in a fluidized bed at such a high temperature is equal to that from a smoldering-type generator.

Smokehouses

Introduction

Meats and meat products can be successfully smoked in very simple devices (e. g., in a barrel inverted over a pile of smoldering sawdust, fitted with supports for spits, rods, or wire mesh, and outlets for the spent smoke). Here, as well as in smoking ovens, but also in kilns or tunnels supplied with smoke from generators, the quality of the products is affected by the duration of the process, the properties of the smoke, and the conditions of heat and mass transfer. The same factors are also important in smokehouses equipped with atomizers of liquid smoke preparations. Additionally, in smoking and steaming chambers, the parameters of the heating steam have to be considered, as do the effects of the high-voltage electrostatic field in electrostatic smoking units.

Smoking Ovens

Smoking ovens, generally built as a cross section of a rectangle, about 1 m wide, 1.2 m deep, and 2 m high, have stony floors and fireproof brick walls. On the side walls, there are two or three pairs of supporting rails at appropriate distances to allow for sliding the frames on which the meat products hang from spits or rods. On the front of the oven there are three doors: two small ones at the

bottom and top to handle the fireplace and enable the access of air and to control the airflow and humidity, respectively. The third, a large door in the middle, is used for manual loading and unloading of the frames, as well as for observing the process. A duct to the chimney connects the coned ceiling. Its coned shape assures that the water and smoke condensate does not drop on the meats but drains down the walls. The smoke duct is equipped with a shutter to control the flow. In order to produce smoked meats of fairly standard quality, the operator may have to rearrange the frames at various distances from the fireplace during smoking to counteract the effect of the differences of temperature, draft, and humidity on the cross section of the oven. Smoking in such conditions is a labor-intensive task if no mechanization of the handling of the loaded frames is available. The process may be improved if two smoking ovens, or batteries of ovens, are arranged so that their common back walls can be lifted. In such an arrangement, the loading and pre-drying of the product takes place in the front row of ovens. After lifting the back wall, the meats are pushed into the back row for further heating, smoking, and finally unloading. This principle may be applied also in operating other types of smokehouses.

Advanced types of smoking kilns, still with a fireplace in the bottom, may extend over two stories of a building. The drying, heating, and smoking conditions in the lower and higher sections of such ovens differ significantly, which is convenient for processing different assortments of meat products.

Smokehouses with External Smoke Generators

Modern smokehouses (Fig. 12.2) make possible full application of the principles of food

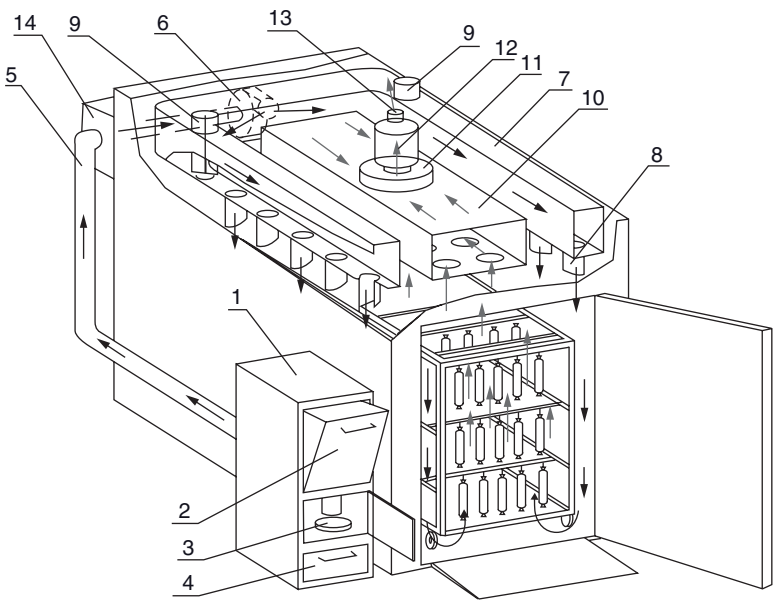

Figure 12.2. A smokehouse with external smoke generator. (1) Smoke generator, (2) sawdust container, (3) electric heater, (4) ashtray, (5) smoke duct, (6) inlet fan, (7) smoke-distributing channel, (8) jet, (9) throttle, (10) smoke inlet collector, (11) outlet fan, (12) afterburner, (13) smoke outlet passage, (14) heater. (Courtesy of Jerzy Balejko.)

engineering regarding heat and mass transfer. They are supplied with smoke of standard quality from an external generator and are heated by steam, gas, or electricity. Sawdust and chips of wood of various species of trees, standardized water content, and mesh size are available commercially. The air and smoke flow or circulation is forced mechanically at controlled velocity. The temperature, humidity, and density of the air/smoke, as well as the process time, are adjusted according to a computer program to requirements depending on the desired properties of the meat products. The smoke is often filtered or conditioned under a water spray to control its temperature and humidity, and to separate some tar fractions and soot. In smokehouses working in a half-open system, the smoke is circulated until its density drops below a critical level. At that point, it is discharged into the chimney, and new smoke from the generator is fed into the kiln. In a closed system, the smoke circulates within the kiln during the whole cycle of smoking, and afterward, a stream of air forces out its residues. The closed system bears the risk of self-ignition of the smoke, which may contain CO at a concentration that is too high.

For smoking with smoke preparations or flavorings, the same smokehouses may be used as in the conventional process. However, additional equipment must be installed for atomization or vaporization of the smoking liquids. Atomization nozzles in the smokehouse create a cloud of smoke droplets in the range of 100 µm, while the smoke flavoring sprayed onto a heated plate turns into vapors. For processing cooked sausages, hot-water shower or steam injection systems must be fitted, so that smoking and cooking can be carried out concurrently in the same kiln.

In modern smokehouses, as well as in advanced types of smoking ovens, the material to be smoked is usually loaded into the smoking chamber on trolleys. Trolleys or conveyors are used also to transport the meat products within tunnel smoke houses, which are designed to operate in a continuous system. In such tunnels, the meats to be smoked are carried through consecutive sections, in which appropriate parameters of temperature, smoke density, humidity, and flow rate are maintained.

For electrostatic smoking, the smokehouse is additionally equipped with a high-voltage section, where the smoke deposition takes place within a few minutes (Sikorski 1962, Tilgner and Sikorski 1962). Because the length of treatment is so short, the density of smoke has to be kept very constant in order to assure a uniform degree of smoking of the product. Electrostatic deposition may also be applied in smoking ovens in which smoke preparations are used instead of smoke. The other phases of the process (i.e., pre-drying and cooking) proceed as in conventional smoking.

Additional Equipment

To reliably operate a smokehouse, several instruments are necessary for measuring and controlling the temperature, relative humidity, and flow rate of the smoke and the temperature in the meat products. Similar instruments are used for control of other processes in the food industry. The density of the smoke, a crucial parameter of smoking, can be easily determined by photoelectric measurement of the intensity of a light beam transmitted through a layer of smoke.

The spent smoke and other exhaust gases, after leaving the smokehouse, should be cleaned before entering the atmosphere. Depending on the contents of the polluting components, different equipment may be used. Some installations comprise three sections: an electro filter, a fibrous filter, and activated charcoal. Other systems use afterburners to oxidize the components of the spent smoke at 800–1500°C. In the presence of catalyzers, the temperature may be reduced to 600°C.

Many smokehouses are equipped with installations for automatic cleaning that are available as standard units. Alkaline detergents may be used for the efficient removal of smoke deposits and tar.

Typical Procedures for Smoking Meats

Meat may be smoked in the raw state or after previous salting, marinating, cooking, or other treatment, which may also be followed by other processing. However, in industrial practice, meat and meat products are usually smoked after salt curing, as described in chapter 6. The pre-treatment of the raw material and the conditions of smoking, mainly the humidity and fat content of the surface layer of the sausage, the temperature, humidity, and density of the smoke, and the duration of the process, affect both the characteristic sensory properties and the shelf life of the products.

Many smoking procedures are used in the industry and in artisan meat processing. They lead to very different sensory properties and shelf lives of various products. In these procedures, the impact of drying, heating, and treating with smoke on the quality of the products may be very different.

Cold smoking is used in manufacturing raw, fermented sausages, made from cured meats. The smoke, at 12–25°C and controlled humidity, is applied for between several hours to about 16 days, depending on the assortment. The loss of water due to drying and the impregnation with smoke components should be equal on the whole cross section of the product. The surface of the freshly cut sausage should be light brown to dark brown, depending on the duration of the process. In smoking salami, the links are first surface dried one day at 12°C in low-density smoke. This is followed by five days of smoking in dense smoke at 15–22°C, and by the last phase of two days in somewhat colder and less dense smoke. After smoking, the salami is ripened for two to three months, as described in chapter 22.

Warm smoking is carried out at 23° to 45°C and relative humidity of 70%–80% for 4–48 hours. The pre-drying and smoke penetration is restricted mainly to the outer layers of the product. It is usually followed by cooking or baking. In smoking frankfurters, the first phase is a tempering period at 32–38°C, aimed at removing the surface moisture to ensure uniform coloring. Smoking proper, lasting usually about 1–1.5 hrs in dense smoke of controlled humidity, brings the internal temperature of the sausages to 60–68°C and imparts a smoky color and flavor. This is followed by cooking in a hot-water spray or steam and by chilling. In smoking cooked sausages, too high temperature may lead to excessive fat and weight loss and thus to creased surfaces of the sausages and nonuniformity of color. Smoking at a temperature not exceeding 40°C is used also for preparation of salted, spiced, dried pork back fat.

In hot smoking, the first stage (lasting about 30 minutes, without smoke, at 40–50°C) results in pre-drying of the surface and is followed by several stages of smoking in dense, hot smoke (at temperatures reaching 85°C) and further surface drying. In manufacturing jagdwurst, the links are kept 2 hours for settling at about 30°C, surface-dried at 40–60°C, smoked about 80 minutes at 45–80°C, dry-heated at 85°C during 25 minutes to reach internal temperature 68–72°C, smoked again at 30°C during 12 hours to a dark brown surface color, and dried at 14–18°C during 5–7 days to a water content of 55%–57% in the product. In manufacturing other assortments, the thermal changes in the meat products are caused by dry heating at about 90°C, steam cooking in the smokehouse, or cooking in water. The internal temperature of the product should be 68–72°C.

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Chapter 13

Meat Packaging

Maurice G. O'Sullivan and Joseph P. Kerry

Introduction

The packaging of muscle-based foods is necessary to ensure that such products reach the consumer in a condition that satisfies his or her demands on a number of levels, namely: nutrition, quality, safety, and convenience, as well as the ability to deliver a product shelf life that will endure the stresses of handling, transportation, storage, sale, and consumer contact. However, in order for such products to be truly commercially successful, consumer desires and demands must be addressed and met with respect to the sensory properties of such products, before other quality dimensions become relevant (Chambers and Bowers 1993). The three sensory properties by which consumers most readily judge meat quality are: appearance, texture, and flavor (Liu et al. 1995). Each food product category presents its own unique challenges in this regard, and meat is no different. Unfortunately, fresh meat color is short-lived and surface discoloration that occurs during chilled and frozen storage is considered a sign of unwholesomeness and product deterioration (Faustman and Cassens 1990). The bright, cherry-red color of fresh beef is used by consumers as an indicator of meat quality (Cassens et al. 1988; Kennedy et al. 2004). In red meats, consumers relate the bright red color to freshness, while discriminating against meat that has turned brown in color (Hood and Riordan 1973; Morrissey et al. 1994). It is because of such sensory quality changes in fresh meat that so much attention has focused on developments within the area of packaging technologies, especially in the last twenty to thirty years. In the case of beef, two important visual clues that determine perceived quality are color and packaging (Issanchou 1996).

There are four categories of preservative packaging that can be used with raw muscle foods. These are vacuum packs (VP), high oxygen modified atmosphere packs (high O_2 MAP), low oxygen modified atmosphere packs (low O_2 MAP), and controlled atmosphere packs (CAP) (Gill and Gill 2005). Over the past number of years, much research has focused on the influence of modified atmosphere packaging (MAP) on meat quality attributes and the purchasing preferences of consumers (Carpenter et al. 2001; Jayasingh et al. 2002). Discoloration in retail meats during display conditions may occur as a combined function of muscle pigment oxidation (oxymyoglobin to metmyoglobin) and lipid oxidation in membrane phospholipids (Sherbeck et al. 1995). MAP is one of the principle methods of maintaining and prolonging meat color sensory quality.

High O_2 concentrations promote the oxymyoglobin (OxyMb) cherry red form of myoglobin (O'Grady et al. 2000) but may impact negatively on the oxidative stability of muscle lipids and lead to the development of undesirable flavors (Rhee and Ziprin 1987; Estevez and Cava 2004). The breakdown products of lipid oxidation have been associ-

ated with the development of off-flavors and off-odors and loss of color in meat (Faustman and Cassens 1989).

A variety of packaging systems and technologies are currently available for muscle foods. Fresh red meats may simply be placed on trays and over-wrapped with an oxygen-permeable film, or placed within a gaseous-modified atmosphere. As the meat industry moves toward central processing that employs MAP and vacuum packaging, they may need to overcome consumer preference for fresh beef that is bright red in color and packaged with the traditional PVC over-wrap (Carpenter et al. 2001).

Finally, the changing faces of ecologically friendly packaging require the addressing of multiple aspects of packaging, including recyclability, simple packaging, reusable, refillable, renewable materials, less materials, less or no plastics, and bulk rather than individual packaging (Doyle 2008). Also, the noncontact preservative effect of active packaging offers the opportunity to producers of prolonging shelf life further, while maintaining the clean label status of meat products.

Modified Atmosphere Packaging of Meat Products

MAP is defined as "a form of packaging involving the removal of air from the pack and its replacement with a single gas or mixture of gases" (Parry 1993). MA packs usually contain mixtures of two or three gases: O_2 (to enhance color stability), CO_2 (to inhibit microbiological growth), and N_2 (to maintain pack shape) (Sorheim et al. 1999; Jakobsen and Bertelsen 2000; Kerry et al. 2006). An example of MA packed meat is presented in Figure 13.1. The capacity for such gases to promote the overall quality of fresh red meat is well established (Gill 1996). Beef steaks are commonly displayed under high oxygen concentrations in MAP in order to promote color stability (Zakrys et al. 2008). The color of lamb may also be extended by storage under MAP conditions (Kerry et al. 2000).

MAP has now been available to producers for many years. As far back as 1933, Killefer (1930), using 100% carbon dioxide (CO_2) at 4–7°C, found that pork and lamb remained fresh for twice as long as equivalent products

Figure 13.1. Modified atmosphere packed meats, beef burgers, and beef steak. Gas mixtures 80% oxygen and 20% CO_2.

stored in air and held at similar temperatures. Also, the shelf-life extension of bacon by packaging in CO_2-enriched atmospheres was investigated by Callow in 1932 (Callow 1932). Additionally, in the 1930s a carbon dioxide-enriched environment was employed to transport refrigerated beef carcasses from Australia and New Zealand (Floros and Matsos 2005). The retail use of MAP did not occur until the 1950s, in the form of vacuum packaging (Floros and Matsos 2005). In 1981, Marks & Spencer introduced to the United Kingdom gas-flushed fresh meat in plastic trays (Inns 1987). It is now used ubiquitously across the meat industry for many different meat products. As previously stated, MA packs usually contain mixtures of two or three gases.

The use of high O_2 concentrations in MA packs promotes oxymyoglobin (OxyMb) formation, the cherry red form of myoglobin (O'Grady et al. 2000). Packaging beef in MA packs and storing at low temperatures extends the product shelf life considerably (Young et al. 1983). Beef and lamb are both red meats and share similar properties, but considerable differences in shelf lives are apparent between them due to their relative susceptibility to chemical and microbial spoilage. In contrast to beef cuts, much of the surface of lamb is adipose tissue, which has a pH close to neutrality and has no significant respiratory activity (Robertson 2006). The pH of beef is lower than that of lamb, thus making it less susceptible to microbial spoilage (Gill 1989; Kerry et al. 2000). In order to optimize shelf life, sensory quality, and microbiological safety using MAP, the packaging system applied must be product specific (Church and Parsons 1995).

High O_2 MAP Meat Packs

The vast majority of meat products have been and continue to be offered in high oxygen pack formats (approximately 80% O_2) in order to maintain bloom, with at least 20% CO_2 to prevent selective microbial growth (Eilert 2005). Whether these gases were placed in the primary package or in a master bag surrounding the primary package, the basic technology has been unchanged for a number of years. This technology has been successful for a number of larger retailers, as the shelf life provided by this package has been sufficient for usage in a controlled distribution system (Eilert 2005). High O_2 MA packs contain atmospheres of O_2 and CO_2, and often N_2. Mixtures of O_2/CO_2 have been used commercially for a considerable time (Brody 1970). A patent in 1970 specified a range of O_2 and CO_2 concentrations suitable for MAP beef (Georgala and Davidson 1970). Results demonstrated that at least 60% O_2 is required to achieve a color shelf life of 9 days, and the patent claims that a mixture of 80% O_2 plus 20% CO_2 keeps meat red for up to 15 days at 4°C (Georgala and Davidson 1970). Typically, fresh red meats are stored in MAP containing 80% O_2:20% CO_2 (Georgala and Davidson 1970), while cooked meat equivalents are stored in 70% N_2:30% CO_2 (Smiddy et al. 2002).

Beefsteaks are commonly displayed under high oxygen concentrations in MAP in order to promote color stability (Zakrys et al. 2008). As previously stated, the major function of O_2 is to maintain the muscle pigment myoglobin in its oxygenated (oxymyoglobin) form (Kerry et al. 2006), but high oxygen levels within MAP also promote oxidation of muscle lipids over time (O'Grady et al. 1998). These high O_2 levels may also impact negatively on the oxidative stability of muscle lipids and lead to the development of undesirable flavors (Rhee and Ziprin 1987; Estevez and Cava 2004). This distinctive off-flavor develops rapidly in meat that has been precooked, chilled-stored, and reheated. The term warmed-over-flavor (WOF) has been adopted to identify this flavor deterioration (Renerre and Labadie 1993). Membrane phospholipids are particularly susceptible to

oxidation processes, thereby causing the rapid development of meat rancidity (Renerre 1990). The oxidation of polyunsaturated fatty acids not only causes the rapid development of meat rancidity, but also affects the color, the nutritional quality, and the texture of beef (Kanner 1994).

High O_2-MAP increases lipid oxidation in meat: beef (Jakobsen and Bertelsen 2000; Zakrys et al. 2008; Zakrys et al. 2009), pork (Lund et al. 2007), and lamb (Kerry et al. 2000). High-oxygen atmospheres (80% O_2) also promote pigment oxygenation, and therefore, prolong the time before metmyoglobin is visible on the muscle surface. The drawback to high O_2 MAP is that although it maintains redness during storage, rancidity often develops in the meat while color is still desirable (Jayasingh et al. 2002). Because consumers use meat color as an indicator of freshness and wholesomeness, recent advances in MAP have focused on finding the correct blend of gases that maximizes initial color, color stability, and shelf life, while also minimizing microbial growth, lipid oxidation, and gaseous headspace (Mancini and Hunt 2005). Jakobsen and Bertelsen (2000) reported that while O_2 levels higher than 20% were necessary to promote meat color, package O_2 contents higher than 55% did not result in additional color stabilizing benefits.

High O_2 concentrations can cause protein oxidation, which has been linked to increased toughness in MAP meat, particularly beef. Thus, protein oxidation may decrease eating quality by reducing tenderness and juiciness, and enhancing flavor deterioration and discoloration (Xiong 2000). Zakrys et al. (2008) showed that high O_2 concentrations in MAP-stored beefsteaks were shown to have increased toughness scores after cooking, as determined by 134 consumers (Fig. 13.2; Zakrys et al. 2008).

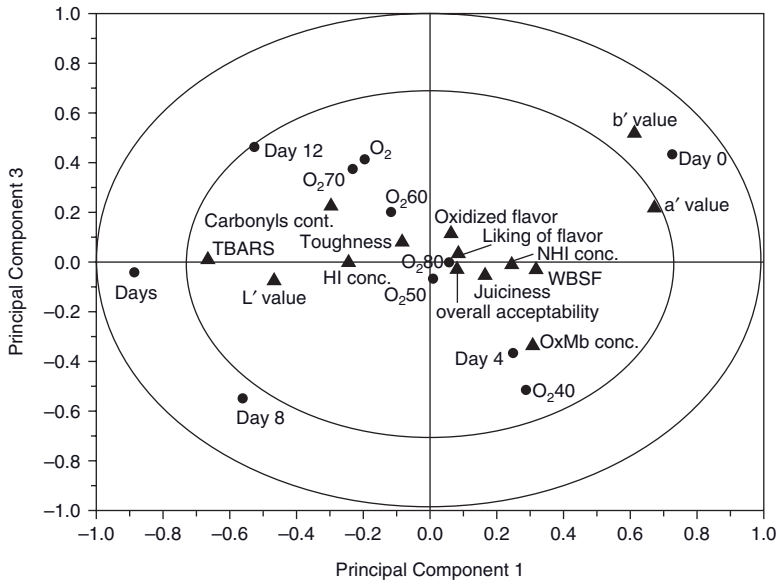

Figure 13.2. An overview of the variation found in the mean data from the ANOVA-partial least squares regression (APLSR) correlation loadings plot for each of the 5 MAP treatment groups: 40%, 50%, 60%, 70%, and 80% oxygen, with all packs containing 20% CO_2 and the make-up gas N_2. Shown are the loadings of the X and Y variables for the first 3 PCs for raw data. • = days and MAP treatments, ▲ = sensory descriptor and instrumental variables. The concentric circles represent 100% and 50% explained variance, respectively. (Adapted from Zakrys et al. 2009.)

Carpenter et al. (2001) showed that consumer preference for beef color was sufficient to influence their likelihood to purchase, but was not enough to bias taste scores. It is likely that once a decision to purchase beef is made in the market, whether the beef is presented in the form of cherry red fresh-bloomed beef, the brown of discounted beef, or the purple of vacuum-packaged beef, consumer eating satisfaction at home will depend only on the beef quality attributes of tenderness, juiciness, and flavor (Carpenter et al. 2001).

Low O_2 MAP Meat Packs

Low O_2 packaging systems have been readily available for usage in the United States, but are not as widely implemented as their high O_2 counterparts (Eilert 2005). Low O_2 MAP are generally packed with CO_2 (usually enough to dissolve into the product) and also N_2, while residual O_2 may be present or included during the packing process. The CO_2 acts as the antimicrobial and N_2 as the pack shape stabilizer (Sørheim et al. 1997). For Low O_2 MAP in the United States, carbon monoxide (CO) may also be used as a gas for meat color enhancement. Within the EU, only Norway adopted the use of CO (0.3–0.5%) in primary packs in the mid 1980s; however, this practice has since ceased, following a decision by the EU Parliament committee in 2004 not to allow the use of CO in meat packaging applications (Sørheim 2006).

Industrially, CO has been added to packages to eliminate the disadvantages of commercial ultra-low O_2 MAP, because CO has a high affinity for myoglobin and forms a bright cherry red color on the surface of beef (Sørheim et al. 1999; Luno et al. 2000; Jayasingh et al. 2001; Hunt et al. 2004). CO is a colorless, odorless and tasteless gas. It is produced mainly through incomplete combustion of carbon-containing materials (Sørheim et al. 1997). Hunt et al. (2004) concluded that the use of 0.4% CO during storage in MAP improved beef color without masking spoilage. Upon removal of product from CO packaging, meat color (likely to be a combination of COMb and OMb) deteriorated during display in a manner not different from product exposed only to air. Thus, the inclusion of 0.4% CO in conjunction with O_2 will not influence color stability, metmyoglobin-reducing activity, or O_2 consumption. This is likely the result of greater formation of oxymyoglobin (oxyMb) in atmospheres containing 20–80% O_2, which dominates or limits the ability of carboxymyoglobin (COMb) to form (Seyfert et al. 2007). COMb is more resistant to oxidation than oxymyoglobin, owing to the stronger binding of CO to the iron-porphyrin site on the myoglobin molecule (Wolfe 1980). However, one of the main consumer fears relating to the use of CO is the possible loss of quality due to a break in the cold chain, causing deterioration in spite of its attractive appearance (Wilkinson et al. 2006). Concern has been expressed in the United States in the past that such a system would mask spoilage that could occur in fresh meat products (Eilert 2005). The FDA noted that while color did not degrade in a package containing CO, offensive odors could still form normally in the product in the presence of CO (FDA 2004). Although there are distinct advantages for the storage and display life of meat with CO in VP or low O_2 MAP, consumers have a negative image of CO because of its hazardous nature and the concern that products may appear fresher than they actually are (Cornforth and Hunt 2008). The declaration of CO for meat as generally recognized as safe (GRAS) in the United States has a legal basis (Boeckman 2006). The use of CO in the primary package of fresh meat in the United States is a major breakthrough. This will allow for the wider distribution of case-ready products and adequate shelf life needed to achieve distribution of these products (Eilert 2005).

Controlled Atmosphere Packaging of Meats

The storage life of chilled meat can be extended by packaging the product under controlled atmosphere packaging (CAP) with N_2 or CO_2 (Gill and Molin 1991). The absence of O_2 in an O_2-free MAP or CAP system results in a significant shelf-life extension, as these packaging formats offer hostile environments to obligate aerobic spoilage microorganisms. CAP packaging has been used commercially for the shipment of chilled lamb to distant markets (Gill 1990).

However, these packaging systems initiate the development of metmyoglobin in the meat, which is unattractive to the consumer (Hunt et al. 1999). The meat will bloom to an attractive bright red color shortly (20–30 min) after opening the pack and exposing the meat to air. Another negative attribute associated with these packaging formats is that the high usage of CO_2 may cause off-flavor or CO_2 taint in the meat, which can be detected upon consumption (Nattress and Jeremiah 2000). CO_2 is highly soluble in water, most of which is contained in the muscle, and also in fat tissue. This solubility is increased with decreasing temperature. When an atmosphere rich in CO_2 is used, the high solubility of the gas in meat tissues must be taken into account (Gill 2003). In an atmosphere of 100% CO_2, meat will absorb approximately its own volume in gas. Thus, the initial gas volume must exceed the required final volume by the volume of the enclosed meat (Gill 2003). When high CO_2 levels are applied in a package headspace, the concentration of CO_2 will decline due to absorption of CO_2 in the meat. CO_2 dissolves in meat until saturation or equilibrium is reached. CO_2 is also suspected of affecting the chemical quality of the meat (Jakobsen and Bertelsen 2002). A lowering of meat pH is a result of CO_2 absorption into the meat and is a consequence of carbonic acid being dissociated to bicarbonate and hydrogen ions (Dixon and Kell 1989). The absorption capacity is related to biological factors (i.e., pH, water, and fat content) (Gill 1988; Jakobsen and Bertelsen 2002), but also to a large extent to packaging and storage conditions, specifically CO_2 partial pressure, headspace to meat volume ratio, and storage temperature (Jakobsen and Bertelsen 2002; Zhao et al. 1995). O'Sullivan et al. (2010) used sensory panelists to assess the preference of steaks packed under atmospheres containing 50% O_2 (50 CO_2), 70% O_2 (30 CO_2), 80% O_2 (20 CO_2), or 100% CO_2. The principal aim of this study was to explore off-flavors developed by CO_2 in commercial MA packs as well as 100% CO_2. Samples were tested by assessors after immediate cooking, upon removal of the respective packaging, and a second identical sample set was served with samples left for 30 minutes in ambient air to let any CO_2 dissipate prior to cooking. From sensory analysis, panelists had a preference for steaks packed under atmospheres containing 50% O_2. The 50% O_2 packed treatments displayed a significant ($P \leq 0.05$) and negative correlation with CO_2 flavor, and this was even more pronounced for samples where the CO_2 was allowed to dissipate (Dis, $P \leq 0.001$). There also appeared to be a directional correlation of the 100% CO_2 samples to CO_2 flavor, although these results were not significant (Fig. 13.3). All other treatments proved to be nonsignificant. One explanation for this may be the leanness of the meat used in this study, which had a very low fat content. In addition, the meat purchased was very stable in terms of composition, with no significant variation in protein, fat, and moisture content. These cuts are typical of those found in Irish supermarkets.

In general, CAP is used for bulk product or items of irregular shape, such as whole lamb carcasses, or as master packs for retail-ready product (Gill 2003). CAP is not suitable for individual trays of retail-ready product because of the undesirable color of

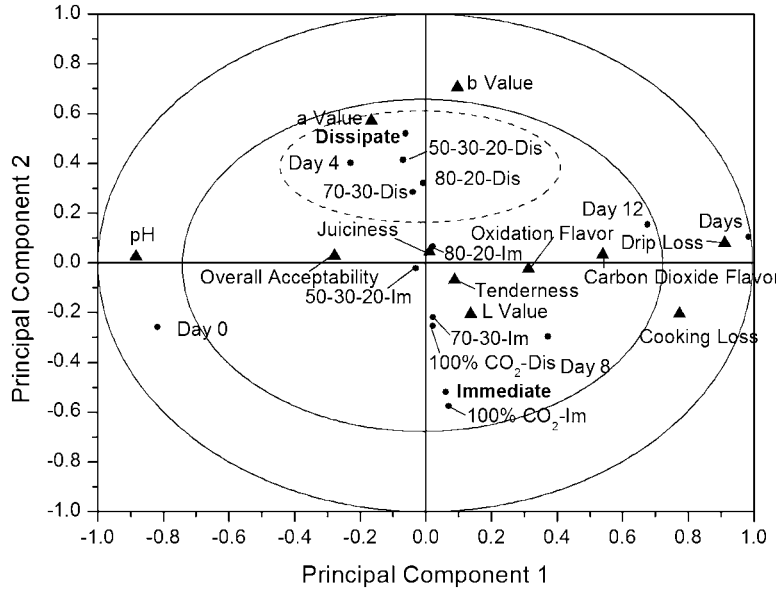

Figure 13.3. An overview of the variation found in the mean data from the ANOVA-Partial Least Squares Regression (APLSR) correlation loadings plot for each of the 4 MAP treatment groups. Shown are the loadings of the X and Y variables for the first 2 PCs for ▲ = days and the individual MAP treatments, • = sensory descriptor and instrumental variables. *Im (Immediate)* = meat samples cooked immediately after opening of the MA packaging and presented to panelists. *Dis (Dissipate)* = meat samples left 30 min in ambient air to let any CO_2 dissipate, then cooked and presented to panelists. The concentric circles represent 100% and 50% explained variance, respectively. (Adapted from O'Sullivan et al. 2010.)

anoxic meat, and because packaging materials that are impermeable to gases are mostly opaque (Gill 2003). The inclusion of O_2 in CAP systems at low levels can have a deleterious effect on meat color. The inclusion of just 100ppm oxygen can cause this discoloration, but this is usually transient, since the metmyoglobin is reduced to myoglobin, usually within four days, as anoxic conditions are established and maintained (Gill and Jones 1994). However, O_2 scavengers may be used in CAP systems to prevent the formation of metmyoglobin, if very low levels of O_2 are accidentally incorporated during pack filling. Buys (2004) found that the inclusion of an oxygen scavenger ensured that retailed bulk-packaged pork chops held in approximately 100% CO_2 were still acceptable to a consumer panel after 14 days of storage.

Vacuum Packaging of Meat

Vacuum packaging (VP) was one of the earliest forms of MAP methods developed commercially and still is extensively used for products such as primal cuts of fresh red meat and cured meats (Parry 1993). An example of vacuum packed meat is presented in Figure 13.4. The first significant commercial application was for vacuum packaging of whole turkeys using rubber stretch bags (Purdue 1997). VP extends the storage life of chilled meats by maintaining an O_2 deficient environment within the pack (Bell et al. 1995). Vacuum packs are comprised of evacuated pouches or vacuum skin packs, in which a film of low gas permeability is closely applied to the surface of the product. Preservative effects are achieved by the development of an anaerobic environment within the pack

Figure 13.4. Vacuum-packed meat products, chorizo and salami.

(Gill and Gill 2005). The objective is that any residual O_2 in the remaining atmosphere, including O_2 dissolved in the product, will be removed by enzymatic reactions within the muscle tissue, or through other chemical reactions with tissue components (Gill and Gill 2005). Respiration of the meat in vacuum packs will also quickly consume the vast majority of residual O_2, replacing it with CO_2, which eventually increases to 10–20% within the package (Taylor 1985; Parry 1993; Gill 1996). However, the amount of O_2 remaining in the pack at the time of closure must be very small if the product is to be effectively preserved, as the capacity of the muscle tissue for removing O_2 is limited (Gill and Gill 2005). The oxygen level is generally reduced to less than 1% under good vacuum conditions. Due to the barrier properties of the film used, entry of oxygen from the outside is restricted (Parry 1993; Robertson 2006).

Vacuum-packaged meat is unsuitable for the retail market because depletion of O_2, coupled with low O_2 permeability of the packaging film, causes a change in meat color from red to purple, due to the conversion of oxymyoglobin to deoxymyoglobin. The consumer has been shown to reject those myoglobin forms that are not acceptable meat colors from their perspective (Parry 1993; Allen et al. 1996). Consumers have demonstrated a bias against the purchase of vacuum packaged beef, which displays the purple color of deoxymyoglobin (Meischen et al. 1987). Also, prolonged storage of meat in vacuum packs results in the accumulation of drip, which is also unappealing to consumers (Jeremiah et al. 1992; Parry 1993; Payne et al. 1997).

VP continues to be used in numerous ways for efficient meatpacking and is still the most cost-effective packaging strategy employed for the packing of meat. A recent innovation in VP has been the evolution of shrinkable films in use with horizontal form-fill-seal machinery (Salvage and Lipsky 2004).

Vacuum Skin Packaging of Meat

Drip formation in vacuum packed meat, as discussed above, can partly be overcome by vacuum skin packaging (VSP), using a film that fits very tightly to the meat surface, leaving little space for the accumulation of

any fluid exudate (Hood and Mead 1993). This style of package uses a polystyrene or polypropylene tray, coupled with the use of a barrier film that can form around the product to reduce any liquid purge emanating from it. An additional web of film or a header can also be added for pre-pricing and pre-labeling. Depending on one's perspective, an advantage or disadvantage of this package is that it gives the product a very unique appearance (Belcher 2006). VSP involves production of a skin package in which the product is the forming mold. It was first introduced using an ionomer film, which softens on heating to such an extent that it can be draped over sharp objects without puncturing (Robertson 2006). The product shelf life can be 15–22 days, depending on the meat cut used. Since the product is displayed in the myoglobin state, there is no loss of color in the display case and oxidation issues are minimized using this packaging format (Belcher 2006).

In summary, VSP eliminates the wrinkled appearance of traditional vacuum-packaged meat products, thus improving the appearance of products, which will have a positive effect on consumer appeal.

Active Packaging

Antimicrobial packaging is a promising and rapidly emerging technology in which antimicrobial agents are incorporated into or coated onto food packaging materials to prolong the shelf life of the packed food, usually by extending the lag phase and reducing the growth rate of microorganisms (Floros et al. 1997; Han 2000; Suppakul et al. 2003). The aim of active packaging is to increase the display life of the contained products, while maintaining their quality, safety, and sensory properties, without direct addition of active agents to the product (Camo et al. 2008). Inclusion of the active agents, be they antioxidants, antimicrobials, or any other, within the packaging material gives rise to active packaging (Camo et al. 2008). An active package was defined by Rooney (1995) as a material that "performs a role other than an inert barrier to the outside environment." They can actively control microbial contamination of foods during storage and distribution. The fundamental concept behind this technology is the incorporation of an antimicrobial agent into the packaging material by either spraying, coating, physical mixing, or chemical binding (Berry 2000). Food manufacturers may be able to maintain the minimum inhibitory concentration of an antimicrobial to prevent growth of pathogenic and spoilage microorganisms by using controlled-release packaging (Koontz 2006). The major potential product applications for antimicrobial films include meat, fish, poultry, bread, cheese, fruits, vegetables, and beverages (López-Rubio et al. 2004).

Antimicrobial (AM) packaging research generally started with the development of antimicrobial packaging materials that contain antimicrobial chemicals in their macromolecular structures (Han 2005). Chemical preservatives can be employed in antimicrobial-releasing film systems, including organic acids and their salts (sorbates, benzoates, and propionates), parabens, sulfites, nitrites, chlorides, phosphates, epoxides, alcohols, ozone, hydrogen peroxide, diethyl pyrocarbonate, antibiotics, and bacteriocins (Ozdemir and Floros 2004). Antimicrobial films can be classified into two types: (1) those that contain an antimicrobial agent that migrates to the surface of the food, and (2) those that are effective against the surface growth of microorganisms without migration (Suppakul et al. 2003). Also, antimicrobial coatings may be developed by incorporating nisin, lactoferrin, sodium diacetate, sorbic acid, and potassium sorbate into a coating material (Limjaroen et al. 2003). Antimycotics and antimicrobials have been added to food packaging films to delay outgrowth of mold. Potassium sorbate release from low-density

polyethylene (LDPE) and high-density polyethylene (HDPE) films has been studied in food systems. In such systems, release rates and migration amounts must be closely monitored for the system to effectively preserve the contents of the package (Han 2000). Looking to the consumers' demand for chemical preservative-free foods, food manufacturers are now using naturally occurring antimicrobials to sterilize and/or extend the shelf life of foods (Han 2005). Present plans envisage the possible use of naturally derived AM agents in packaging systems for a variety of processed meats, cheeses, and other foods, especially those with relatively smooth product surfaces that come in contact with the inner surface of the package. This solution is becoming increasingly important, as it represents a perceived lower risk to the consumer (Nicholson 1998). Various bacteriocins, such as nicin, pediocin, lacticin, propionicin, etc., can be incorporated into foods and/or food packaging systems to inhibit growth of spoilage and pathogenic microorganisms (Daeschul 1989). The extracted bacteriocins, which are generally small molecular weight peptides, can be utilized in various ways; however, it is very important to characterize their resistance to thermal treatment and pH (Han 2005). The storage temperature may also affect the activity of AM packages. Several researchers have found that the protective action of AM films deteriorated at higher temperatures, due to high diffusion rates in the polymer (Vojdani and Torres 1989). The diffusion rate of the AM agent and its concentration in the film must be sufficient to remain effective throughout the shelf life of the product (Cooksey 2000).

Antioxidant packaging is a recent development in active packaging technologies that has had some success. Nerín et al. (2006) described the promising results of a new antioxidant active packaging system; a plastic film with an embodied rosemary extract was able to inhibit both myoglobin and lipid oxidation in beef, leading to the enhanced display life of the meat. Additionally, Camo et al. (2008) investigated and compared the effect of two natural antioxidant sources (rosemary and oregano extracts) incorporated into an active package filled with a modified atmosphere on the display life of lamb steaks. These workers found that a rosemary extract, a rosemary active film, or an oregano active film resulted in enhanced oxidative stability of lamb steaks. Also, active films with oregano were significantly more efficient than those with rosemary, exerting an effect similar to that of the direct addition of the rosemary extract and extended fresh odor and color from 8 to 13 days compared to the control.

Active packaging has the advantage of maintaining the preservative effects of various compounds (antimicrobial, antifungal, or antioxidant), but without being in direct contact with the food product. This is an important development, considering the consumer drive toward clean labeling of food products and the desire to limit the use of food additives.

Summary and Future Trends in Meat Packaging

In recent years, much attention has focused on the shift from consumers buying meat at the family butcher shop to purchasing it at the local supermarket. More and more traditional butcher shops have closed because they cannot compete on price, offer the same supermarket one-stop shop opportunity, or provide the extended shelf life of MAP meats to the consumer that are available on refrigerated supermarket shelves. This is the situation in most developed countries, particularly within the EU, where sales of fresh meat have increased in supermarkets at the expense of the specialized butcher's store (Mannion 1995). However, recently, consumers have become very much more discerning with respect to the origins of the food they

consume. Poor labeling by the supermarkets has resulted in a swing back toward the local butcher, where meat traceability is transparent and promoted as a selling point; in addition, green issues relating to product movement to markets (air miles) and support for local product producers has encouraged this same trend. The impact of such developing trends on the pre-pack sales of meat at the supermarket level remains to be seen.

Mize and Kelly (2004) reported the trends in fresh meat packaging at retail level in the United States. They found that in 2002, 69% of the linear footage of the self-service meat case was occupied by fresh meat and poultry. This figure declined to 63% in 2004, reflecting a growing conversion of meat items to products with greater consumer convenience, such as fully cooked entrees and marinated meats, as well as hams and sausages. They also reported an increase in packages that were case ready, from 49% in 2002 to 60% in 2004.

As stated earlier, high O_2 MAP is now used ubiquitously across the meat industry for many different meat products. Alternatively, low O_2 packaging systems have been readily available in the United States, but not as widely implemented as the high O_2 counterparts. Vacuum packaging continues to be, in many cases, the most cost-effective packaging strategy. A relatively recent innovation in vacuum packaging has been the evolution of shrinkable films in use with horizontal form-fill-seal machinery (Salvage and Lipsky 2004). This packaging format uses a polystyrene or polypropylene tray and uses a barrier film that can form around the product to reduce any amount of purge coming out of the product. An additional web of film or a header can also be added for pre-pricing and pre-labeling (Belcher 2006).

As the meat industry moves toward central processing that employs MAP and Vacuum-Skin Packaging (VSP), processors may need to overcome consumer preference for fresh beef that is bright red in color and packaged with the traditional PVC overwrap. Nevertheless, it is encouraging that the initial perceptions of quality will likely not bias eating satisfaction once a decision to purchase is made and the meat is taken home, thereby hastening the acceptance of the newer packaging technologies (Carpenter et al. 2001). Additionally, meat processing and packaging technologies that are accepted by the market and adopted by the industry will have to become more efficient, consistent, and leaner in activity if future global challenges are to be met. Low-oxygen packaging technologies will continue to evolve as long as they can successfully and economically enable the wider distribution of centrally packaged fresh meat (Eilert 2005).

It is critical that we understand the factors that will have the largest influence on the evolution of meat packaging. The demand for convenience foods will continue to be fueled by the aging of our population, the diminished cooking skills of the typical consumer, and the reduced time available for home preparation of meals. The ability of materials to offer flexibility in primary processing as well as reheating at home will be critical (Eilert 2005).

The volatility of oil prices has a direct effect on the cost of traditional petrochemical-based packaging materials. Also, the environmental considerations of disposing of traditional packaging after use have become center stage in recent years with respect to green solutions to modern living. The increased costs of petroleum will continue to drive the demands for bio-based packaging materials. Consumer demand for more environmentally friendly packaging and more natural products will also create increased demand for packaging from biodegradable and renewable resources (Cutter 2006). Even though food manufacturers cannot eliminate packaging, they can redesign packages to reduce the amount of material used or to incorporate newly developed materials such

as biodegradable plastic in their products' packaging. This is particularly important in the European Union, where many countries are considering tougher legislation to encourage the use of less packaging material (Dodds 2007). Biopolymer films may serve as potential replacements for synthetic films in food packaging applications to address strong marketing trends toward more environmentally friendly materials, but hydrophilicity is a central limitation to replacement and full-scale commercial utilization of biodegradable films (Han et al. 2005). However, a variety of bio-based materials have been shown to prevent moisture loss, reduce lipid oxidation, improve flavor, retain color, and stabilize microbial characteristics of foods (Cutter 2006).

Additionally, any assessment of the environmental impact of food packaging must consider the positive benefits of reduced food waste in the supply chain (Marsh and Bugusu 2007). The demand for pre-packaged fresh meat will continue to grow, but it is critical that packaging formats that enable wider distribution of these products evolve (Eilert 2005). Foamed food trays made of polylactic acid (PLA) resin, the corn-based biopolymer, have recently been trialed in the United States and Europe for commercial meat packaging applications (Schut 2008). The polymers are said to biodegrade when packages that are made from them are composted. Moisture and heat in the compost pile break the PLA polymer chains to smaller chains, and then ultimately to lactic acid. Microorganisms in the compost consume the lactic acid for nourishment (Nachay 2008).

An Italian company, Coopbox SpA in Reggio, which is a major producer of foam food packaging, introduced the first foamed PLA trays, called Naturalbox, in 2005, which can be used for the packaging of meat, fish, or poultry. Also Cryovac Inc. (Reading, Pennsylvania) has commercialized a PLA foam tray in the U.S. market with its "NatureTray" product aimed at fresh meat. Both these companies use a foam form that is derived from 100% annually renewable resources. In the future, we can hope to see even more applications for renewable packaging materials in the packaging of meat products. These products will address the various technical challenges of MAP and vacuum packaging and overcome the gas permeability issues required to make such packaging effective.

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Chapter 14

Novel Technologies for Microbial Spoilage Prevention

Oleksandr Tokarskyy and Douglas L. Marshall

Introduction

Storage of chilled meats in air leads to rapid spoilage by psychrotrophic bacteria, predominantly *Pseudomonas* spp. and *Brochothrix thermosphacta*. As shown in Chapter 13, traditional packaging systems for meat products have been very successful in slowing the rate of microbial spoilage and extending the shelf life of meats. These systems are designed to manipulate the gas environment surrounding the product. Such systems include oxygen-permeable overwrap for short-term retail display to maintain the bloom color of red meats. For long-term storage, vacuum packaging (complete removal of headspace gases) or modified atmosphere packaging (MAP)/controlled atmosphere packaging (CAP) is employed. The success of these packaging systems is such that the majority of red meat produced in the United States is vacuum or MAP packaged (Siragusa et al. 1999).

In the present chapter we review a number of recent non-thermal technological developments for the control of meat spoilage microorganisms and extension of shelf life. The types of microbes found on meats and conditions that lead to spoilage can be found in recent reviews (Marshall and Bal'a 2001; Nychas et al. 2007). We present here new information related to revolutionary packaging innovations such as gas scavenging and antimicrobial impregnation systems. Also discussed are recent developments in natural product biological interventions (phage, bacteriocins, chitosan, essential oils, and enzymes), chemical interventions (organic acid salts, acidified sodium chlorite, phosphates, ozone, and electrolyzed water), and physical interventions (ionizing irradiation, high pressure, hydrodynamic shockwave, pulsed electric fields, high intensity light, and cold plasma). Brief commentary on novel thermal processing interventions (high frequency and ohmic heating) and novel carcass decontamination methods is also provided. Many of the interventions remain at the theoretical stage and will require extensive validation and economic analysis before practical introduction to industry. Others, however, have found widespread use and will likely remain a mainstay in industry.

Packaging Systems

As introduced in Chapter 13, several new packaging systems have been developed that hold promise to extend the shelf life of meats. Traditional MAP/CAP usually involves removal of headspace air, followed by reintroduction of gases that have a different composition than air (Kerry et al. 2006). Incorporation of elevated CO_2 levels into packaging inhibits pseudomonads in chilled meats (Marshall et al. 1991, 1992; Zhao et al. 1992). Complete oxygen removal by vacuum packaging or 100% CO_2/N_2 substitution inhibits spoilage bacteria while favoring growth of lactic acid producing bacteria, resulting in extended shelf life of the product (Nychas et al. 2007). The need for low

(<0.15%) or high (80%) oxygen level in MAP is to prevent rapid browning of red meats due to metmyoglobin formation (MacDougall and Taylor 1975; Mancini and Hunt 2005). Complete removal of oxygen is desirable to prevent meat oxidation but results in a purplish meat color (Cornforth and Hunt 2008). In the remainder of this section we briefly summarize the antimicrobial and commercial aspects of these systems.

Anaerobic CO-MAP, which uses low levels of carbon monoxide (CO, 0.4%), CO_2 (20% to 30%), and nitrogen (remainder), inhibits growth of aerobic spoilage psychrotrophic bacteria due to the absence of oxygen. Carbon monoxide fixes a strong red color associated with fresh red meats, resulting in longer freshness perception by consumers. Consumers have historical familiarity with the red color of fresh meats as an indicator of freshness. Because CO fixes this red color, products stored beyond their useful shelf life may visually appear fresh yet have high bacterial counts resulting in spoilage. Consumers also may be concerned with the use of CO for this application because of the well-known association of this gas with human fatalities following inhalation exposure from faulty combustion systems (Cornforth and Hunt 2008).

With conventional MAP, the headspace gas originally introduced changes during product storage. For example, carbon dioxide dissolves in liquids at refrigerated temperatures, and its permeability as with most plastic films is three to five times greater than that of oxygen, so it is difficult to continuously maintain its concentration during shelf life (Ozdemir and Floros 2004). Also, any residual or acquired oxygen may lead to *Brochothrix* growth and undesirable changes in meat color. As a result of these changes, a new generation of active packaging systems has emerged. To extend meat product shelf life, active packaging involves the incorporation of certain additives into packaging systems, such as antimicrobial agents, oxygen scavengers, carbon dioxide emitters, or chlorine dioxide emitters (Kerry et al. 2006).

Oxygen scavengers are mostly available as sachets attached to the package and utilize one of several technologies: ascorbic acid oxidation, iron powder oxidation, photosensitive dye oxidation, or biological scavenging systems (Coma 2008). Carbon dioxide emitters usually rely on either ferrous carbonate or ascorbic acid/sodium bicarbonate mixture. Not surprisingly, commercially available systems often contain both an O_2 scavenger and a CO_2 emitter (Table 14.1).

Table 14.1. Commercial active packaging with scavenger/emitter gas-based systems

Technology	Format	Trade name	Manufacturer
CO_2 emitter/O_2 scavenger	Sachet, label, card	Ageless®	Mistubishi Gas Chemical Co. (Japan)
O_2 scavenger	Sachet	Oxysorb®	Pillsbury Co. (USA)
CO_2 emitter	Sachet	FreshPax®	Multisorb Technologies Inc. (USA)
O_2 scavenger	Label	ATCO®	Standa Industrie (France)
O_2 scavenger	Label	FreshMax®	Multisorb Technologies Inc. (USA)
O_2 scavenger	Film[1]	OS2000®	Sealed Air Corporation (USA)
O_2 scavenger	Film, Sachet	Bioka®	Bioka Ltd. (Finland)
O_2 scavenger	Film[1]	ZerO2®	CSIRO and VisyPak (Australia)
O_2 scavenger	Film[2]	Ageless-OMAC	Mistubishi Gas Chemical Co. (Japan)
CO_2 emitter/O_2 scavenger	Sachet	Freshilizer®	Toppan Printing Co. (Japan)
Chlorine dioxide generator	Sachet	Microsphere®	Bernard Technologies (USA)
CO_2 emitter		Verifrais®	SARL Codimer (France)
CO_2 emitter		Vitalon®	Toagosei Chemical Co. (Japan)

[1] UV light activated
[2] Heat activated

Biological O_2 scavenging systems contain viable respiring innocuous microorganisms entrapped in alginate, gelatin, or agar (Tramper et al. 1983; Doran and Bailey 1986; Gosmann and Rehem 1986), or incorporated into hydroxyethyl cellulose or polyvinyl alcohol films (Altieri et al. 2004). Several reviews are available that provide more detailed scavenger and emitter information (Floros et al. 1997; Suppakul et al. 2003; Kerry et al. 2006; Coma 2008).

Payne et al. (1998) examined the effect of packs flushed with CO_2, packs flushed with CO_2 and containing Ageless® oxygen scavenger, and packs containing oxygen scavenger alone on microbial counts of beef stored for up to 20 weeks at −1.5°C. Although packs with the scavenger system had the greatest microbial counts at the end of week 16, all three treatments were acceptable to consumers, with flush-scavenger and scavenger alone having lowest oxygen levels (<0.1% v/v) compared with flush alone (0.9%). Ellis et al. (2006) studied the quality of refrigerated chicken breast stored under MAP (100% N_2 or 75 : 25 N_2 : CO_2), with or without slow- and fast-release sachets containing antimicrobial chlorine dioxide (ClO_2). Samples containing ClO_2 sachets had 1.0 to 1.5 $\log_{10}$ CFU/g lower total plate count on day 6 and day 9 of storage compared to MAP controls alone. Tewari et al. (2001) showed that commercial oxygen scavenger (Ageless® or FreshPax®) combined with N_2 CAP slowed discoloration and metmyoglobin formation of beef stored at 1°C compared to N_2 CAP alone.

A potential major drawback of complete oxygen removal from packaging by oxygen scavengers or other means is the possibility of nonproteolytic *Clostridium botulinum* growth and neurotoxin production during temperature abuse storage conditions (Coma 2008). The threat of nonproteolytic strains is that outgrowth and toxin production by the bacterium may occur despite the absence of sensory defects. Thus, a consumer could have no visual, aroma, or taste indicators that the product is toxic. We believe further research is needed to address this concern. The use of time-temperature indicators, such as 3M™ brand MonitorMark™ Time Temperature Indicators, may be useful for consumers to predict whether a product has been exposed to temperature abuse during transport, storage, or retail display (Shimoni et al. 2001).

Ionizing Irradiation

Irradiation is a safe and effective method to improve food safety and quality. Ionizing irradiation employs gamma rays (cobalt-60 and cesium-137 as radioactive sources), x-rays (machine-generated), and e beam (high-energy electrons, machine-generated) as treatments to successfully kill microbes in foods. Irradiation damages microbial DNA, resulting in cell death. According to Aymerich et al. (2008), viruses are most resistant to irradiation, followed by bacterial spores, yeasts, molds, Gram-positive bacteria, and Gram-negative bacteria. This technology has excellent penetration power. For example, x-rays and gamma rays can penetrate 80 to 100 cm while e beams have less penetrating power, ranging from 8 to 10 cm. None of these ionizing treatments make food radioactive, making questionable negative consumer fears about the technology. Irradiated foods should bear the internationally recognized radura symbol (Fig. 14.1) together with a "treated with irradiation" statement on the label to inform consumers.

Two levels of irradiation processes are recognized based on absorbed dose: radurization (1 to 10 kGy) and radapperdization (20 kGy and up) (Murano 2003). Reviews of irradiation treatment of meats and meat products are available elsewhere (Thayer et al. 1986; Newsome 1987; Monk et al. 1995; Farkas 1998). A summary of most recent meat gamma irradiation studies is presented in Table 14.2.

Figure 14.1. Radura logo (international and U.S. FDA versions).

Use of irradiation of up to 4.5 kGy for refrigerated red meats, up to 7 kGy for frozen meats, and up to 3 kGy for poultry is permitted in the United States (FDA 1999) and is reflected in the U.S. Code of Federal Regulations (9 CFR Parts 381 and 424). Unfortunately, this technology has not been widely accepted due to logistics challenges, opposition by activist groups, and resistance from processors (Lazar 2006b). In the United States, the main reason for lack of use is logistics, with few irradiation facilities available with sufficient capacity for large-scale processing. For example, Food Technology Service runs a Cobalt-60 facility in Florida, which is far removed from major animal production areas in the Great Plains. SureBeam in Iowa ran an e-beam facility in the heart of animal production areas but went bankrupt in 2004. Sadex Corp. now manages the former Surebeam facility. Smaller research irradiation facilities are housed at Iowa State University, Kansas State University, and Texas A&M. Lazar (2006b) mentioned that irradiation facilities are expensive to erect and only around forty of them exist in the United States today, mostly specializing in treating medical equipment, animal feed, and spices, while lacking capacity for meat processing.

Phage Technology

Bacteriophages (also known as phages), from "bacteria" and Greek *phagin*, "to eat," are viruses that infect bacteria. Phages consist of an outer protein shell with enclosed DNA or RNA. Phages infect, grow, and multiply only inside bacterial cells. Lytic phages cause bacterial lysis (cell death), which leads to the spread of more phage in the environment. Some phages lyse only a fraction of infected cells and keep other cells alive while continuously shedding new phages. Phages capable of lysogeny integrate phage DNA into the bacterial host DNA without causing cell death. Most reports on the use of phage technology focus on applications to control meat-derived bacterial pathogens. For example, specific phages have been investigated against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Salmonella enterica* Typhimurium (Bigwood et al. 2008). In 2006, *L. monocytogenes* phage was approved by the FDA as a food antimicrobial (Stahl 2007).

Table 14.2. Efficacy of gamma irradiation on meat products

Meat product	Microorganism	Dose for 5 $\log_{10}$ reduction, kGy	Reference
Marinated beef rib	*Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium*	3 to 4	Jo et al. 2004
Fresh broiler chicken	Total aerobic plate count	>5	Javanmard et al. 2006
	Coliform count	3 to 5	
Raw chicken breast and thigh	*B. cereus, Enterobacter cloacae, Alcaligenes faecalis*	<2	Min et al. 2007
Ground pork	*Listeria monocytogenes*	3	Bari et al. 2006
Fresh pork loins	*Pseudomonas* spp., psychrotrophs	3	Dogbevi et al. 1999

Several advantages of phage technology for meat spoilage control are described by others (Greer 2005; Hudson et al. 2005). For example, phages are self-reproducible and release more phage after bacterial lysis. Phage specificity may be an advantage if selective for spoilage microflora only. On the other hand, specificity may diminish phage activity against broad-spectrum spoilage microflora. Whitman and Marshall (1971a) noticed that phages from bacteriophage-host systems isolated from refrigerated food products usually attacked only those hosts upon which they were isolated. Phages are generally more stable than their hosts and can survive processing (Koo et al. 2000). Greer (1988) showed that phage concentration remained stable (5 to 6 $\log_{10}$ PFU/cm^2) on the surface of refrigerated (4°C) beef rib-eye steaks during 14 days of storage in air. Whitman and Marshall (1971b) showed that some *Pseudomonas* phages isolated from beef may remain infectious after heating to 60°C, pH change to 4.0, and exposure to 4 M NaCl. Phages are naturally present entities and constitute part of the environment. Whitman and Marshall (1971a) isolated a total of 38 host-phage pairings from ground beef, sausage, chicken, raw milk, and oysters. Phage concentration as high as 6.3×10^6 PFU/g was found on chicken skin. Not surprisingly, most isolated were invaders of *Pseudomonas* spp., followed by Gram-positive cocci and members of the Enterobacteriacea family. Similarly, Atterbury et al. (2003) isolated 34 *Campylobacter* phages from retail chicken meat.

Because of the naturally wide prevalence of phages on raw meats, one may conclude that phage addition to food products is not adding a foreign entity. Phages used to control food spoilage bacteria can be derived from corresponding foods and food-processing environments. Because phages are highly specific to prokaryotes, it is unlikely that they can target human eukaryotic cells. To our knowledge, sensory data demonstrating that phage treatments do not alter the appearance or flavor of foods have not been published.

Among the many potential drawbacks of phage technology, limited host range is considered most important. Most reported studies were performed on experimentally inoculated refrigerated meats and demonstrated efficacy against spoilage psychrotrophs and psychrophiles (Greer 1986, 1988; Greer and Dilts 2002; Greer et al. 2007). Greer (1988) showed that homologous phage can replicate and limit bacterial growth on inoculated and refrigerated phage-treated beef. Maximum shelf life extension was seen with an initial bacteria density in excess of 3 $\log_{10}$ CFU/cm^2 and phage concentrations above 7 $\log_{10}$ PFU/cm^2. Greer and Dilts (2002) were able to extend the shelf life of inoculated (*B. thermosphacta*) and homologous phage-treated refrigerated pork adipose tissue from 4 to 8 days. *Leuconostoc gelidum* phage isolated from vacuum-packaged pork loin was able to limit growth of the corresponding host on inoculated pork adipose tissue stored at 4°C in air or vacuum (Greer et al. 2007). However, the same research group (Greer and Dilts 1990) showed that a pool of seven phage strains failed to lyse 43.8% of 1,023 strains of naturally isolated pseudomonads from beef, pork, and lamb. When added to refrigerated, naturally contaminated beef rib-eye steaks, the same phage pool remained viable throughout the study and significantly reduced *Pseudomonas* growth, but did not significantly affect shelf-life duration. The authors concluded that phage inability to prolong shelf life of meat was caused by the narrow host range of the phage pool.

Development of phage-resistant bacterial mutants is another point of concern that may be overcome using a pool of phage strains, with each targeting different receptors on the bacterial cell-wall surface. On the other hand, phages continuously co-evolve with bacteria,

adjusting themselves to regain ability to infect mutated bacteria (Weitz et al. 2005). Another major drawback of phage technology is the difficulty of getting phages to kill mesophilic and psychrotrophic microorganisms in a food product stored at refrigeration temperatures. Phage activity requires actively growing and dividing hosts, which for many mesophiles generally does not occur in a chilled stored product. Although psychotrophs can grow at chill temperatures, their growth rate is slow. For phage to be effective against spoilers their activity must be certain at low storage temperatures.

Another issue is that large initial bacterial populations (3 to 5 $\log_{10}$ CFU/g) are needed for phage activity (Greer 2005; Hudson et al. 2005). Since phage attack on bacteria initiates upon contact, the probability of this encounter increases if populations of both counterparts are large. If spoilage bacterial numbers are low on a product due to the effectiveness of other control measures, then phage treatment may not be valuable. However, in the case of refrigerated meat spoilage, where generally large population numbers are present, phage technology may have merit.

The nature of the environment can play a significant role in phage attack, with viscous product physically reducing the possibility of phage-host interaction. Other drawbacks include the possibility of phage-mediated virulence gene transfer between bacteria. Lysogenic conversion of lytic phage to temperate phage could result in host protection from other phages. As a last argument, the average consumer associates viruses with disease, and their perception of adding viruses to foods has yet to be investigated.

To summarize, the biggest challenge in utilizing phage technology in modern meat spoilage control might be high phage specificity, which makes it difficult to achieve the desired results of shelf life extension for naturally contaminated meat products. Genetic manipulation of available phages and selective breeding may be tools to decrease phage specificity, which would increase the range of bacteria susceptible to control. Further steps may also include development of multistrain phage cocktails with complementary activity aimed at different cell structure targets. In addition, little information is available on lactic acid bacteria spoilage control with phage. This bacterial group contains common spoilers of vacuum-packaged products. Most current research focuses on *Pseudomonas* spp. and *B. thermosphacta*.

High Pressure Processing

High hydrostatic pressure (HHP) treatment involves placing packaged meat in a pressure vessel and applying isostatic water pressure of 100 to 900 MPa. HHP processing is considered nonthermal, since temperatures increase only 3°C for every 100 MPa applied (Aymerich et al. 2008). Equipment for HHP is commercially available, including manufacturers Avure Technologies (United States) and Nicolas Correa Hyperbaric (Spain). HHP kills bacterial cells through a combination of actions, with the bacterial membrane the primary site of damage. Gram-negative bacteria are more susceptible, followed by Gram-positive bacteria and spores (Hugas et al. 2002). Linton et al. (2004) reported that the microflora of chicken mince became less diverse and shifted to Gram-positive bacteria after HHP treatment. Regarding cell shape, rods (elongated) are more susceptible than cocci (round). It is generally believed that HHP does not significantly change the sensory quality of meats, although cooked color (at 150 MPa), oxidation of ferrous myoglobin (at 400 MPa), and lipid oxidation has been reported in fresh and marinated meats (Hugas et al. 2002). Results of studies showing prevention of meat spoilage with HHP treatment are summarized in Table 14.3.

Table 14.3. High hydrostatic pressure treatment of meat products

Product	Target bacteria	Results	Process	Reference
Minced beef muscle	Total microflora	3 to 5 $\log_{10}$ reduction	450 MPa, 20 min, 20°C	Carlez et al. 1994
Mechanically recovered poultry meat	Mesophilic bacteria	3.6 $\log_{10}$ reduction	450 MPa, 15 min, 2°C	Yuste et al. 2001
Marinated beef loin	Aerobic total count	>4.5 $\log_{10}$ reduction	600 MPa, 6 min, 31°C	Garriga et al. 2004
Dry cured ham		>2.5 $\log_{10}$ reduction		
Cooked ham		>6 $\log_{10}$ reduction after 60 days at 4°C		
Minced chicken	Aerobic plate count	1 $\log_{10}$ reduction	500 MPa, 15 min, 40°C	Linton et al. 2004

Hydrodynamic Shockwave Treatment

Hydrodynamic shockwaves (HDS) are generated either electrically (capacitor discharge system) or by using explosives in water. Besides tenderizing meat products by disrupting the myofibrillar structure (Schilling et al. 2003), HDS might influence bacterial counts as well, resulting in extended product shelf life (Raloff 1998). Explosively produced HDS are not commercially feasible because it is a batch-type process, has specific packaging requirements, and has potential worker safety concerns. In contrast, electrically generated HDS has been commercialized by Hydrodyne, Inc. (Claus et al. 2001).

Mixed results are found in the literature on the effectiveness of HDS to inactivate microbes on meats. Williams-Campbell and Solomon (2002) showed that explosively generated shockwaves caused immediate reduction of aerobic plate counts by 1.5 to 2.0 $\log_{10}$ CFU/g in fresh beef. After 14 days of storage, treated beef counts were 4.5 logs less than control samples. Schilling et al. (2003) showed that blade-tenderized beef treated with HDS had lower standard plate counts (0.5 log difference) compared to controls after 14 days of storage. On the other hand, Moeller et al. (1999) found no significant difference in aerobic plate counts and coliform counts between explosive HDS-treated pork muscle and control. Thus, aside from the obvious increase in tenderness, HDS treatment as a tool to decrease microbial loads and prolong the shelf life of meat products remains undetermined, and additional research is needed to support this concept.

Antimicrobials

Bacteriocins

Bacteriocins are cationic and hydrophobic peptides produced by lactic acid bacteria, with antibacterial activity against related Gram-positive bacteria (Chen and Hoover 2003). In addition to bacteriocins, lactic acid bacteria produce other antimicrobials, such as lactic acid, acetic acid, diacetyl, ethanol, and carbon dioxide among others (Davidson and Hoover 1993). Bacteriocins, usually named after the bacterium that produces it, can be classified into four major classes, with class I and class II being the most investigated (Hugas 1998). A brief summary of bacteriocins is presented in Table 14.4.

Of a variety of bacteriocins discovered, only nisin (commercially available as Nisaplin®) is on the Generally Recognized as Safe (GRAS) list as a direct food additive approved by the U.S. FDA (Siragusa et al. 1999). This heat-stable, easily digestible bacteriocin is especially effective for control

Table 14.4. Summary of bacteriocins and their producing bacteria

Bacteriocin	Producer	Bacteriocin	Producer
Nisin, lacticin	*Lactococcus lactis*	Sakacin	*Lactobacillus sakei*
Lactocin	*Lactobacillus sakei*	Curvacin	*Lactobacillus curvatus*
Pediocin	*Pediococcus acidilactici*	Curvacitin	*Leuconostoc curvatus*
Enterocin	*Enterococcus faecium*	Bavaricin	*Lactobacillus bavaricus*
Brevicin	*Lactobacillus brevis*	Leucocin	*Leuconostoc gelidum*
Divergicin	*Carnobacterium divergens*	Carnobacteriocin/Piscicolin	*Carnobacterium piscicola*

Adapted from Hugas 1998

of the foodborne pathogen *L. monocytogenes* (Scannell et al. 1997; Murray and Richards 1998; Pawar et al. 2000). In addition, nisin is currently used in the United States as an anti-botulinum agent for pasteurized cheese products and pasteurized liquid eggs.

A drawback of bacteriocin usage is that effectiveness is only against closely related bacteria, which usually excludes Gram-negative bacteria. For example, nisin was effective against *L. monocytogenes* on cooked pork under MAP, but not against *Pseudomonas fragi* (Fang and Lin 1994). In order to sensitize Gram-negatives to nisin, chelators or bacterial membrane disruptors, such as EDTA, Tween-80, or high hydrostatic pressure (HHP), might be required (Natrajan and Sheldon 2000b; Galvez et al. 2007).

Application of nisin in meat products is somewhat challenging due to its binding ability to meat components, low solubility (hydrophobic nature), and loss of efficacy at pH > 5 (Scannell et al. 1997; Murray and Richards 1998). For example, Rose et al. (1999) showed that glutathione, which is present in raw ground beef, can inactivate nisin. Scott and Taylor (1981) showed the need for greater nisin concentration to inactivate *Clostridium botulinum* in cooked meat compared to microbiological medium. Finally, Chung et al. (1989) showed a 70% loss in nisin activity in raw meat during storage at 5°C for 4 days.

Hugas (1998) mentioned that pediocin might be more effective than nisin in meat applications, since it is derived from the meat-fermentation bacterium *Pediococcus acidilactici*. Another approach for biopreservation might be use of lactic acid-producing bacteria that also produce bacteriocins as direct protective cultures on meats (Hugas 1998) due to the fact that lactic acid bacteria do not induce significant spoilage until large population numbers are reached (Nychas et al. 2007). Bloukas et al. (1997) extended shelf life of vacuum-packaged frankfurters stored at 4°C by one week using commercially available protective culture of *Lactobacillus alimentarius*.

Government approval is needed in order to take advantage of the variety of bacteriocins present in nature. To date, nisin is the only approved bacteriocin. Nisin can be used to control Gram-positive bacteria, such as the pathogen *L. monocytogenes* and the spoiler *B. thermosphacta*. Although methods of sensitizing Gram-negatives are well known, the major drawback of nisin is its loss of activity in meat products, such that large concentrations are needed. This increases application costs.

Lactic Acid, Sodium Lactate, Diacetate, and Acetate

The U.S. government allows the use of lactic acid, sodium lactate (4.8%), sodium diacetate (0.25%), and sodium acetate (0.25%) on meat products as extensive research has shown their safety for human consumption (FDA 2000). Whether produced by lactic acid bacteria or chemically derived, the listed compounds are antagonists to food-borne pathogens and to general spoilage microflora

due to nonspecific mechanisms of action (Kim et al. 1995a, b; Marshall and Kim 1996; Bal'a and Marshall 1998; Kim and Marshall 2000). Numerous publications have documented the effectiveness of these compounds against *L. monocytogenes*, *E. coli* O157:H7, *Clostridium perfringens*, and *Salmonella* spp. (Glass et al. 2002; Porto et al. 2002; Juneja 2006; Michaelsen et al. 2006; Paulson et al. 2007). Lactate efficacy can be improved by combining with diacetate (Jensen et al. 2003; Serdengecti et al. 2006). The main drawback of using straight organic acids instead of their salts is lowered pH and the pale/watery appearance of fresh meats (Kotula and Thelappurate 1994; Lin and Chuang 2001). A summary of organic acid applications (with an emphasis on lactate) for meat product shelf life extension is presented in Table 14.5.

A suggested alternative to lactic acid, low molecular weight polylactic acid, was capable of releasing free lactic acid for extended periods of time, which helped maintain and resist pH change better than free lactic acid (Mustapha et al. 2002). Their results showed

Table 14.5. Lactic acid-derived antimicrobials

Product	Antimicrobial	Result	Reference
Sliced poultry sausage	2% Na lactate	3× to 4× shelf-life extension, 5 to 7°C, air 7× shelf-life extension, 5 to 7°C, N_2	Cegielska-Radziejewska and Pikul 2004
Pork chops	Na acetate Na lactate Na lactate/diacetate	Na lactate/diacetate treatment had lowest APC and least discoloration after 96-h display	Jensen et al. 2003
Low-fat Chinese-style sausage	3% Na lactate	Lower microbial counts after 12 weeks storage at 4°C	Lin and Lin 2002
Retail beef cuts	1.2% acetic acid, 120 s 1.2% lactic acid, 120 s	Paler meat, but small sensory difference; 1 to 2 $\log_{10}$ CFU/g reductions in *Escherichia coli* and APC count within 9 d storage	Kotula and Thelappurate 1994
Pork loin chop	2% Acetic acid	Pale soft exudate appearance, >9 day shelf-life.	Lin and Chuang 2001
	10% Na lactate dip	Extended shelf-life by 3 days compared to control (9 vs. 6)	
Vacuum packaged fresh pork sausage	1% Na lactate	1 to 2 weeks shelf-life extension	Brewer et al. 1993
	2% Na lactate	2 week shelf-life extension	
Vacuum packaged cooked beef loins	4% Na lactate	Lower APC after 7 days at 10°C	Maca et al. 1999
Vacuum packaged beef bologna	3% Na lactate	Lower APC after 10 weeks storage at 4°C	Brewer et al. 1992
Vacuum packaged frankfurters	2% Na lactate	2 to 3 week shelf-life extension at 4°C	Bloukas et al. 1997

APC = aerobic plate count

that 2% polylactic acid behaved the same as 2% lactic acid against *E. coli* O157:H7 in raw vacuum-packaged beef stored at 4°C.

Chitosan

Chitin is the second-most abundant natural biopolymer after cellulose and is a starting material for chitosan (deacetylated derivative of chitin) manufacturing. Since biodegradation of chitin is slow, its accumulation during crustacean processing (mainly shrimp and crab shell wastes) is a disposal challenge. The production of value-added chitin by-products, such as chitosan, could provide a solution to crustacean processing waste accumulation (Shahidi et al. 1999). Chitosan is reported to have antimicrobial properties. Factors that improve antimicrobial activity are a low degree of acetylation and a low pH, both of which increase solubility (Shahidi et al. 1999). Due to the highly reactive nature of polycationic chitosan, which readily interacts with proteins, fats, and other anionic compounds, chitosan antimicrobial activity is less in foods than *in vitro* (Rhoades and Roller 2000). Chitosan has achieved self-affirmed GRAS status (FDA-CFSAN 2004), removing regulatory restrictions on its use in some foods.

Studies by Darmadji and Izumimoto (1994) showed that 1% chitosan addition to minced beef stored at 4°C for 10 days inhibited growth of spoilage bacteria, reduced lipid oxidation and putrefaction, and resulted in better sensory quality. Specifically, an initial reduction of total bacterial count by $0.5\log_{10}$CFU/g was observed, with average count reductions after 10 days storage at 4°C of 1.0, 2.6, 1.0, 1.4, >2.0, and $>2.0\log_{10}$CFU/g for total bacterial, pseudomonad, staphylococci, coliform, Gram-negative bacteria, and micrococci counts, respectively. Sagoo et al. (2002) showed that the addition of 0.3 and 0.6% chitosan to an unseasoned minced-pork mixture reduced total viable counts, yeasts and molds, and lactic acid bacteria by up to $3\log_{10}$CFU/g for 18 days at 4°C compared with an untreated control. Juneja et al. (2006) found that addition of 3% chitosan to ground beef and ground turkey prevented growth of inoculated *C. perfringens* after cooking and inadequate cooling. Their results showed a 4 to $5\log_{10}$CFU/g reduction in *C. perfringens* spore germination and outgrowth over 12-, 15-, and 18-hour cooling cycles and a $2\log_{10}$CFU/g reduction during a 21-hour cooling cycle. Three treatments of fully cooked grilled pork (air packaged, vacuum packaged, or treated with chitosan and vacuum packaged) were investigated for the duration of shelf life (Yingyuad et al. 2006). The authors found significant shelf life extension of 2°C stored product, with standard plate counts of 6.8, 3.8, and $1.6\log_{10}$CFU/g for air, vacuum, and vacuum-2% chitosan stored samples on day 14, respectively.

Essential Oils

Plant-derived essential oil components may be active against bacteria but are difficult to apply in foods due to significant changes in sensory quality (Davidson 2001). Seydim and Sarikus (2006) compared the antimicrobial activity of oregano, rosemary, and garlic essential oils in whey protein isolate films (1.0 to 4.0% wt/vol) against *E. coli O157:H7, Staphylococcus aureus, Salmonella* Enteritidis*, L. monocytogenes*, and *Lactobacillus plantarum* on agar plates. Film with 2% oregano essential oil was the most effective compared to films with garlic (effective at 3% and 4%) or rosemary extracts (no effect). Oussalah et al. (2004, 2006) also showed that alginate-based or protein-based edible films containing oregano essential oil were more effective than cinnamon or pimento in the extension of shelf life of whole beef muscle. They found that application of oregano oil edible film caused 0.9 and $1.1\log_{10}$CFU/g reductions in *Pseudomonas* and *E. coli O157* counts, respectively after 7 days of storage at 4°C (Oussalah et al. 2004).

Likewise, Skandamis and Nychas (2002) found that oregano essential oil extract extended shelf life of refrigerated MAP-stored fresh meat.

Allyl isothiocyanate is one of many volatile natural antimicrobials found in cruciferous plants, such as horseradish, black mustard, cabbage, and turnip. Nadarajah et al. (2005a) prepared paper disks containing 1 ml of 65% allyl isothiocyanate mixed with corn oil. They then applied the paper disks to ground beef patties that were then vacuum packaged and stored for 15 days at 4°C. Results showed a delay in natural microflora growth and significant population reduction in inoculated *E. coli O157:H7*. They argued that the antimicrobial might have use as a vapor. When 5% to 20% mustard flour was used as a natural source of allyl isothiocyanate in ground beef, inoculated *E. coli O157:H7* population declined but no effect on spoilage microflora was noted (Nadarajah et al. 2005b). Sensory evaluation results showed that panelists could detect mustard treatment, but considered mustard-treated meat to be acceptable.

The influence of various herb decoctions to control the major poultry spoiler *Yarrowia lipolytica* was investigated by Ismail et al. (2001). Basil, marjoram, oregano, or rosemary decoction-saturated cellulose disks showed no inhibition zones on lawn-inoculated *Y. lipolytica* agar plates, compared with sage and thyme decoctions. These latter decoctions were capable of initial 0.45 $\log_{10}$ CFU/g *Y. lipolytica* reduction on chicken wings, although the effect was diminished after 3 days of storage. No effect on aerobic plate count was observed.

Ha et al. (2001) incorporated 0.5 to 1.0% grapefruit seed extract in multilayered polyethylene films and investigated its activity against spoilage microflora of wrapped ground beef. The authors observed that total plate count was lower in grapefruit seed extract wrapped beef compared to wrapped beef throughout the study, with a 1.0 $\log_{10}$ CFU/g difference on day 13 of storage at 3°C.

We believe essential oils and plant extracts probably have limited application for shelf life extension of fresh meats due to flavor changes associated with the quantities needed to achieve meaningful results. On the other hand, in applications where flavoring is expected, such as with seasoned or marinated products, use of essential oils may be beneficial to extend shelf life.

Enzymes

Lysozyme is a naturally occurring (human saliva, egg white), 14.6 kDa, single-peptide protein that has antimicrobial activity due to its enzymatic ability to hydrolyze β(1–4) glycosidic linkages in bacterial cell walls (Proctor and Cunningham 1988). It is more active against Gram-positive bacteria, and activity against Gram-negatives can be increased by use of membrane disrupting agents (detergents and chelators), such as EDTA (Padgett et al. 1998). Because of this narrow activity range, most studies use lysozyme in combination with other antimicrobials.

Gill and Holley (2000) showed that combined lysozyme, nisin, and EDTA treatment of ham and bologna sausages reduced populations of *B. thermosphacta* to nondetectable levels for up to 4 weeks, while during storage at 8°C, growth of *Lactobacillus curvatus, Leuconostoc mesenteroides,* and *Listeria monocytogenes* was slowed for up to 3, 2, and 2 weeks, respectively. Cannarsi et al. (2008) showed that the combination of 0.5% lysozyme and 2% EDTA extended the shelf life of chilled buffalo meat, with an antimicrobial affect on all microflora present, including *B. thermosphacta*. Nattress and Baker (2003) combined nisin and lysozyme as an antimicrobial treatment on pork loins, with successful inhibition of lactic acid bacteria and preferential growth of Enterobacteriacea. However, the authors

noticed that aerobically displayed nisin-lysozyme treated meat spoiled sooner than untreated meat. They attributed this to inhibition of lactic acid bacteria and a resultant shift to putrefactive bacterial spoilers. In summary, a combined lysozyme/nisin/EDTA mixture may be a promising tool for extension of the shelf life of anaerobically packaged meats by inhibiting lactic acid bacteria, which is the predominant bacterial spoilage group capable of growth in such conditions.

Other Antimicrobials

There are a few other novel antimicrobial agents that have been reported to eliminate food-borne pathogens and prevent meat spoilage. Examples include acidified sodium chlorite, trisodium phosphate, ozonated water, and electrolyzed water. Acidified sodium chlorite solution is a mixture of sodium chlorite and a GRAS food-grade organic acid. A chemical reaction between the two produces chlorous acid, which is the main active agent. Numerous studies have shown acidified sodium chlorite activity against *L. monocytogenes, S. aureus, Bacillus cereus, Salmonella* Enteritidis, *E. coli*, *C. jejuni*, and *Yersinia enterocolitica* (Castillo et al. 1999; Beverly et al. 2006; Özdemir et al. 2006; del Rio et al. 2007). Bosilevac et al. (2004b) evaluated the influence of 300 ppm acidified sodium chlorite spray in 50/50 and 90/10 lean beef trimmings and ground beef made from those trimmings on aerobic plate count. They found that acidified sodium chlorite was most effective on 50/50 lean trimmings, reducing counts by 1.1 $\log_{10}$ CFU/g. Counts in ground beef chubs were reduced by 1.0 to 1.5 $\log_{10}$ CFU/g until day 20 at 2°C, while maintaining acceptable sensory ground beef quality. Gill and Badoni (2004) compared 0.02% peroxyacetic acid, 0.16% acidified sodium chlorite, 2% lactic acid, and 4% lactic acid on the natural flora of beef brisket from two slaughtering plants. They found that peroxyacetic acid and acidified sodium chlorite were less effective than 4% lactic acid against aerobes and coliforms. They also found that activity was influenced by plant location. Acidified sodium chlorite is approved by the USDA for poultry and red meat applications at 500 to 1200 ppm (21CFR173.325).

Phosphates are known to inhibit spoilage microorganisms (Marshall and Jindal 1997; Kim and Marshall 1999). Castillo et al. (2005) showed that a 7.6% trisodium phosphate dip reduced initial aerobic mesophilic count of chicken wings by 1.5 $\log_{10}$ CFU/g, resulting in a shelf life extension of 2 to 3 days during storage at 4°C. Numerous other investigations showed mixed results for trisodium phosphate effectiveness, both against meat spoilers and pathogens (Ismail et al. 2001; Lin and Lin 2002; Pohlman et al. 2002; Fabrizio and Cutter 2005; Özdemir et al. 2006; del Rio et al. 2007). Because trisodium phosphate requires high concentrations for effectiveness, the resultant cost and soapy meat surface and flavor may limit its use.

Ozone is a highly oxidative gas that easily decomposes (especially under UV light) to produce oxygen. Ozone is on the FDA GRAS list and its current use in meat processing is limited to water and surface sanitizer (oxidative power) and degreaser roles. According to Lazar (2006a), the ability of ozonated water to both continuously clean and sanitize eliminates the need for a sanitation shift break during production, making meat processing plants productive 24/7. Several researchers investigated the possibility of using ozonated water to decontaminate meat (Kim et al. 1999; Castillo et al. 2003; Kalchayanand et al. 2008), although most agree that a major drawback is its ineffectiveness in the presence of organic matter (Moore et al. 2000). The inactivity of ozone in the presence of organics and its short half-life makes meat decontamination difficult. These

drawbacks coupled with worker safety issues related to ozone inhalation hazards limit widespread adoption of ozone technology.

Electrolyzed water is produced by passing 12% NaCl solution across a bipolar membrane with an electrode on each side, resulting in an acidic solution called electrolyzed oxidizing water and an alkaline solution (Fabrizio and Cutter 2004). Electrolyzed oxidizing water has a low pH (2.3 to 2.7), high oxidation-reduction potential (ORP, >1000 mV), and free chlorine (25 to 80 ppm) (Huang et al. 2008). Thus, electrolyzed oxidizing water antimicrobial effect is due to the combined action of low pH, high ORP, and free chlorine. Fabrizio and Cutter (2005) investigated the influence of electrolyzed oxidizing water (pH 2.3 to 2.7, 1150 mV ORP, ~50 ppm free chlorine) on *L. monocytogenes* inoculated on beef frankfurters stored for 7 days at 4°C. Electrolyzed oxidizing water caused only a slight reduction (<0.5 $\log_{10}$ CFU/g) in pathogen numbers, but was more effective than 2% acetic acid and 10% trisodium phosphate. Similarly, Fabrizio and Cutter (2004) showed no significant influence of electrolyzed oxidizing water applied to fresh pork inoculated with *L. monocytogenes* and *Salmonella* Typhimurium. As with most antimicrobials, complex organic composition of meat tends to lessen the ability to inactivate bacteria. Novel approaches are needed to retain the antimicrobial activity of these agents in meat matrices.

Active Antimicrobial Packaging

Antimicrobial packaging can involve utilization of several concepts (Table 14.6). Quintavalla and Vicini (2002) noticed that microbial contamination of intact fresh muscle occurs mostly at the surface and antimicrobials applied directly on the surface could be easily inactivated by meat components. Therefore, antimicrobial packaging could be protective for antimicrobials, allowing them to slowly migrate to the product surface over extended periods of time without deactivating them. Siragusa and Dickson (1992) investigated the possibility of organic acid use in bioactive edible packaging in the form of calcium alginate gels. On lean beef tissues inoculated with *L. monocytogenes*, they showed that lactic acid (1.7% v/v) immobilized in alginate reduced pathogen counts by 1.3 $\log_{10}$ CFU/g compared to a 0.03 log decrease from the acid treatment alone. Similarly, acetic acid (2% v/v) reduced counts by 1.5 (alginate) and 0.25 $\log_{10}$ CFU/g, respectively. Cutter and Siragusa (1996, 1997) investigated the impact of nisin alone and nisin in calcium alginate gels as a surface treatment antimicrobial on refrigerated beef lean and adipose tissues inoculated with the spoiler *B. thermosphacta*. Untreated, alginate-treated, or nisin alone did not suppress bacterial growth (>6 $\log_{10}$ CFU/cm^2 by day 7), while treatment with nisin-alginate did suppress growth (2.4 $\log_{10}$ CFU/cm^2 by day 7). Bacteriocin titers from both tissues were greater in nisin-alginate vs. nisin-only samples after day 7 of incubation. Active packaging in the form of edible films is advantageous because it retains antimicrobial activity and steadily delivers the antimicrobial to the contaminated meat surface.

Table 14.6. Types of active antimicrobial packaging

- Addition of sachets/pads containing volatile antimicrobial agents (contact with product through headspace)
- Incorporation of volatile/non-volatile compounds directly into packaging
- Coating or absorbing antimicrobials onto polymer surfaces
- Chemical bonds (ion or covalent linkages) between antimicrobials and packaging material
- Using polymers that are inherently antimicrobial
- Edible packaging containing antimicrobials

From Appendini and Hotchkiss (2002)

Table 14.7. Commercial active antimicrobial packaging

Technology	Antimicrobial	Trade name	Manufacturer
Silver substituted zeolite paper, plastic	Silver	AgIon®	AgIon Technologies LLC (USA)
Triclosan plastic	Triclosan	Microban®	Microban Products (UK)
Allylisothiocyanate labels, sheets	Allylisothiocyanate	WasaOuro®	Lintec Corp. (Japan)

Commercial antimicrobial packaging is available (Table 14.7). Silver-substituted zeolite technology developed in Japan introduces a thin layer (3 to 6 μm) of Ag-zeolite on the surface of common food contact polymers. Zeolite slowly releases antimicrobially active silver in the food, provoking an antimicrobial effect. AgION® Silver Ion Technology received U.S. FDA approval for use on all food-contact surfaces (FDA 2008). Triclosan-impregnated food packaging materials recently have been approved in the European Union as long as migration into food products does not exceed 5 mg per 1 kg (Quintavalla and Vicini 2002). Triclosan is a nonionic, broad-spectrum antimicrobial agent commonly used in personal hygiene items, such as soaps and detergents. Cutter (1999) investigated triclosan-incorporated plastic (1,500 ppm triclosan, Microban®, Microban Products Co., United States) against bacteria on irradiated, inoculated, and vacuum-packaged beef surfaces. Except slight reduction in *B. thermosphacta*, triclosan failed to control populations of *Salmonella*, *Escherichia*, or *Bacillus.* The lack of triclosan activity was speculated to be due to triclosan inactivation by fatty acids and adipose tissues.

Some inherently antimicrobial polymers include chitosan (discussed previously) and irradiated nylon (Quintavalla and Vicini 2002; Yingyuad et al. 2006). Irradiated nylon has surface-bound amine groups that are effective against numerous pathogens, although we are unaware of its use in meat applications. Ouattara et al. (2000) investigated chitosan as a food-packaging matrix for the incorporation of acetic and propionic acids and their slow release into bologna, cooked ham, and pastrami. Chitosan films inhibited indigenous Enterobacteriaceae and surface-inoculated *Serratia liquefaciens*, but failed to affect growth of lactic acid bacteria.

Scannell et al. (2000) investigated the immobilization of lacticin and nisin in cellulose-based paper and polyethylene/polyamide plastic for spoilage prevention of cooked sliced ham. Lacticin was unsuccessful in binding to plastic, while nisin bound well and retained its activity for 3 months. Nisin-treated cellulose paper applied to cooked sliced ham packaged in MAP and stored at 4°C had a slight influence on total plate count over a 24-day storage period (1 $\log_{10}$ CFU/g lower counts compared to control at the end of the trial). In contrast, this treatment successfully controlled lactic acid bacteria (not detectable for nisin-treated vs. 4 $\log_{10}$ CFU/g increase for control at the end of the trial). Ming et al. (1997) used pediocin-coated cellulose casings to control *L. monocytogenes* growth on surface-inoculated fresh turkey breast, fresh beef, and ham. *L. monocytogenes* counts on pediocin-treated casing did not increase (3 $\log_{10}$ CFU/ml in rinsates) over a 12-week storage time, but increased to 5.5, 6.0, and 4.0 $\log_{10}$ CFU/ml on untreated casing for ham, turkey breasts, and beef, respectively. Franklin et al. (2004) showed that packaging films coated with cellulose-based solution containing 7,500 and 10,000 IU/ml nisin significantly inhibited *L. monocytogenes* growth in vacuum-packaged, surface-inoculated hot dogs. Counts remained at a constant 3 $\log_{10}$ CFU/package level compared to controls, which increased to 9 logs

within 60 days of storage at 4°C. Similar antimicrobial effects may be seen with lactic acid spoilage bacteria, since the behavior of *L. monocytogenes* is similar to this group; however, confirmation studies are needed.

Siragusa et al. (1999) incorporated nisin in a polyethylene-based plastic film and observed its activity against inoculated *B. thermosphacta* on vacuum-packaged beef surface tissue sections. An initial reduction of 2.0 $\log_{10}$ CFU/cm^2 of *B. thermosphacta* was observed within 2 days of refrigerated storage at 4°C. After 20 days of storage, samples with nisin-containing plastic showed significantly fewer bacterial numbers compared with control, 5.8 vs. 7.2 $\log_{10}$ CFU/cm^2, respectively.

Edible antimicrobial coatings, besides their main function, also hold meat juices, reduce rancidity and myoglobin oxidation (oxygen barrier), restrict volatile compound loss, and reduce off-odor absorption during the storage of refrigerated meats (Kerry et al. 2006). A calcium alginate edible film containing nisin, citric acid, EDTA, and Tween-80 was effective against *Salmonella* Typhimurium on chicken skin, with count reductions ranging from 2 to 3 $\log_{10}$ CFU/ml after 72-hour exposure at 4°C (Natrajan and Sheldon 2000b). Dipping of uninoculated chicken drumsticks in the same solution followed by overwrap packaging in foam tray packs containing nisin-treated film/absorbent tray pads and storage at 4°C caused projected shelf life extension up to 2.2 days based on initial psychrotrophic plate count reduction of up to 2.3 $\log_{10}$ CFU/g (Natrajan and Sheldon 2000b). Marcos et al. (2007) demonstrated the effectiveness of enterocin-containing biodegradable films (alginate, zein, and polyvinyl alcohol) to control *L. monocytogenes* on air-packed and vacuum-packed sliced cooked ham. Millette et al. (2007) incorporated nisin in alginate film and beads through covalent links and were able to reduce *S. aureus* numbers on beef muscle slices (film) or ground beef (beads) at 4°C. A summary of investigated antimicrobial active packaging is presented in Table 14.8.

Other NonThermal Technologies

We are aware of a few other novel food processing technologies, but meat industry applications might be technically or economically challenging. Some equipment-intensive applications may have future potential if the cost of production becomes economically viable. For example, pulsed electric fields use bursts of high-intensity electric pulses to inactivate microorganisms. The main problem with this method for meat applications is the requirement for the food to be liquid (Marth 1998). Pulsed high-intensity light treatment consists of xenon flash-lamps capable of producing brief (<2 ms) flashes of wide-spectrum (200 to 1,100 nm) light to kill bacteria. Pulsed high-intensity light has been approved for treatment of foods and food contact surfaces (FDA 1996). Its potential for meat industry applications is yet to be determined. Marth (1998) mentioned low penetration power and the possibility of lipid oxidation as drawbacks of this technology. Cold plasma technology has reported antimicrobial effects but has not yet been applied to meat products (Critzer et al. 2007; Niemira and Sites 2008).

Novel Thermal Technologies

High-frequency heating is considered a thermal process where the meat product is heated through microwave or radiofrequency energy, which causes oscillation of water molecules, friction, and resultant heat generation (Hugas et al. 2002). The permitted frequency bands include 13.56, 27.12, and 40.68 MHz for radiofrequency heating and 433, 915, 2,450, and 5,800 MHz for microwave usage (Aymerich et al. 2008). All microwave ovens share a similar design that includes a magnetron device as a power source and a waveguide to bring radiation to

Table 14.8. Active antimicrobial packaging

Meat product	Target	Antimicrobial	Packaging	Results	Reference
Air and vacuum packed sliced cooked ham	*Listeria monocytogenes*	Enterocin incorporation	Biodegradable/ edible matrices of alginate, zein, and polyvinyl alcohol	Reduction in growth during storage	Marcos et al. 2007
Beef slices, ground beef	*Staphylococcus aureus*	Nisin incorporation	Alginate, as film or beads	Population reduction during storage	Millette et al. 2007
Chilled vacuum-packed grilled pork	Aerobic plate count, oxidation (peroxide value)	Chitosan coating	Chitosan	APC and peroxide value reductions	Yingyuad et al. 2006
100% N_2 MAP ground beef patties	Mesophilic aerobic bacteria	Allyl isothiocyanate soaking	Cellulose (filter paper) disks	Reduction in growth during storage	Nadarajah et al. 2005b
Whole beef muscle	*Escherichia coli* O157:H7, *Salmonella typhimurium*	Oregano essential oil incorporation	Alginate film	Pathogen population reduction during storage	Oussalah et al. 2006
Whole beef muscle	*Escherichia coli* O157:H7, *Pseudomonas* spp.	Oregano essential oil incorporation	Milk protein-based film	Pathogen population reduction during storage	Oussalah et al. 2004
Ground beef	Aerobic and coliform bacteria	Grapefruit seed extract co-extrusion or solution coating	Polyethylene	Reduction in growth during storage	Ha et al. 2001
Vacuum-packed bologna, cooked ham, pastrami	Enterobacteriaceae, *Serratia liquefaciens*	Acetic and propionic acid incorporation	Chitosan matrix	Reduction in growth during storage	Ouattara et al. 2000

Vacuum packed beef carcass surface tissue sections	*Brochothrix thermosphacta*	Nisin co-extrusion	Polyethylene	Initial population reduction	Siragusa et al. 1999
Lean and adipose beef carcass tissues	*Brochothrix thermosphacta*	Nisin	Alginate gel	Reduction in growth during storage	Cutter and Siragusa 1996
Lean beef surfaces	*Brochothrix thermosphacta, Salmonella typhimurium, Escherichia coli* O157:H7	Triclosan incorporation	Plastic	No or little effect	Cutter 1999
Fresh broiler skin	*Salmonella typhimurium,* mesophiles, psychrotrophs	Nisin, EDTA, citric acid, Tween 80 incorporation	Polyvinyl chloride, polyethylene, nylon Alginate film, agar film	Salmonella population reduction during storage, Mesophiles/ psychrotrophs reduction in growth during storage	Natrajan and Sheldon 2000a, 2000b
Fresh turkey breast, ham, and fresh beef	*Listeria monocytogenes*	Pediocin coating	Cellulose casings	Growth inhibition	Ming et al. 1997
Lean beef tissue	*Listeria monocytogenes*	Lactic acid, acetic acid	Alginate gel	Population reduction during storage	Siragusa and Dickson 1992
Hot dogs	*Listeria monocytogenes*	Nisin	Methylcellulose	Growth inhibition	Franklin et al. 2004
Ham	Lactic acid bacteria	Nisin	Cellulose inserts	Growth inhibition	Scannell et al. 2000

a heating chamber. A radiofrequency oven is equipped with a generator coupled with a pair of electrodes and is known as an RF applicator. According to Murano (2003), microwaves can penetrate to a depth of 5 to 7 cm, which results in faster cooking and fewer nutrient changes compared with conventional ovens. Meat spoilage is prevented by simple cooking (thermal inactivation of spoilage microorganisms). The alternative nonthermal effects of microwaves have not been confirmed (Aymerich et al. 2008). For example, Yilmaz et al. (2005) showed 2 $\log_{10}$ CFU/g reductions in total microbial flora after microwaving meat balls at 2,450 MHz frequency, in an 800 W oven, for 300 seconds. The major technical drawback of high-frequency heating is nonuniform heating. Aymerich et al. (2008) noted that radiofrequency heating and microwaves tend to create cold and hot spots within meat products due to differences in geometry, fat distribution, and dielectric properties. Another drawback is the high cost of equipment and maintenance.

Ohmic heating involves heating foods between two electrodes by passing electrical current through the product. The heating rate is directly proportional to the electrical current and electrical conductivity of the food product. According to Piette et al. (2004), ohmic heating is currently successfully applied to liquid products, with industrial usage for solid meat products yet to come. The main disadvantage of ohmic heating is the significant energy cost with recommended density of treatment intensity of 4,000 A/m^2 (Hugas et al. 2002)

Novel Carcass Decontamination Techniques

Several post-harvest decontamination techniques have been authorized by USDA-FSIS to reinforce zero fecal contamination, as well as to extend shelf life, improve microbiological quality, and remove pathogens from carcasses (USDA-FSIS 1996). Hot water and/or steam (minimum 82°C) treatment of carcasses followed by vacuuming has been approved for fecal decontamination, if contamination is no more than one inch. This process can achieve 3.3 $\log_{10}$ CFU/cm^2 reduction in total bacterial counts (initial 6.4 $\log_{10}$ CFU/cm^2) and 5.5 $\log_{10}$ CFU/cm^2 reduction in *E. coli O157:H7* counts (initial 7.6 $\log_{10}$ CFU/cm^2) (USDA-FSIS 1996). Moreover, additional testing showed that this technology was more effective than convenient knife-trimming by 0.5 $\log_{10}$ CFU/cm^2. Others have found that a novel vacuum-steam-vacuum cycle treatment reduces microbial counts on smaller animal carcasses such as fish (Kozempel et al. 2001).

Skinned carcasses can be rinsed with aqueous solutions of food-grade organic acids (1.5 to 2.5%), such as acetic, lactic, or citric acid (USDA-FSIS 1996). Snijders et al. (1985) showed that lactic acid treatment, when applied to a hot carcass surface, reduced aerobic plate count by 1.5 $\log_{10}$ CFU/cm^2. Goddard et al. (1996) showed that beef loins treated with lactic and acetic acids had no difference in color or odor compared to untreated samples. A potential concern of acid application is the possibility of pathogens adapting to an acidic environment and eventually surviving in the meat-processing environment or human gastrointestinal tract (Samelis et al. 2002; Yuk and Marshall 2004, 2005). Other approved antimicrobial rinses include trisodium phosphate (8% to 12%, 32°C to 43°C) and hot water/steam (min 74°C for more than 10 seconds).

Naidu (2002) utilized activated lactoferrin (a natural iron-binding protein) as a spray treatment of carcasses. The authors claimed activity not only against bacterial pathogens, but also against meat spoilers such as *Pseudomonas* spp. and *Klebsiella* spp. Several researchers (Kim and Slavik 1996; Cutter et al. 2000; Bosilevac et al. 2004a) demonstrated the effectiveness of cetylpyridinium chloride treatment of poultry and beef to reduce pathogens and aerobic plate counts.

Cetylpyridinium chloride (1%) rinsed pre-evisceration beef carcasses decreased aerobic plate counts from 4.9 to 3.2 $\log_{10}$ CFU/100 cm^2. Chlorine dioxide (Ashland Specialty Chemical Company), 1,3-dibromo-5,5-dimethylhydantoin (Albermarle Corporation), and a mixture of peroxyacetic acid, acetic acid, hydrogen peroxide, and 1-hydroxyethylidene-1,1-diphosphonic acid (Ecolab, Inc) also have been approved as poultry or beef carcass decontamination treatments (FDA 2008).

Summary

When considering novel technologies to control microbial spoilage of meats and cutting-edge improvements in shelf-life extension, manufacturers usually consider cost of implementation and consumer acceptance. For example, plant-extract essential oils may be a low-cost, low-tech solution that is accepted in developing countries but may not be favorably viewed in developed markets due to consumer reluctance to buy flavored meat products. Conversely, some high-tech interventions may be too costly to implement even in the most developed markets or may meet with substantial consumer purchase reluctance. An example here could be the slow adoption of irradiation technology in the United States. Nevertheless, continued research in developing novel thermal and nonthermal technologies promises to offer industry and consumers a variety of processes and products. Prudent manufacturers will embrace those applications that provide a competitive advantage in the marketplace. Therefore, we believe that the evolution of meat-processing technologies will continue to add shelf life to packaged products and enhance the eating quality of this food sector.

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Chapter 15

Plant Cleaning and Sanitation

Stefania Quintavalla

Introduction

Sanitation is broadly defined as the formulation and application of procedures that establish an environmental state that promotes cleanliness and protects public health. It has many implications when applied to the food-processing environment. A safe water supply and distribution system, unpolluted air, sound construction of facilities, effective pest control, proper waste handling, disposal, and treatment are critical elements of environmental sanitation (Quintavalla and Barbuti 2007).

Cleaning and sanitation are among the most important activities in the meat products plants, as these measures provide the necessary environment for proper meat handling and processing. There are direct links between inadequate sanitation and the contamination of meat and poultry products with pathogenic bacteria. Proper cleaning and sanitation is becoming increasingly important in modern meat processing as more perishable and hygienically sensitive meat products come on the market, particularly convenience foods such as prepackaged portioned chilled meat, vacuum- or modified-atmosphere packaged sliced meat products, both cooked and uncooked (ham, sausage, etc). These products are examples of "ready-to-eat" products or RTE, as they are usually taken right from the package and consumed as they are, with little or no heat treatment. Sanitation is critical for ensuring that RTE products do not become cross-contaminated. Cross-contamination is the transfer of bacteria and possible pathogens to the exposed RTE product before packaging. These bacteria may come from the environment, from the employees, or from the equipment. This is the reason why factors like housekeeping, personal hygiene, training and education of the personnel, plant layout, design of equipment and machines, characteristics of material selected, and the maintenance and general condition of the plant should be addressed when deciding on cleaning and disinfection procedures.

Sources of Contamination in Meat Processing Plants

Raw Materials

The first source of contamination is the raw meat. The muscle tissues of healthy living animals are nearly free of microorganisms. Contamination of meat comes from external surfaces, such as hair, skin, and the gastrointestinal and respiratory tracts during slaughtering. Initial microbial contamination of meats results from the introduction of microorganisms into the vascular system when contaminated knives are used for the exsanguination; the vascular system rapidly disseminates these microorganisms throughout the body. Raw meat characteristic pathogens are *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter* (in poultry), and *Escherichia coli* O157:H7. Contamination subsequently occurs with the

introduction of microorganisms on the food-contact surfaces in operations performed during slaughtering, cutting, processing, storage, and distribution of meat.

Equipment

Contamination of equipment occurs during production; even with hygienic design features, equipment may collect microorganisms and other debris from the air, as well as from employees and materials during production.

Employees

Plant personnel are among the most significant reservoirs and vectors of microorganisms, chemical residues, and foreign material in a food facility. The skin, hands, hair, nose, and mouth harbor microorganisms that can be transmitted through direct routes to food-contact surfaces during processing, packaging, and preparation. The transfer of contaminants can also occur indirectly via personal equipment, such as clothing, footwear, and tools used in daily tasks.

Air and Water

Dust, pollen, and mold spores, as well as airborne microorganisms, are present in ambient air, and these contaminants can easily find their way into the product. Air withdrawn from the room to use in food-processing areas, such as preparation and packaging areas, should require filtration or some other means of removing particulates. Moreover, the reduction of airborne mold spores content in ripening and aging rooms typical of dry-meat and fermented dry-meat products processing plants is of paramount importance.

Water is used as a cleaning medium during the sanitation operation and as an ingredient added in the formulation of various processed meats. Water may contain enteric pathogens (bacteria, viruses, and parasites), as well as chemicals and other toxic substances that can be transmitted to humans through food products. The importance of water quality will be outlined later in this chapter.

Cleaning and Disinfection Procedures

Cleaning is the removal of dirt and organic substances such as fat and protein particles (commonly called soil) from surfaces of walls, floors (nonfood-contact surfaces), and tools and equipment (food-contact surfaces), leaving surfaces clean. With cleaning procedures, high numbers of microorganisms (90% or more) will be removed. However, many microorganisms can persist on these surfaces, and their inactivation requires antimicrobial treatments, carried out through physical means (hot water, steam, UV) or with the application of chemical substances (sanitizers or disinfectants), which should be effective against microorganisms but should not affect human health through hazardous residues or cause corrosion of the equipment.

Cleaning and sanitation (or disinfection) procedures in the meat industries are complex processes depending on the surfaces to be treated and the kind of contamination to be removed. Selection of suitable chemicals for cleaning or for sanitation may require special knowledge and could represent a difficult task for the personnel involved. However, staff must be made aware that efficient cleaning and disinfection is of utmost importance for product safety and quality.

Detailed Standard Operating Procedures (SOP) should be prepared to describe specific methods, types of cleaning/sanitizing solutions to be used, the timeline for performing the tasks on different pieces of equipment, and areas for routine cleaning and sanitation operations. Records should be kept to show

that each step has been performed according to the procedure.

The level and frequency required for additional periodic cleaning and sanitation primarily depends upon the effectiveness of the routine program. Even with a rigorous routine program, food residues can accumulate over time, particularly in hard to reach areas. SOP documentation needs to clearly describe methods for each piece of equipment or work area. When needed, periodic cleaning and sanitizing should follow the same steps as the routine procedures, except for changes to detergent and sanitizer use.

For the whole process, a few distinct operations are involved. These are clearly distinct operations but are linked together in such a way that the final result will not be acceptable, unless all are carried out correctly.

Figure 15.1 shows the different operations that should be included in a complete cleaning procedure.

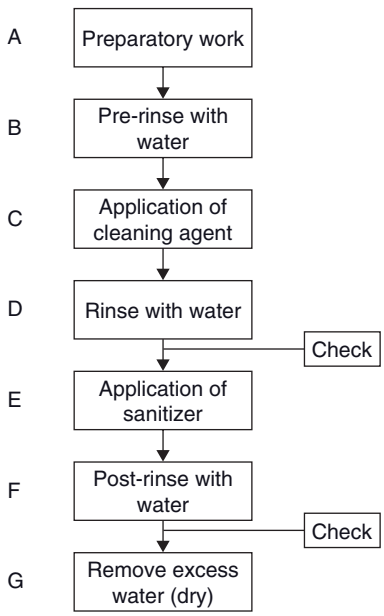

Figure 15.1. Recommended operations for an effective cleaning and sanitizing procedure.

Step A: Preparatory Work

In this phase, the processing area should be cleared of remaining products, containers, and other loose items. Machines and conveyors should be dismantled so that all locations where microorganisms can accumulate become accessible for cleaning and disinfection. All electrical connections and other sensitive systems should be protected against water and the chemical used. Before use of the cleaning agent, food debris should be removed manually with a dry brush or broom or shovel. In this step, using large amounts of water to remove this material would be extremely wasteful and eventually cause the clogging of the drains and the overloading of waste-water treatment facilities.

Step B: Pre-Rinse with Water

All surfaces should be further prepared for the use of cleaning agents for pre-rinse activity, preferably with water at 43°–55°C, starting at the top of all processing equipment and directing all soil down to the floor.

Step C: Application of Cleaning Agent

The effectiveness of a cleaning procedure in general depends upon: (1) the type and amount of material to be removed; (2) the chemical and physicochemical properties of the cleaning agent at the concentration, temperature, and exposure time used; (3) the mechanical energy applied (impact of waterjet, manual work, stirring effect, etc); and (4) the condition of the surface to be cleaned.

In meat plants, the type of residues to be removed are mainly organic matter (proteins and fats) and, to a lesser extent, inorganic matter such as salts and additives. The first are most effectively removed by strongly alkaline detergents (especially caustic soda, NaOH). In addition, combinations of acidic detergents (especially phosphoric acid) and

nonionic surfactants have proved to be effective against organic matter. Inorganic matter is most effectively removed by acid cleaning agents.

Cleaner application can be handled by using brushes or scrapers for dismantled equipment, or in general, for smaller surfaces to be cleaned. Application through a centralized or portable high pressure-low volume system, using 50° to 55°C water could be a solution for large surfaces such as floor and wall areas, as well as working tables, containers, and equipment. The pressure should be between 30–70 bar and the spraying nozzle ≤15 cm from the surface to be cleaned; otherwise, the pressure being applied decreases rapidly. If hot water is used, the temperature should be 55°C at the nozzle, in order to achieve sufficiently high temperatures at the surfaces, in particular for fat removal.

Soak time prior to rinse-down should not exceed 20 minutes.

To be effective, detergents should be able to wet and penetrate soil, emulsify fat, disperse and suspend soil, and counteract water hardness. In addition, they should prevent soil from redepositing on clean surfaces and be noncorrosive to equipment. No single detergent combines all these traits, so formulations of compounds are tailored for each type of cleaning operation. Commercially available cleaning agents in modern cleaning practices are complex compositions of alkaline, acid, or neutral chemical substances. In order to improve their soil-loosening properties, surface-active agents, also called surfactants or detergents, are added. Detergents decrease the surface tension of water, so water can penetrate into the small spaces between soil particles and surfaces, where those particles are attached, thus facilitating their removal.

Alkaline cleaning compounds are used for the removal of organic soil, protein residues, and fats. Strongly alkaline compounds (pH greater than 13), for example sodium hydroxide, are used to remove heavy burnt-on soil, encountered in high-temperature processing such as ovens and smokehouses. These compounds are very corrosive, and inhalation of the vapors can cause respiratory damage, so they are used in specialized cleaning operations such as CIP (Cleaning-In-Place) procedures. The moderately alkaline compounds (pH 10–12), such as sodium metasilicate, have good dissolving powers and are formulated in detergents to aid the removal of fats and grease. Mildly alkaline compounds (pH 7–10) are used for manual cleaning. An example is sodium carbonate, which is used in many manual and heavy-duty detergent mixtures as a buffering agent and for its water softening capabilities.

Acid cleaning agents are used particularly for removal of encrusted residues of dirt or protein, or inorganic deposits (“scaling”). Strong inorganic acids, hydrofluoride and hydrochloride, because of their corrosiveness to stainless steel, are usually used to remove heavy scale deposits found on steam-producing equipment or boilers. Organic acids, such as citric and hydroxyacetic acid, are less corrosive and are used in manual cleaning formulations.

In practice, alkaline and acid cleaning substances should be used alternatively. The alkaline agent should be the substance used for routine cleaning, but every few days an acid substance should be employed instead in order to remove encrusted residues, scaling, etc.

A relatively new cleaning method in the food industry, in particular for larger-scale plants, is foam or gel cleaning. Water foam containing detergents and other cleaning agents is sprayed on wetted walls, floors, and surfaces of equipment. The foam does not immediately run off but clings to the surfaces. It allows a longer-term contact on the surfaces to be cleaned. After a sufficient contact time (min. 15 min), the foam is washed down with water (usually low-pressure water spray).

Step D: Rinse with Water

Complete removal of cleaning agents is important, since residues may completely inhibit the effect of the sanitizer that is applied next. All equipment should be rinsed within 20 to 25 minutes after cleaning compound application, using the same pattern as the pre-rinse and detergent application. A common procedure may consist in the use of water at 45°–55°C with a pressure of 30 bar and 20 L water per minute. After this step, a first inspection of the equipment surfaces, even touching as necessary, is recommended.

Step E: Application of Sanitizer (Disinfection)

Cleaning reduces a substantial amount of microorganisms but it does not have the potential to eliminate all surface contamination. Persistent microorganisms will continue to grow in number by using the remaining protein as nutrients, and they pose a further risk to the foods to be processed. Following the initial rinse that removes gross soil, detergent application, mechanical scrubbing, and a final wash to remove detergent, sanitizers (or "disinfectants") are applied to complete the procedure. This step is called "disinfection" and can be accomplished with physical treatments such as hot water, steam, or UV irradiation, or by means of chemical compounds.

Hot-water sanitizing is commonly used where immersing the contact surfaces is practical (e.g., small parts, utensils). Both time and temperature are important. Depending upon the application, sanitation may be achieved by the immersion of parts or utensils in 77°C to 85°C water for 5 minutes to 45 seconds, respectively.

For example, many countries still require the sanitation of knives used in meat processing by brief immersion in water at no less than 82°C. However, many current international regulations allow science-based equivalent alternative procedures to 82°C dipping to be used (Eustace et al. 2007; USDA 2004). A 15-second immersion time in water at 82°C or warm water (approximately 50°C) containing quaternary ammonium compounds was suggested as being effective in reducing bacterial numbers by about 3 log CFU/cm^2 (Taormina and Dorsa 2007).

A recent study (Goulter et al. 2008) demonstrated that dipping knives in water for shorter times at higher temperatures, for example, 82°C for 20 seconds, or for longer times at lower temperatures (70°C for 45 s), can produce equivalent inactivation of the tested bacteria (more than 5-log reduction against *Escherichia coli* and *Listeria monocytogenes*). Pre-rinsing knives at 40°C increased the performance of the subsequent dipping step. As such, a pre-rinse should be implemented where possible to increase the general status of knives in those meat-processing operations in which it is recommended.

Hot-water sanitation is easy to apply, readily available, effective for a broad range of microorganisms, and noncorrosive. However, it can contribute to the formation of biofilms.

In meat-processing plants, chemical sanitizers are preferred; concentration, exposure time, temperature, pH, water hardness, surface cleanliness, and bacterial attachment are the most important factors affecting sanitizer activity (Marriott 1999). Important properties of a sanitizer are: ability to provide rapid antimicrobial activity against a range of organisms; easy availability, inexpensive cost, and readiness to use; stability and resistance to the presence of organic matter, detergent, and soap residues; ability to work in a wide range of pH, water hardness, and temperatures; lack of toxicity to humans; and noncorrosive and water-soluble action (Guthrie 1988).

The following table (Table 15.1) provides information about appropriate chemicals for sanitizing in meat-processing facilities.

Table 15.1. Chemicals for sanitizing meat-processing facilities

Compound	Use concentration (ppm)*	Optimum use temperature	Advantage	Disadvantage	Relative cost
Sodium hypochlorite	100–200	Room temperature	Effective against a wide range of bacteria	Corrosive if not used properly, organic matter reduces activity	+
Iodophor	25	Room temperature (<35°C)	Stable, long shelf life, less corrosive	Can stain, less effective against bacterial spores	+++
QUATS	100–200	Room temperature	Stable, long shelf life, less corrosive, additional cleaning properties	Leaves residues, high foaming, low efficiency on bacterial spores	++
Peroxyacetic acid	100–200	Chilled to room temperature	Effective against a wide range of organisms, biodegradable	Hazardous in concentrate form	++
Acid-anionic	200–400	Room temperature to hot	Less corrosive	Narrow spectrum of antimicrobial activity	+++
Isopropyl alcohol	70% v/v	Room temperature	Fast acting	Not effective against spores, flammable	++

*Sources: Giese (1991); Cords et al. (2005)

Chlorine is the most commonly used sanitizer in food production facilities, followed by quaternary ammonium compounds or QUATs (the only sanitizer group with true residual activity) and peroxides.

In order to achieve a maximum disinfection effect, it is recommended to alternate periodically the type of chemical sanitizer applied: this procedure will help to counteract the development of resistant bacteria in the meat-processing plant.

Frequency of disinfection depends on the necessary requirements:

- Several daily disinfections (by hot water or chemicals) are necessary for hand tools, meat saws, and cutting boards.
- Daily disinfection is useful for dismantled equipment, such as parts of grinders, fillers, stuffers, etc.
- Disinfection once a week is recommended for other equipment and the floors and walls of processing and chilling rooms.

Step F: Post-Rinse with Water

Post-rinsing after the sanitation step may be required to avoid residues of sanitizers on surfaces and to counteract corrosion. Post-rinsing should be carried out with water from a water hose or from a low-pressure system. Because surface recontamination must be avoided, water of potable quality must be used.

Step G: Remove Excess Water (Dry)

As every microorganism needs moisture to grow, drying is important to reduce the opportunity for microorganisms to grow on surfaces. Also, floors should be kept drained of standing water and as dry as possible.

Ensuring Effectiveness of Cleaning and Sanitation Programs

A surface can be considered "clean" when all visible soil or residues have been removed (physically clean); when all compounds used in cleaning and sanitizing and all the deposits (i.e. scale) are removed by rinsing (chemically clean); and when the number of present microorganisms has been reduced to acceptable levels (microbiologically clean).

Monitoring steps in the cleaning program should include:

(1) post-cleaning visual inspection to ensure that any visible food residue has been removed;
(2) temperature checks for water and chemical solutions to ensure they are within the ranges specified in the procedure;
(3) concentration checks for detergents and sanitizers to ensure they are used according to directions.

Verification of the effectiveness of the program should be scheduled and may range from simple procedures that must be performed as a preoperative action on a daily schedule to more complex testing that may need to be outsourced:

- Processors often rely on sensory inspection (looking, touching, and smelling) to determine the effectiveness of their sanitation program: a flashlight (torch) can be used to inspect the inside of semi-enclosed equipment. Surfaces such as stainless steel should be smooth and nongreasy, and there should be no unpleasant odors.
- ATP (adenosinetriphosphate) fluorescence testing can be used to detect the presence of both microorganisms and food residues. The ATP test is a rapid test; the results are available immediately and give a pass or fail for the sanitizing procedure.
- Microbiological testing of equipment and surfaces is required to verify if the level of microorganisms present post-sanitizing is within acceptable limits. Each plant should determine the points to sample and the frequency of sampling based on results over time.

Water Quality

Water used for food processing, cleaning, and sanitizing activities must be potable. Knowing the water source available to a food-processing plant is a must when designing a sanitation program. Water functions as a carrier for detergents and sanitizers. It also carries soil or contaminants away from the surface that has been cleaned and sanitized. Water quality varies greatly, and its ability to function in cleaning is determined by several factors: total alkalinity, calcium hardness, iron, pH, silica, total dissolved solids, and standard plate count. Often problems in plant sanitation programs are a direct result of the raw water supply. These sanitation problems can be prevented or minimized through advanced knowledge of the raw water quality. One factor of prime importance is water hardness. Water hardness is responsible for excessive soap and detergent consumption (with hard water, more cleaners will be required as minerals precipitate out with the alkali cleaners), mineral deposits, undesirable films, and precipitates. When hard water (>120 ppm $CaCO_3$) must be used for cleaning, the addition of chelating or sequestrating reagents is necessary.

Biofilm Formation

A biofilm can be described as a group of bacteria that have colonized a surface. The biofilm not only includes the bacteria but also a complex polysaccharide-like material produced on the surface and any material trapped within the matrix. It is generally assumed that the biofilms take hours or days to develop. They are self-regulating; that is, pieces may dislodge from the surface, allowing other cells to attach and to entrap more food soil inside (Hood and Zottola 1995) and contaminate the food as it passes by. There is evidence that usual sanitation practices are less effective on attached microorganisms compared with free cells (Holah et al. 1990a). Resistance of treated biofilms to sanitizing agents seems to be due to the characteristics of extracellular polymeric substances rather than intrinsic attributes of the cells in the biofilm (Pan et al. 2006).

The macro-cavities of porous surfaces may play an important role in the ability of a microorganism to colonize food-contact surfaces. Stone and Zottola (1985) demonstrated that the number of cracks and irregularities may allow the surface to harbor bacteria and reduce the effectiveness of cleaning procedures.

Cleaning is the most important step for minimizing microbial colonization of meat-processing equipment. Microorganisms are far more sensitive to disinfectants once they have been detached from the surfaces to which they were adhering. Mechanical action with brushes and medium- and high-pressure jets is recognized as being highly effective in eliminating biofilm (Holah et al. 1990b; Carpentier and Cerf 1993). Acid products are no more effective than hot water, while chlorinated alkaline detergents are the most effective for detaching biofilm, followed by nonchlorinated alkaline detergents (Carpentier and Cerf 1993). Peroxides have been reported to be effective for the removal of bacterial biofilms and are widely used in the food industry (Fatemi and Frank 1999; Stopforth et al. 2002).

Usual recommendations for choosing materials and food equipment design are useful to avoid biofilm formation: use of smooth, nonporous material and of equipment without inaccessible corners; cleaning at short intervals to prevent excessive buildup of soil and biofilm; and, since water is indispensable for biofilm production, drying of the surfaces after the cleaning-disinfection procedures whenever possible.

Control of *Listeria monocytogenes* in Meat-Processing Plants

Listeria monocytogenes is a pathogenic bacterium that is widely spread throughout the environment. It has been isolated from soil,

water, silage, and many other environmental sources. Listeriosis may develop in birds, animals, and humans. *L. monocytogenes* is especially pathogenic to high-risk populations, including newborns, pregnant women, elderly, and immunocompromized persons. The symptoms of the disease include fever, chills, headache, abdominal pain, and diarrhea.

Epidemiological studies have confirmed that meat products have been involved in sporadic cases and outbreaks of listeriosis in many countries. Contamination of ready-to-eat (RTE) meat and poultry products by *L. monocytogenes* has represented a major source of foodborne illness in the United States in recent years. The lethality treatment received by many processed RTE meats and poultry products generally eliminates the pathogen; however, when the disinfection is inadequate, products can be contaminated by exposure to the environment after the lethality treatment, during peeling, slicing, and repackaging operations. In general, the contamination sites in meat- and poultry-processing plants are mainly the food-contact surfaces and processing machines: conveyors, slicers, peelers, dicers, and brining and packaging machines.

Experience in cold-smoked pork products processing establishments indicates that contamination of the finished product appears to mostly be due to *L. monocytogenes* strains already present in the plant environment (Bērziņš et al. 2007). This experience confirmed the results obtained by other authors (Norton et al. 2001; Hoffman et al. 2003). Both persistent and nonpersistent strains were isolated from pork products produced in seven different plants.

Previous studies have shown that persistent *L. monocytogenes* strains can often be found in various parts of food processing equipment. Lundén et al. (2002) evaluated the possibility of transferring persistent *L. monocytogenes* contamination from one plant to another with a dicing machine for cooked meat products. After the transfer of the dicing machine, *L. monocytogenes* originally found in plant A was soon also found in plants B and C, and was frequently isolated from the diced products. This particular strain of *L. monocytogenes* proved to have a high level of adherence to stainless steel surfaces and a significant resistance to QUAT.

Research and experience indicates that *L. monocytogenes* tolerates well various stress factors that it encounters in food-processing plants and has a very good ability to attach to different surfaces and thus to persist in food plants for years (Senczek et al. 2000; Lundén et al. 2003). Insufficient manual scrubbing during the cleaning process and an inadequate disinfection have been linked to the presence of *L. monocytogenes* in finished products.

Sanitizers that are most effective against *L. monocytogenes* are quaternary ammonium compounds (QUATs), chlorine solutions, and products containing peroxyacetic acid (Table 15.2).

Rotating sanitizers periodically is usually a good practice, as it will provide more effectiveness against *L. monocytogenes* as well as other bacteria. The frequency of cleaning and sanitizing the equipment and the environment of a plant depends upon the experience and the microbiological data. Suggested frequencies are shown in Table 15.3.

It has been shown that *L. monocytogenes* can become established and persist in floor drains. Therefore, drains should be cleaned and disinfected in a manner that prevents contamination of other surfaces in the room. Solid forms of disinfectants (e.g., blocks of QUATs) can be placed in drains to help control bacterial growth. Floor drains should not be cleaned during production. High-pressure hoses should not be used to clear or clean a drain, as aerosols will be created that spread contamination throughout the room. Employees who have been cleaning drains should not contact or clean food contact surfaces without changing clothes and washing

Table 15.2. Sanitizers most effective against *L. monocytogenes*

Medium	Sanitizer	Concentration (ppm)	Inactivation (n–Log)	Source
Deionized water	Iodophor	12.5–25	>5-Log	Lopes (1986)
Deionized water	QUAT1	100–200	>5-Log	Lopes (1986)
Deionized water	QUAT2	200	>5-Log	Lopes (1986)
Deionized water	Sodium hypochlorite	100–200	>5-Log	Lopes (1986)
Stainless steel	Sodium hypochlorite	200	4-Log	Mustapha and Liewen (1989)
Stainless steel	QUAT	50	>4-Log	Mustapha and Liewen (1989)
Stainless steel	Ethanol	70% v/v	<4-Log	Best et al. (1990)
Biofilm on stainless steel	Peroxyacetic acid PAA	160	5-Log	Fatemi and Frank (1999)
Biofilm on stainless steel	Peroctanoic acid POA	160	5-Log	Fatemi and Frank (1999)

Table 15.3. Meat-processing area and frequency of cleaning

Area	Frequency
All processing equipment	Daily
Floors/Drains	Daily
Waste containers	Daily
Storage areas	Daily
Wall	Weekly
Condensate drip pans	Weekly/monthly
Coolers	Weekly/monthly
Freezers	Semiannually

Source: Henning and Cutter, 2001

and disinfecting hands. Utensils for cleaning drains should be easily distinguishable and be dedicated to that purpose to minimize the potential for contamination.

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Part II

Products

Chapter 16

Cooked Ham

Fidel Toldrá, Leticia Mora, and Mónica Flores

Introduction

The processing of cooked ham involves the use of brine that is either injected or infused through soaking, followed by the application of thermal treatment. The final quality depends on both the raw materials and the processing. The most outstanding factors are the type of meat cut, the type and amounts of ingredients, the injected volume of brine, the rate and extent of tumbling, and the cooking time and temperature (Delahunty et al. 1997). The goal is to obtain a product with high sensory quality that is microbiologically safe, usually based on minimum temperature-time treatments. The best-quality products are generally produced with a low-brine injection level and no addition of polyphosphates. In general, the more water is injected into the ham, the poorer is the quality, since water retention is facilitated by some compounds like polyphosphates and starches.

The consumption of cooked ham is relatively high, since it is a very popular meat product. For instance, it may account for as much as 26% of the delicatessen products sold in Europe, with France, Spain, and Italy being major consumers (Casiraghi et al. 2007). There are different manufacturing technologies for the production of cooked ham, depending on the raw materials and the processing conditions, which are discussed in this chapter.

Types of Products

There is a broad range of types of cooked ham, which generally are classified depending on different characteristics. In general, cooked hams can be classified according to the raw material used for the processing, the composition of brine ingredients (like the use of polyphosphates, starches, and carrageenan), the technological yield (from 85% to higher than 110%), and finally, the ham presentation (boneless, bone-in, pieces, whole legs, and so on) (Frentz 1982).

The manufacture of cooked ham has been evolving in order to solve different problems such as the increased proportion of exudative meats or the reduction of the salt content and processing time. The intensive selection for leaner pigs in response to consumer demands resulted in an increased proportion of exudative pork meat. The protein of this meat exhibited poor texture, lower water-holding capacity, and poor cooked cured color (Schilling et al. 2004). In order to improve the functionality of these meats, several binders have been used, including starch, carrageenan, and soy proteins (Motzer et al. 1998).

In this regard, the appearance of modern tumbled hams is essential in the industry. Modern hams are characterized by a milder flavor than traditional hams, such as Wiltshire, Bradenham, and York (Nute et al. 1987). The modern hams contain less meat and more

water, although the final quality mainly depends on the raw material and the processing conditions used.

Generally, modern tumbled hams are manufactured by injecting the pork meat with brine, after which the ham is tumbled and then cooked. The Wiltshire method consists of an old, traditional method of curing, whereby the pork leg is immersed for several days in brine, which yields a high-quality product (Delahunty et al. 1997). In addition, there is a method for producing sweet-cure hams that have a low salt content and a blander flavor, where sugar is generally added to the brine (Delahunty et al. 1997).

Many other cooked hams are manufactured in, and receive the name of, the region where they are produced. This is the case of Italian prosciutto cotto or French hams (Jambon de Bourgogne, Jambon de Reims, etc.) (Frentz 1982).

Raw Materials

Refrigerated hams or frozen/thawed hams must be carefully controlled when received at the factory and before further processing. These hams must be hygienically manipulated and controlled in order to have a product with a good shelf life. The water-holding capacity of the hams used as raw material is essential in order to minimize the cooking losses. Water-holding capacity is linked to the pH of the ham; pH values within the range 5.8 to 6.2 may assure good water retention. Incidence of PSE (pale, soft, exudative) and DFD (dark, firm, dry) hams must be registered. In the case of PSE hams, they have a low pH value and a low water-holding capacity that will give higher cooking losses and drier hams. DFD hams may be used; their high pH can facilitate water retention but makes them prone to microbial growth. These hams are heat-treated, avoiding risks for microbial growth, but they may present some preservation problems compared with normal hams (Toldrá 2006a). The fat is very important for flavor development, while the composition in fatty acids mainly depends on the feed given to pigs (Jiménez-Colmenero et al. 2006) and the crossbreed used (Armero et al. 2002). Any undesirable aroma or oxidative development (i.e., rancidity) must be detected before processing. Hams are usually boned before brine injection.

The salt added in the brine may reach a final content of around 2% in the ham. Salt contributes to a reduction in water activity, partial solubilization of myofibrillar proteins, and typical salty taste. Nitrite is also added at levels of 120–150 mg/kg and contributes to typical light pink color formation, antioxidant activity, and preservation effect against pathogens (Pegg and Shahidi 2000). In order to avoid risks of nitrosamine formation, the addition of ascorbates or erythorbates at levels of 200–400 mg/kg is recommended. Some sugar (dextrose) may be added for taste purposes. Phosphates, at levels from 0.15% to 0.3%, given as P_2O_5, may be allowed in some countries, depending on the quality of the product. Polyphosphates contribute to improvements in ham's water retention. Poor-quality hams may also contain some nonmeat ingredients like milk powder, caseinate, soy proteins, potato flour, or carrageenan, to act as thickeners and to improve water retention.

Processing Technology

Cooked ham is also known as canned ham. The scheme for the main processing stages is shown in Figure 16.1. Main stages are the reception of hams, the brine injection, tumbling and massaging, cooking, and cooling. All these stages are described below.

Reception

Refrigerated hams or frozen/thawed hams are received and controlled by weight. The pH is measured in order to detect any PSE or DFD

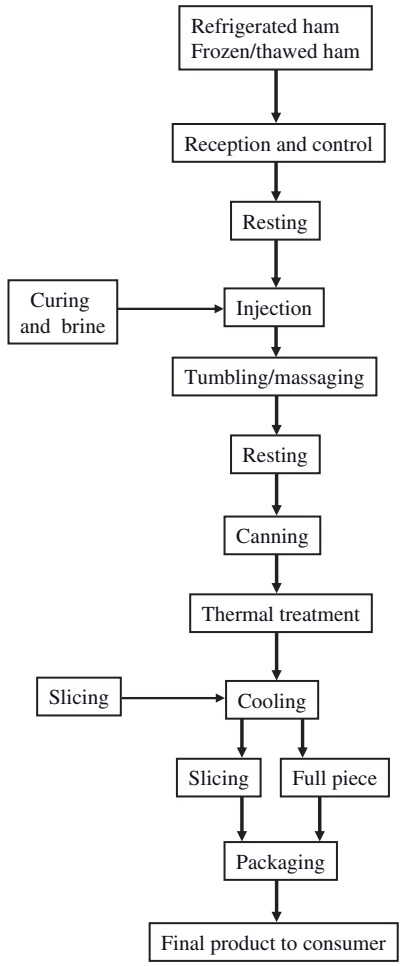


Figure 16.1. Process flow diagram for the processing of cooked hams (Toldrá 2007).

ham. The skin is removed as well as the bone (optional), since hams may be cooked as entire pieces or after boning, which facilitates the brine injection and diffusion.

Brine Injection

Salt is the main ingredient of brines, and its amount varies depending on the type of product. Final salt amounts in cooked hams are around 2%. Salt can be accompanied by sugars such as sucrose, dextrose, or corn syrup, to give a pleasant mild taste. Nitrite is added as the main preservative. The generation of nitric oxide assures the preservation but also contributes to the formation of the typical pink color. Sodium ascorbate or sodium erythorbate may be added to assure rapid nitrite disappearance and avoid the potential generation of nitrosamines. Other ingredients may be added to the brine, depending on the desired final quality of the hams. For instance, different amounts of phosphates, polyphosphates, or pyrophosphates can be added to increase the amount of retained water; these amounts are regulated and controlled. Phosphates increase the pH of the ham, far from the isoelectric point of meat proteins, but also increase the ionic strength and contribute to protein solubilization.

The brine is injected into both boneless hams and bone-in hams through multineedle systems. Pumping speed and volume of injection are controlled. The brine favors the binding of muscles and protein solubilization, and it gives a better yield and a higher final weight. After the brine injection, the current practice is to hold the hams for resting for a brief period of time to help the diffusion of salt and additives throughout the entire piece.

Massaging and/or Tumbling

This is a mechanical operation operated under refrigeration conditions that aims to distribute the brine through the entire piece and to extract the meat proteins from the fibers. In this way, a uniform distribution of ingredients like sodium chloride, nitrite, sugars, and spices may be achieved. This operation also facilitates improved tenderness and juiciness. Hams can be either massaged or tumbled. Massaging consists of holding hams in mixers for a few hours under mild agitation (low-speed paddles) to avoid physical disruption or damage to the full muscles' appearance. During tumbling, hams are located inside rotary tumblers that operate

under vacuum to avoid further undesired oxidations and improve salt diffusion. The treatment may be continuous, or it may alternate tumbling and resting. The tumblers may have inner baffles to optimize the homogenization of the brine inside the hams.

Cooking

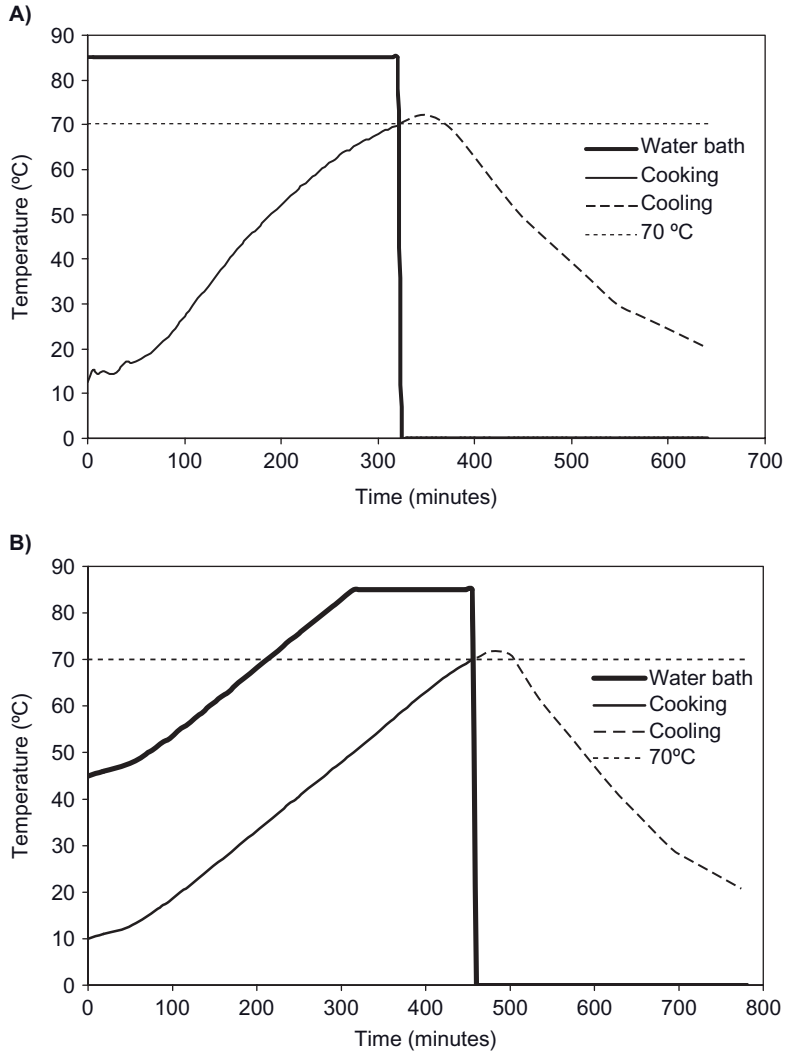
This is a delicate stage that requires a rigorous control of time and temperature to achieve the final desired effect and ensure the wholesomeness of the product (Ponce-Alquicira 2005). The aim of heat treatment is microbial destruction and enzyme inactivation. The heat treatment is calculated in order to combine the maximal inactivation of pathogen and spoilage microorganisms for an extended shelf life of the product with minimal effect on the sensory characteristics (Guerrero-Legarreta 2001).

Boned hams are canned in special molds that will give the final shape. Hams can optionally be packaged in special plastic that allows a better water retention (zero water loss) and hygienic storage and distribution. Cooking can be considered as pasteurization, since internal temperature of the ham reaches up to 72°C for 30 to 60 minutes. Cooking is usually performed in hot-water baths. There are two heat transfer mechanisms: convection (heat transfer from the heating medium to the ham surface) and conduction (heat transfer mechanism from the ham surface to the inner areas) (Guerrero-Legarreta 2006).

The speed of temperature increase and its control during cooking is important. So, cooking can be operated in three ways (Toldrá 2007): (1) heating at a fixed temperature where the ham may not reach the wished internal temperature; (2) heating until reaching a determined temperature inside the ham (usually 68°C), but this process may give some excessive heating of the surface of the ham by over-exposure to the heating medium (an example of the heating profile for this type of cooking is shown in Figure 16.2A); and (3) heating by steps (ΔT) that consist of an increase in the internal temperature of the ham through steps, not exceeding 25–30°C each time. This process avoids excessive heating of the surface of the ham. An example of the heating profile for this type of treatment is shown in Figure 16.2B. The cooking efficiency, weight loss, and yields may be different for the last two cooking methods (cooking at a fixed internal temperature and cooking by steps) as shown in Table 16.1. It has been reported that slow heating rate forms a protein network with better water binding and less jelly losses. This means low cooking damage, a more tender product, and better slice cohesion (Desmond and Kenny 2005). Several enzymatic reactions, oxidations, Maillard reactions, etc., take place in the hams during cooking, and all of them contribute to the final development of sensory characteristics typical of cooked hams (Toldrá 2006b). So, the conversion of creatine into creatinine was reported to be proportional to the heating intensity, and the creatinine-to-creatine ratio in the surface of the ham could be used for control purposes as an effective indicator of the internal temperature reached within the ham (Mora et al. 2008a, b).

Cooling

Cooling is also a delicate stage to ensure the wholesomeness of the hams. Cooling must achieve temperatures of the ham below 4–5°C, and this may be achieved by air blast, immersion in cold water, or with cold water showers. Examples of cooling profiles of hams are shown in Figures 16.2A and 16.2B. The final cooling from 40 to 15°C is considered the most critical period and should be restricted to less than 4 hours when possible (Desmond et al. 2000). Slow cooling conditions may be dangerous, due to the long periods at relatively high temperatures when microorganisms might grow. Vacuum cooling was reported to offer reduced cooling



Heating and cooling curve for a piece of ham cooked under constant temperature (A) and ΔT (B) method conditions.

Figure 16.2. Heating and cooling profiles, as measured by temperature in the center of the ham, through cooking under two different conditions: (A) heating under constant temperature and (B) heating by steps, ΔT method.

Table 16.1. Effect of different cooking methods on cooking efficiency, weight loss and yield in cooked ham

Cooking methods	Cooking efficiency[a] (min/°C)	Process losses (%)	Yield (%)
Constant T	4.90	15.7	101.55
ΔT	6.96	13.6	105.05

[a] Calculated as the slope of the curve (time versus temperature) of cooking and cooling processes

rates as compared with air blast, water immersion, or cold room, but it affected the yield and quality, especially toughening (Desmond et al. 2000).

Final Product

Once cooled, hams are taken out of the molds and packaged. Hams may be smoked to acquire a typical color and smoke flavor (Ellis 2001). Cooked hams may be sold as either entire pieces for slicing at the retailer shop at consumer request or as packaged slices ready to be consumed. A wide variety of vacuum and modified atmosphere packages containing different numbers of slices are typically found in supermarkets.

Quality Aspects of the Finished Product

Color

Cooked ham has a typical light pink cured color as a consequence of nitrite addition. Nitrite is reduced to nitric oxide that reacts with myoglobin, forming nitrosylmyoglobin that gives a reddish color. This color changes from red to pink during the heating process, especially at temperatures above 65°C, because the generation of nitrosylhemochrome has a typical light pink cured meat color. This color is also known as cooked cured-meat pigment (Pegg and Shahidi 2000). Of course, the intensity of the color depends on the initial content of myoglobin, which is associated with the type of muscle and the age of the animal, being higher in older animals and in muscles with oxidative pattern (Aristoy and Toldrá 1998). The color coordinate characterizes the red color and color stability. It was reported to be negatively correlated with sensory analysis by consumers so that more redness was considered less acceptable (Válková et al. 2007). This means consumers prefer cooked hams with light color and less red color.

In Europe, all muscles of the ham are kept in the product, even though they may have different color intensities. Examples of high- and low-quality cooked hams are shown in Figures 16.3A and 16.3B, respectively. The uniformity of color is very important in the United States, where some muscles with darker and more intense color are removed, and silverside and topside muscles are preferred. The color of smoked hams may be darker due to the deposition of dark colors from the pyrolytic decomposition of wood.

Texture

The texture of cooked hams depends on several factors, such as the extent of heating (structure breakdown), the moisture content, the extent of proteolysis (degree of myofibrillar protein breakdown), and the content of connective tissue. The content of intramuscular fat also exerts a positive influence on some texture and appearance traits.

There are different types of starches that can be added to hams. Commercial starches are usually modified by pregelatinization to be cold-water swellable, cross-linking to impart stability for cooked ham processing, increasing the water-holding capacity (Martin 2001). They will enhance texture and bind water, as well as improve the mouth feel of the cooked product.

However, several processing factors such as the different cooling methods may affect the tenderness, juiciness, overall texture, acceptability, and cured color (Desmond et al. 2000). Also, the pigs' genotype can affect cooked ham quality. For instance, cooked hams produced from nn pigs were reported to be drier, tougher, stringier, and less smooth than those produced from NN and Nn pigs (Fernandez et al. 2002).

Flavor

Cooked ham experiences some biochemical changes as a consequence of enzymatic reac-

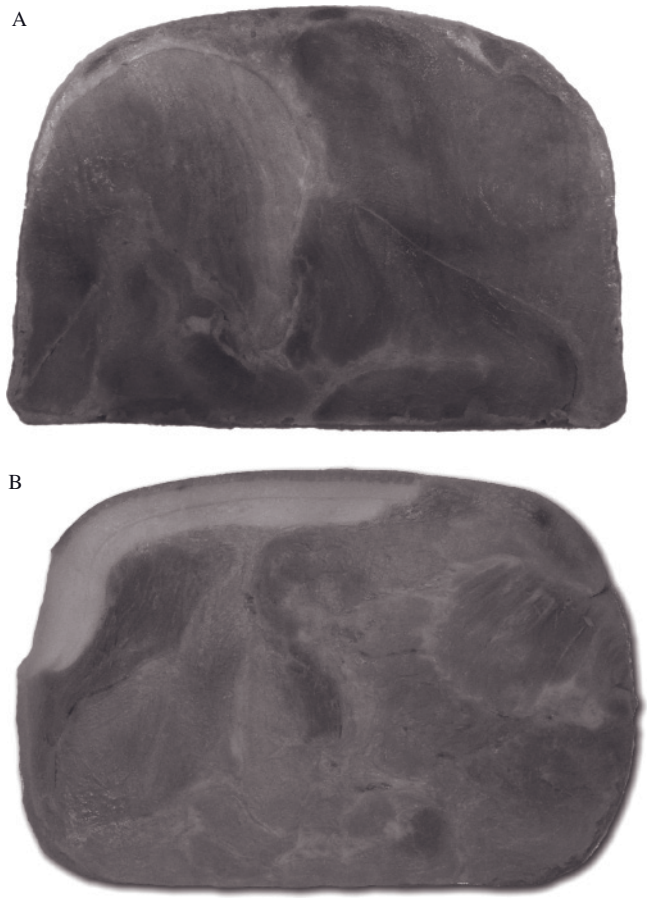


Figure 16.3. Cross-section of cooked ham: (A) high-quality cooked ham in which the muscular integrity has been respected and (B) low-quality where muscle integrity has been partly lost.

tions, mainly through proteolysis and lipolysis, even though these enzymes have a reduced time for action. Muscle proteases and lipases contribute to the generation of free amino acids and fatty acids, which have some influence on taste and aroma.

The conditions within the hams (high water activity, low salt content) are favorable for proteolysis, but muscle protease enzymes are sensitive to temperatures above 50°C and thus, are rapidly inactivated during cooking because their stability decreases rapidly (Toldrá et al. 1992). In any case, there is some generation of free amino acids by muscle aminopeptidases that contribute to taste (Toldrá et al. 1995; Flores et al. 1998), but the amount of released amino acids depends on the extent of resting before cooking.

Lipolysis is also favored by conditions prior to cooking, especially when the the pH is near neutral conditions. Fatty acids are released during resting and initial cooking of hams (Toldrá 2007). One or two days of resting, prior to cooking, allows longer enzymatic action and larger amounts of released amino acids and fatty acids that will act as substrates for further chemical reactions (i.e., Strecker reactions) responsible for the generation of volatile compounds. As in the case

of proteases, lipases are also inactivated during cooking. It must be taken into account that fatty acid composition is a key aspect in flavor generation. An excess of linoleic acid may impart some off-flavor during cooking. Further chemical reactions (i.e., Maillard reactions) are accelerated during cooking and contribute to the generation of aroma volatile compounds. The extent and characteristics of flavor will depend on the time and intensity of heating.

Cooked ham has a highly appreciated flavor, which is mostly due to the processing conditions, brining, and spices added. The flavor of cured cooked pork is completely different from that of uncured cooked pork (Ramarathnam et al. 1991, 1993) due to the lower generation of carbonyl compounds in cured cooked pork. However, no unique compound has been identified as responsible for the characteristic cured aroma in cooked ham. Therefore, the cured cooked aroma is reported to be a mixture of many volatile compounds. In this sense, the flavor of cooked ham was studied by the extraction and identification of its volatile compounds (Baloga et al. 1990; De Winne and Dirinck 1997; Guillard et al. 1997; Leroy et al. 2009). Although many volatile compounds such as alkanes, alkenes, aldehydes, ketones, alcohols, aromatic hydrocarbons, carboxylic acids, esters, terpenes, sulfur compounds, furans, pyrazines, amines, and chloride have been identified, only a few of them directly contribute to cooked ham aroma (Toldrá and Flores 2007a).

In order to determine the impact of a specific volatile compound on the total aroma, it is necessary to study several factors, such as odor threshold, concentration, interaction with the food matrix, and temperature. The contribution of the volatile compounds to the aroma of cooked ham was studied through olfactometry techniques (Guillard et al. 1997). Several compounds were described as odor-active compounds in cooked ham, such as terpenes (1,8-cineole, linalool, L-carvone, cinnamaldehyde, menthol) derived from spices; also, sulfur-compounds (methional, dimethyl disulfide, allyl isothiocyanate) and a branched acid (3-methyl-butanoic acid) originated from the Strecker degradation of amino acids (Guillard et al. 1997). However, of the many volatile compounds detected, none had an aroma similar to the aroma of cooked ham.

On the other side, microorganisms do not contribute to the desirable flavor of cooked ham as occurs in other meat products, such as fermented sausages (Toldrá and Flores 2007b). The contribution of microorganisms to the flavor of cooked ham is insignificant, but they are responsible for acidification and formation of off-flavors generally during storage (Samelis et al. 1998). The refrigerated storage of sliced cooked ham under vacuum or modified atmospheres leads to alterations in the sensory characteristics of the product, such as color defects, off-odors, and slime formation. Therefore, the refrigerated storage of cooked ham modifies the volatile composition due to the metabolism of lactic acid bacteria that generates typical fermentation products, such as methyl branched alcohols and aldehydes (Leroy et al. 2009). In addition, lipid oxidation is another phenomenon that generates oxidation products, such as unsaturated aldehydes (De Winne and Dirinck 1997) that are not present in fresh cooked ham.

Safety Aspects

The use of nitrite in meat products, and therefore in cooked ham, is due to its antimicrobial effect, which prevents the growth of *Clostrodium botulinum*; in addition, nitrite is responsible for the characteristic pink color and prevents lipid oxidation, increasing meat stability (Pegg and Shahidi 2000). However, the post-heat handling of cooked ham is essential to avoid recontamination, because this will determine its shelf life. The slicing of cooked ham prior to packaging recontami-

nates the product mainly with lactic acid bacteria (Samelis et al. 2000). The metabolic activity of lactic acid bacteria under vacuum or modified atmosphere produces acidification or sourness, slime, gas, and generates off-odors and color deterioration (Samelis et al. 2000). Generally, the dominant LAB are *Lactobacillus*, *Carnobacterium,* and *Leuconostoc*; when oxygen is present in modified atmosphere packages, *Brochotrhrix termosphacta* is also detected (Vasilopoulos et al. 2008). Moreover, the pathogen *Lysteria monocytogenes* is able to develop in cooked ham under refrigerated storage (Cabedo et al. 2008). In the past few years, many different strategies have been developed to protect cooked ham and extend its shelf life, such as the use of protective cultures (Vermeiren et al. 2004), the use of additives such as lactate and salts (Stekelenburg & Kant-Muermans 2001), and antimicrobial packaging (Marcos et al. 2007, 2008; Jofré et al. 2008a, b). In addition, several authors have used microbial metabolites or chemical compounds as indicators of cooked ham spoilage (Laursen et al. 2009). Also, traditional cooked hams are more sensitive to spoilage, due to their lower salt content and lower additive content, than are conventional cooked hams (Vasilopoulos et al. 2008).

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Chapter 17

Cooked Sausages

Eero Puolanne

Introduction

For producers and consumers, cooked sausage is an ideal meat product: it can be made by many different formulations and in many forms (Fig. 17.1). All edible parts of the carcass can be used in an efficient way, thus making it possible to utilize its entire nutritional capacity. It is a ready-to-eat food that can be eaten cold or heated, as a part of a meal or on its own. It requires little preparation time, and no skills are needed to prepare a meal from it. In addition, salt, nitrite, and heating improve the safety and keepability of meat far beyond that of fresh meat. Sausage is presumably the first multicomponent food product that was prepared by an industrial process.

Main Types of Products Worldwide

In his booklet *Principal Characteristics of Sausages of the World Listed by Country of Origin*, Kinsman (1980) separates cooked sausages into two categories: (1) cooked sausages made from uncured meats, ground, seasoned, stuffed into casings, and cooked but not smoked, usually served cold (examples: Braunschweiger liver sausage, liver-cheese), and (2) cooked, smoked sausages made from cured meats, chopped or ground, seasoned, stuffed into casings, smoked slightly, and then fully cooked, which do not require further cooking before consuming but are often heated before serving (examples: Berliner, bologna, Cotto salami, frankfurters, smokie links, and wieners). Kinsman presents hundreds of different sausages, including basic formulations and processing principles, from around the world, listed by country of origin. The second category, cooked smoked sausage, is most commonly regarded as cooked sausage. In his extensive textbook, Feiner (2006) also presents examples of cooked sausages from around the world with preparation details.

Some common types of sausages as examples will be described below. Sausages with the same name or of the same type are prepared in different countries from meat of different species, with or without added phosphate, with or without extenders, with varying seasoning, and so on. A detailed catchall coverage of world sausages is therefore not possible, as for example, in Germany only there are more than a thousand different kinds of sausages and in the United Kingdom more than four hundred! The Internet is an endless source of information on these different varieties.

Frankfurters (Wieners)

Frankfurters are typically made of beef and pork, but poultry and other meat sources are also used; they are 30%–40% lean meat and 15%–30% fat. Sausage is usually finely chopped, but the finely chopped mass may also contain grain-size meat particles. As frankfurters are eaten hot, the salt content is usually relatively low, 1.6%–1.8%. They are

Figure 17.1. Vacuum-packaged cooked sausages. (Photograph courtesy of Pertti Leino.)

stuffed into 18–22 mm natural casings, but artificial casings are also widely used. Sometimes an artificial casing is removed before packaging (skinless products). Usually, products are smoked. There have been many attempts to prepare nonmeat frankfurters of soya or other nonmeat ingredients, but they have not been very successful.

Grill Sausages

Sausages similar to frankfurters but of larger diameter (25–40 mm) are made especially for grilling. The formulas may or may not contain potato starch or other extenders. Grill sausages are eaten hot.

Bratwurst

A bratwurst is a sausage composed of pork, beef, or veal. The name is German, derived from Old High German brätwurst, from *brät-*, which is finely chopped meat, and *-wurst*, sausage. Though the "brat" in bratwurst describes the way the sausages are made, it is often misconstrued to be derived from the German verb "braten," which means "to pan fry or roast." Etymology aside, frying and roasting are far from the most common methods of preparation. Bratwurst are usually grilled and sometimes cooked in broth or beer.

The original probably comes from the region of Thuringia, where it is traditionally known as Thüringer Rostbratwurst. The oldest known recipe is from 1432. In Germany, there are also other regional variations. In Nuremberg, the bratwurst are considerably smaller, approximately the length and thickness of an adult's thumb. Perhaps the most popular sausage in Germany is Nürnberger Bratwürste (Nürnberger Rostbratwürste). Traditionally soaked in milk, roasted, and served three abreast on a bun with mustard, this pork-based wurst is recognized in markets and restaurants across Germany and prepared according to taste (boiled, smoked, grilled, etc.). Fresh marjoram is often attributed as one of the important flavors in this distinctive sausage. In the Franconia region, the bratwurst are long and thin, often served in pairs.

How the sausage is served varies by region. In Thuringia, the sausage is often eaten with hot German mustard in a bread roll or Brötchen. There and farther south, the bratwurst are often served "pinched" in a bread roll, much like a forerunner of the

American hot dog bun. It is a very popular form of "fast food" in German-speaking countries, cooked and sold from small stands and street vendors. Recipes for the sausage can also vary; some sources list over forty different varieties of German bratwurst. In other countries bratwurst is also popular, for example, in the United States, where bratwurst are typically grilled, rather than boiled. Sometimes they are boiled in beer prior to grilling. They are usually eaten on a hot dog bun, brat bun, or a hard roll, topped with mustard or many of the other condiments often eaten with hot dogs. These may include ketchup, onions (grilled or raw), sauerkraut, pickle relish, shredded cheese, and mayonnaise. The bratwurst is occasionally served as a pair of links nestled in a buttered hard roll with these same toppings; this is called a "double brat" (Wikipedia).

Bologna

Bologna sausage is a finely chopped pork sausage with no visible pieces of fat. Bologna can alternatively be made out of chicken, turkey, beef, or pork.

In addition to meats, nonmeat binders and extenders (such as nonfat dry milk, cereal, or dried whole milk) or isolated soy protein may be used. Bologna is usually served in round uniform slices pre-cut in a package or sliced at a deli. There are many bologna producers, including local delis and grocery store meat counters.

Ring bologna or ring sausage is an ambiguous term with regional dependencies. One form is produced in 2-inch (5-cm) diameter sausages that are normally about a foot long (30 cm). These can often be found pickled in a combination of vinegar, salt, sugar, and spices. One typical national variety is sauna ring sausage that is most popular in Finland.

Bologna type sausages can be made by mixing coarsely ground cured meat particles (usually pork) into the batter at the very end of chopping (Wikipedia).

Mortadella

Mortadella is a large Italian sausage or cold cut made of finely chopped pork sausage that incorporates at least 15% small cubes of pork fat (principally the hard fat from the neck of the pig). It is delicately flavored with spices, including whole or ground black pepper, myrtle berries, nutmeg, coriander, and pistachios. Traditionally, the pork filling was ground to a paste using a large mortar and pestle (Wikipedia).

Mettwurst

Mettwurst is a strongly flavored German sausage made from raw minced pork, which is preserved by curing and smoking. The southern German variety is soft and similar to Teewurst. Braunschweiger mettwurst is smoked somewhat but still soft and spreadable, while other northern German varieties such as the Holsteiner are harder and more similar to salami, due to longer smoking. The Low German word *mett*, meaning minced pork without bacon, is derived from the Old Saxon word *meti* (meaning food) and is related to the English word "meat." Mettwurst can be cooked or fried or spread on rye bread with onions and eaten raw (Wikipedia). In some countries, mettwurst means sausage where meat has been chopped still frozen to get a grainy structure, but the sausage is cooked in steam or in hot smoke rather than fermented (Wikipedia).

Breakfast Sausage

A breakfast sausage (or country sausage) is a type of fresh pork sausage found in the United States, usually served at breakfast. Breakfast sausage is not cured or smoked. It is essentially highly seasoned ground meat, so it does not keep and should be stored and handled appropriately. Variations made from pork and beef mixtures as well as poultry can

now be found. There are also vegetarian varieties that use textured vegetable protein in place of meat. In America, the predominant spices used for seasoning are pepper and sage. Some breakfast sausage is flavored with ham (Wikipedia).

Blood Sausage

Black pudding or (less often) blood pudding is an English term for sausage made by cooking blood with a filler until it is thick enough to congeal when cooled. It is also called blood sausage (in German, *Blutwurst*). Blood sausage is also a useful term for similar blood-based solid foods around the world.

Pig or cattle blood is most often used; sheep and goat blood is used to a lesser extent. Blood from poultry, horses, and other animals is used more rarely. Typically, fillers include meat, fat, suet, bread, sweet potato, barley, and oatmeal (Wikipedia).

Weisswurst

Weisswurst, literally, "white sausage," is a traditional Bavarian sausage made from very finely minced veal and fresh pork bacon. It is usually flavored with parsley, lemon, mace, onions, ginger, and cardamom, though there are some variations. The mixture is then stuffed into fresh, clean pork casings and separated into individual sausages about 10 to 12 cm in length and about 2 cm in thickness. As it is very perishable, Weisswurst is traditionally manufactured early in the morning and prepared and eaten as a snack between breakfast and lunch; there is a saying that the sausages should not be allowed to hear the church bells' noon chime. The sausages are heated in water, broth, or white wine just short of boiling, for about 10 minutes, which will turn them grayish white because no preserving nitrite is used in Weisswurst preparation (Wikipedia).

Liver Sausage

Liverwurst is an anglicization of the German *Leberwurst* (Dutch *leverworst*, Hungarian *ken_májas*, Swedish *leverkorv*, Finnish *maksamakkara*), literally meaning "liver sausage." It is a typical sausage served in Germany, Hungary, the Netherlands, Finland, and Sweden.

Most liverwurst varieties are spreadable. The sausage is usually made with pork. Only about 10%–20% of the sausage is actually pork liver, which is enough to give it a distinctive liver taste. Other ingredients are meat, fat, and spices, such as ground black pepper, marjoram, allspice, thyme, ground mustard, or nutmeg. Many regions in Germany have their own recipes for liverwurst, often adding ingredients like pieces of onion or bacon. Recently, more exotic additions such as cowberries and mushrooms have gained in popularity. Though the German name Kalbsleberwurst is translated as "calf liver sausage," it normally contains pigs' livers, rather than calves' livers. It also contains veal. Braunschweiger is a spreadable liver sausage that is sometimes called liverwurst, or just liver sausage, in North America (Wikipedia).

Processing Stages

The processing of cooked sausages is basically very similar all around the world. The level of technology and the size of industrial plants naturally differ from country to country and plant to plant. The basic processing stages are given in Figure 17.2.

Ingredients and Additives

The main ingredient of sausages is always meat that is derived from the deboning of carcasses. Other carcass-derived materials include edible byproducts, pork skin emulsion, and also sometimes blood or plasma. The deboned meat is sorted based on fat

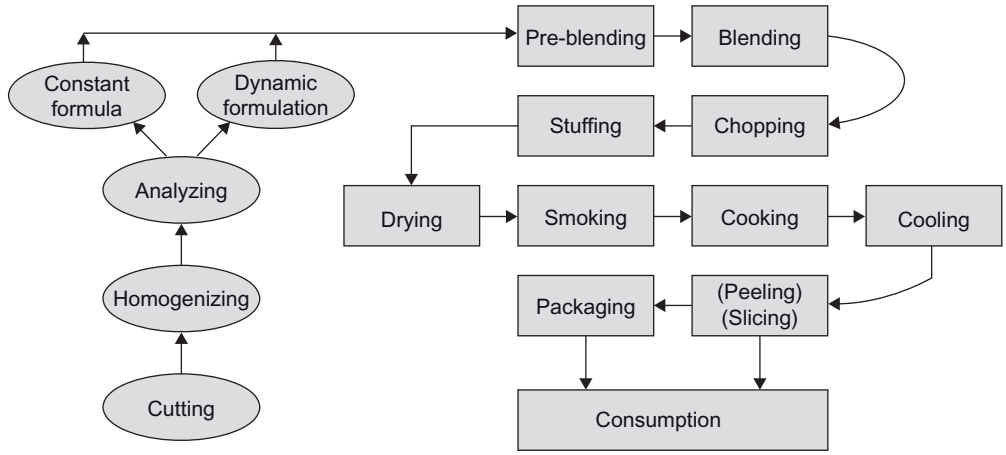

Figure 17.2. Unit operations in the preparation of cooked sausage.

content and connective tissue content, which may or may not be positively correlated. According to industrial practices, usually five to ten different assortments (trimmings) are sorted out from one carcass type. They differ from each other based on connective tissue content and/or fat content, starting from lean meat without thicker connective tissue membranes, to fat trimmings, in many cases also containing much connective tissue. This allows the preparation of a wide range of different types of sausages just by varying the relative proportions of different assortments from different animals and carcass types. When carcasses are deboned, usually the largest and most valuable muscles (like loins and rounds) are not included in sausage assortments but sold as such in raw state or used in whole-meat products like hams.

The varieties have previously only been visually sorted, but modern large-scale production requires standardized ingredients. Therefore, batches of assortments are first coarsely ground, mixed in large blenders, and the fat content is quickly determined. The fat content can then be adjusted to the standard value by adding fat or lean. Alternatively, the fat content given will be used in dynamic formulation, and the fat content of the final product is calculated on the basis of the actual (varying) fat contents of the assortments. Water (ice) is a substantial ingredient. It is added at a level of 20%–30% of the total weight of the batter.

The ideology of making sausages has always required a wide range of ingredients to be added to the sausage. The booklet compiled by Donald Kinsman (1980) lists a great variety of different ingredients: meat from different species, edible byproducts, milk constituents, vegetables, mushrooms, cereals, potato starch, and spices are used, depending on cultural traditions and the role of sausages in the diet. In some countries, there are mainly all-meat sausages, and their prices are higher than average carcass meat price, but in many other countries, sausages contain lower-quality meats and nonmeat ingredients as extenders, and those sausages offer an inexpensive option for meat consumption.

About a fifth (salt only) or a third (salt and phosphates) of salt-soluble myofibrillar proteins become solubilized (Grabowska and Hamm 1979), and the remaining myofibrillar system swells and keeps much of the water provided by the ingredients and water added in formulation (Hamm 1972). Salt,

pH, and phosphates play a central role in this (Ruusunen and Puolanne 2005). When cooked, these ingredients coagulate (dissolved proteins) or aggregate (proteins in the remaining myofibrils) to form a gel (Tornberg 2005). In contrast, connective tissue membrane proteins partly dissolve at around 65°C, partly swell during cooking, and the solubilized collagen forms a gel only when cooled. The same occurs with connective tissue that can be added as homogenized rind or dried powder. It should be noted, however, that collagen, once dissolved, slowly forms a gel at lower temperatures (20–30°C), but the gel melts at 45–59°C (hysteresis) (Puolanne and Ruusunen 1981). This behavior is somewhat similar for fat, but other proteins do not behave like this.

Sausages may also contain edible byproducts, like liver, tongue, blood or blood plasma, meat that is not included in the carcass (e.g., *masseter, diaphragm*), and organ fat. Other organs are also used, but the use of all meats and edible byproducts is tied to cultural aspects as well as to possible uses for the edible byproducts elsewhere (e.g., as animal feed).

Cooked sausages contain salt (NaCl), which is not an additive, according to EU regulations, but an ingredient, which can be, due to health reasons, partly replaced by other salts (for instance, potassium chloride) (Desmond 2005; Puolanne and Ruusunen 2005). The salt content is usually between 1.5% and 2.5%, but lower and higher contents are frequently seen. Salt contributes to water/fat binding, gel formation, taste, and keepability. Nitrite is also added to most cooked sausages at levels of 120–150 mg/kg, but there is a constant trend to reduce these levels. There are also, however, sausages made without nitrite, like German Bratwurst. It is recommended that when nitrite is used, ascorbates should be added to reduce the risk of nitrosamine formation. Nitrite also has antioxidative capacity and improves color stability (Pegg and Shahidi 2000). The usual level of ascorbates ranges from 200 to 400 mg/kg. In most countries, the use of phosphates is allowed and the levels used vary from 0.15% to 0.3%, given as P_2O_5 (in the EU, the maximum is 0.5%). Lactate and acetate are used for microbial safety (Bedie et al. 2001), and citrate can replace phosphate. Sodium glutamate is also frequently used as a taste enhancer. Glucono-delta-lactone is used as a color enhancer.

For economical reasons, nonmeat ingredients are also added to sausages. There is also a wide range of other ingredients that are added to increase water and fat holding; some of them are gelling agents, while others are thickeners. Milk powder, caseinate, and soy protein hold water as well as have emulsifying properties. Potato flour, carrageenan, and other polymers are added to hold water. Various plant materials are added for flavor or as extenders, such as mushrooms, beans, onions, cheese, vegetables, and spices. They do not have a technological function but may require some extra measures to be incorporated into the sausage batter production.

Formulation

Sausage preparation starts in the carcass cutting line or when meat assortments are purchased from the market. Formulations of sausages were originally designed for optimal use of all the carcass meat and edible byproducts. In practical terms, the prices/values of different assortments were determined by the technological and sensory properties, that is, how the sausage quality resulted from their relative proportions in the formulation. Therefore, as the customer pays for quality, the assortment prices are calculated on the basis of the prices of the sausages sold, and not vice versa. Later, in the long run, when the formulations have become stable, the relative assortment of prices also hardens in the market. Therefore, it is also possible to use stable formulation and stable prices, although most modern factories utilize

dynamic linear programming, in which the availability of assortments and their daily prices are used as variables, and the detailed chemical composition of the product is fixed. The optimal target function is that the end-product price will be minimized.

When linear programming is used, there are also technological limitations involved. Within the set chemical composition, the batter should hold all the formulation water and fat and maintain firmness within the acceptable limits. Usually, the bind values have been determined using meat, fat, and water mixtures. Since Hansen (1960) presented the emulsion hypothesis for finely chopped cooked sausages, salt-solubilized protein content and the emulsifying capacity of proteins have been used as technological traits for meat trimming (Carpenter and Saffle 1964). The bind value is also determined by firmness (Tuominen and Honkavaara 1982) or by added fat or water binding using centrifuging or cooking (Grabowska and Hamm 1978). Pouttu and Puolanne (2005) presented a method to determine the water-holding capacity of sausage ingredients in a multicomponent system. Whatever method is used, it should be kept in mind that the ingredients do not have a constant bind value, as it will vary along with such factors as the proportion of the ingredient in the formulation, added water and fat levels, other ingredients, salt content, whether or not phosphates are added, and freeze storage of assortments, in addition to the natural variation of a biological material (Pouttu and Puolanne 2005). Therefore, certain safety margins for water/fat holding must always be used. Regardless, the quality, indicated as desired firmness rather than water/fat-holding, is usually the most critical characteristic.

Comminution

The main determinant of structure in sausages is the extent of comminution, that is, the size of the meat particles in batter. The batter is prepared by chopping with a bowl cutter (Fig. 17.3) or by an emulsifier-type continuously working cutter (Fig. 17.4). The phrase "emulsion" is incorrect in this context, as there is not a liquid-liquid situation; actually, the batter structure is a "suspension," a mixture of protein particles and solid fat particles that are dispersed in a continuous water phase with solubilized protein. The particle size varies from coarsely ground (kidney blade) to finely comminuted batter. These are not regarded as sausages in all cases, although

Figure 17.3. Bowl chopper. (Photograph courtesy of Pertti Leino.)

Figure 17.4. Continuous chopper. (Photograph courtesy of Pertti Leino.)

they are of the same format: the coarsely ground products are not sausages, since the meat has been cured in a tumbler and stuffed before being cooked in the casing. Between these two extremes are combination products. One type is batter where the meat has been chopped into a grain the size of a couple of millimeters. Another type is produced when the basic batter has been finely chopped, but then larger meat particles (or other foodstuffs) have been added into the basic batter.

The linear speed of bowl cutter knives is about 140 m/s. The sharp blades (the flat cutting edge is about 20–30 µm, i.e., the cutting breadth is about ten times the sarcomere length) cut connective tissue and muscle fibers into pieces, but fractioned myofibrils can be seen through a microscope. The average cutting coverage, calculated on the basis of chopping time, number of rotations/minute, knife number, and cutting flat edge, usually equals the mean circumference of the bowl of an industrial chopper (8 min * 3000 rpm * 6 * 25 µm = π * 1.15 m = ~3.6 m; Puolanne 1999). Consequently, industrial experience tells us that a finely chopped sausage batter is ready, theoretically, when, on average, "all points of the sausage batter have been hit once by the knife's flat edge." The knife velocities of today are faster than ten years ago, reaching 5,000–6,000 rpm (Feiner 2006).

The chopping knives also hit fat cells. The diameters of the fat cells vary between 2 and 120 µm (Aberle et al. 2001). It has been estimated that when a knife moves through the batter at a speed of 140 m/s, friction heats the contact surface in the channel up to 80°C for less than 1 msec. This may result in a transient melting of fat and, consequently, the formation of emulsion-like structures in the batter. This requires some kind of emulsification capacity in the raw batter; otherwise, the fat would leak out. When sausage batter is then cooked, the gel that is formed traps the fat. If there is too much fat, a weak batter gel and intense chopping with a high end-temperature allows for fat to be separated. In larger, visible fatty tissue particles, the fat is kept in fat cells, which would suggest that emulsification is not actually required in all cases. Microscopic studies have shown that there are, in addition to emulsified fat, intact fat cells also in finely chopped sausages (Hansen 1960), the relative amount of which depends on the extent of chopping. In some particular sausages (e.g., liver sausages), the ingredients are cooked before preparation and chopped hot, and the product is actually a water-in-oil emulsion. These products may also contain larger meat particles, which means that the product has multiple internal structures.

The chopper is also a very efficient mixer. Chopping brings salt, phosphate (if used),

Figure 17.5. Stuffing of frankfurters. (Photograph courtesy of Pertti Leino.)

and water into immediate contact with the myofibrillar system, which results in a swelling of myofibrils as well as a partial solubilization of myofibrillar proteins (Hamm 1972; Offer and Knight 1988). When the batter has been stuffed into casings for cooking, the solubilized myofibrillar proteins form a gel structure that glues the meat particles together and encases the fat. With cooking, the solubilized proteins form a gel that traps the fat, as well as the muscle fiber and myofibril particles, making the batter a homogeneous structure (Tornberg 2005). In coarsely ground products, brine injection, curing of the meat prior to mincing, and tumbling are required, since the diffusion of curing ingredients is so slow in meat. The curing solution dissolves some myofibrillar proteins from the cut surfaces, and they form a gel, on heating, which glues the particles together.

Smoking/Cooking

According to industrial practices, when the sausage batter has been prepared, it will be

Figure 17.6. Sausage cooking and the measurement of end temperature. (Photograph courtesy of Pertti Leino.)

stuffed into natural or artificial casings and linked (Fig. 17.5). Then the sausages are moved to a smoking chamber, where they first are dried at 50–60°C. Excessively high temperatures or long times must not be used in order to avoid temperatures >60°C, which may reduce smoke absorption or cause fat separation in the surface layer. Immediately after drying, sausages are smoked at 65–70°C until the desired surface color and aroma have been reached, and the temperature is about 50°C (Bøgh-Sørensen et al. 1981). Then the sausages are cooked in steam (75°C) until a core temperature of 72–73°C is reached (Fig. 17.6).

Quality Aspects of the Finished Product

Quality includes taste and flavor, structure, color, nutritional value, and microbial quality. The taste and flavor of sausage is a combination of the savory taste of cooked cured meat and spices. The flavor is influenced by the formulation (what meat animals have been used, what the proportions are, and especially the content of fat from the various sources). An integral part of the flavor is the effect of nitrite, which is based not on the direct taste of the salt itself but on an indirect effect on various components of the meat (Pegg and Shahidi 2000). Much chemical research has been performed on the effects of nitrite on flavor, but this extremely complex system is still far from fully elucidated.

The sensory and nutritional quality is mainly based on lean meat content, fat content, and the amount of water added. In addition, if other foodstuffs are added, they may improve the overall nutritional quality, or as extenders, they may dilute the nutritional density and taste. The use of phosphates facilitates the use of less lean meat and more water and fat, thus exerting a negative effect on nutritional value (unless low-fat/low-sodium products are targeted). The almost infinite number of possible formulation combinations allows the designing of the sausages according to needs: sometimes strong-tasting and firm sausages are required, while other times milder and softer are needed.

Microbes also exert a substantial effect on the sensory properties of meat, although the hygienic quality of sausages has improved tremendously. It is not possible to cover the microbial effects exclusively. Salt (NaCl) decisively influences the microbial pattern in meat, as it strongly reduces the proteolytic metabolism of the flora. The inhibitory effect of salt is based on its content in the water phase of the product. In countries where the product has a high fat content and phosphate is not added, the salt content in the water phase ranges from 4% to 5%; but with lower fat and higher levels of water (with phosphates), the content in the water phase could be even as low as 2.5%. It should be remembered that about 10 percentage units of lean meat water is strongly bound to the polar parts of proteins and thus not included in the free water phase of the product (3%–6% units of the product water, depending on the lean meat content of product; Hamm 1972).

Nitrite also has a very strong inhibitory effect on microbes, but the effect is strain specific. The effect is especially important against the most pathogenic bacteria within the contents used in cooked meat products. As the pK_a-value of the effective form, nitrous acid (HNO_2) is 3.4, nitrite is more efficient at lower pH values, but on the other hand, low pH values increase the degradation of nitrite (Honikel 2007).

The nutritional quality of sausages directly reflects the formulations of the sausages, which can be most variable. As the maximum temperature used in cooking is 72–74°C, meat proteins do not lose their nutritive value, but on the contrary, collagen turns digestible via denaturation (Bailey and Light 1989). Since sausage does not lose liquid by

cooking, minerals and vitamins remain in the batter. The vitamins are destroyed to a certain extent, depending on the vitamin and the process. About 10%–20% of the vitamins of the B group are destroyed during the preparation. Vitamin A is quite resistant to cooking, but about one-third can be lost by chopping (Niinivaara and Antila 1972; Lawrie and Ledward 2006). Smoking may reduce the biological value of proteins, the significance of which depends on the relative surface area and the intensity and length of smoking. In most cases however, the reduction is not substantial.

Connective tissue proteins (collagens) denature when sausage is cooked, and they are also largely comminuted by chopping, especially in finely chopped sausages. Ninety percent of the denatured collagen will be hydrolyzed in the human digestive track and, consequently, used as energy or for protein synthesis (Bailey and Light 1989). The biological value of collagen is, however, very low as such, but in combination with other proteins, it may have some value. A protein efficiency ratio (PER) of 2.5 for good-quality proteins allows for a collagen content of up to 30% of the meat proteins (Bailey and Light 1989). Therefore, most cooked sausages fulfill this requirement.

The oxidative changes in fats or membrane phospholipids can cause rancidity. Also, oxidative changes may cause polymerization of fats as well as proteins. Heme iron is a strong prooxidant, and particularly in freeze-stored meat and cooked meat, the oxidation may be very fast (the worst combination is food prepared from freeze-stored meat). Microbes may also increase oxidative changes in meat. In sausages, however, there are efficient antioxidative agents. Nitrite stabilizes heme iron, which results in a much lower oxidation rate. Phosphates also have an antioxidative effect as they chelate prooxidative cations. Ascorbates are also antioxidants, although they are primarily used as color enhancers. Finally, the use of packaging that excludes oxygen can reduce oxidation. Consequently, sausages are not, or they need not be, susceptible for rancidity, provided that nitrite and ascorbates are used, and eventually phosphates. Also, by avoiding a long freeze storage of meat raw materials, using appropriate packaging, and avoiding microbial spoilage, the risk of rancidity can be reduced (Pegg and Shahidi 2000).

Safety Aspects

With a hygienically well-organized production process and a satisfactorily cooked product, the sausage contains only a few hundred living microbes per gram, and those usually are not particularly capable of proliferating at cold storage temperatures. Common salt inhibits proteolysis, and nitrite and other antioxidative agents inhibit oxidation. The main role of nitrite, however, is to inhibit pathogenic bacteria. Most of these bacteria are strongly influenced by nitrite at the levels used in sausages (Pegg and Shahidi 2000). The primary reason for the use of nitrite in meat products is its specific capacity to inhibit the growth of *Clostridium botulinum*. This is increasingly important, as the trends are for the use of vacuum packaging (anaerobic), lowering the salt content, improved hygiene (less competing flora), and in-package pasteurization (hardly any vegetative flora). In these circumstances, the strictly anaerobic spore-forming *C. botulinum* may start growing and become toxic, if the packages are stored for longer period of times, and especially if temperature abuse is involved (Korkeala 2006). However, nitrite is by no means a guarantee for full safety.

Recent and Future Trends

The salt intake in modern industrialized countries has been connected to elevated blood pressure and consequently to an increase in coronary heart disease. Only the

salt that is detected by the taste receptors will be considered salty. Therefore, there have been considerable efforts to reduce salt (i.e., sodium) intakes, especially via industrial food. This reduction cannot be achieved quickly, but over a period of years, so that consumers gradually get used to lower salt content in their foods. In Finland, for example, the reduction of the average salt content in cooked sausages from 2.3%–2.4% to 1.5%–1.7 % took about twenty years (Ruusunen and Puolanne 2005). In many countries the same development has been experienced, but all countries have not done it yet.

The use of nitrite has been debated for more than thirty years, and during that period, the levels added have been lowered through legislative actions and voluntary decisions in the industry from about 200 mg/kg to 80–120 mg/kg. The industry is still looking for possibilities for further reductions, and the number of nitrite-free cooked sausages is even increasing. However, no single substance that would replace all the positive effects of nitrite (inhibition of pathogenic bacteria and spoilage flora, color formation, antioxidant capacity, effects on taste) has been found (Cassens 1990). In-package pasteurization would allow a reduction of nitrite, but then extra measures for pathogen safety must be performed. In-package pasteurization might increase the risk of spore-forming *C. botulinum*, as it is not competitive when high numbers of other bacteria are present (Korkeala 2006).

Health food, or even functional food, is a worldwide trend, and there have been attempts to use sausages as vectors for functional ingredients, including several methods for manipulating sausage properties. Making changes in the lipid portion of sausages does not necessarily fulfill the criteria of functional food, but it belongs to the same trend. There are low-fat varieties, down to 6%–10% fat. Also animal fat has been replaced with vegetable fat or even conjugated linoleic acid (Martin et al. 2008). Vegetable fats usually melt at lower temperatures than meat fats, and they are liquid at chopping temperatures. This causes a fat separation during preparation and also during cooking. This separation can be reduced by making a preemulsion using an emulsifier (e.g., soy protein or caseinate). As most sausage fat is pork fat or poultry fat, which are close to or within the dietary recommendations, the real benefit of the replacement could be questioned.

Sodium has been targeted by replacing sodium chloride with different mineral salt mixtures. Most of them contain potassium chloride. The bitterness of potassium ion limits the total replacement of sodium with potassium. Also, lactate as potassium salt has been used as a partial salt replacer. As the contents required are rather high (from 1%–2%), the bitterness caused by potassium is a limiting factor.

Accelerated processing (i.e., prerigor curing) has been extensively studied over the years. Hot boning with prerigor curing allows a very fast processing of carcass without cooling of sausage meats. Salt is required at levels of 1.5% or more (in prerigor curing as well as in the sausage batter) in order to achieve the desired water/fat binding (Puolanne and Terrell 1983). Prerigor curing would replace the use of phosphates. Despite the research and demonstrated positive results, the practice has not been widely adopted in the industry.

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Chapter 18

Bacon

Peter R. Sheard

Introduction

Originally developed as a method of preserving pork before the widespread use of refrigeration, bacon remains a popular product in its own right. Its production varies from country to country (Feiner 2006), but typically involves the treatment of boneless pork cuts with curing salt, usually added as a brine. As practiced in North America, bacon is produced from boneless pork belly that is hot smoked, sliced thinly, and vacuum packed (Andersen 2004), while in Ireland and the United Kingdom, the most popular bacon is made from cured pork loins. In continental Europe, bacon lardons (cubes) are used mainly as a cooking ingredient.

Salt (sodium chloride) and nitrite are essential for curing, although nitrates (sodium or potassium) are still used in some brines. Both major ingredients, salt and nitrite, are multifunctional. Salt acts as a preservative by lowering water activity, gives bacon its characteristic salty flavor, and increases the water-holding capacity of meat by solubilizing myofibrillar protein and increasing the myofibrillar lattice spacing (Offer and Trinick 1983). Although nitrite chemistry is relatively complex, the basic functions of nitrite are well known (e.g., Pegg and Shahidi 2000; Honikel 2008). It (1) acts as a preservative, (2) promotes the formation of the cured meat color, (3) contributes to cured meat flavor, and (4) acts as an antioxidant. The principles of curing have been described in an earlier chapter. This chapter focuses on the manufacture of bacon, as practiced in Europe, particularly in the UK where annual per capita consumption is about 8 kg (Fisher 2006).

Processing Stages

Traditional Wiltshire Curing

Fifty years ago, nearly all bacon was made by a traditional Wiltshire process (Fig. 18.1). This involved three distinct stages: injection, immersion, and maturation. The process has been described elsewhere (Hughes 1988; Varnam and Sutherland 1995; Ranken 2000; Lawrie and Ledward 2006).

Injection of Pork Sides

Whole sides, usually bone-in and rind-on, were injected with a brine containing salt, nitrate, and nitrite. Injection was carried out manually, using a single needle. The brine was introduced at multiple points (25–30) along the carcass in order to obtain a reasonably uniform distribution.

Immersion in a "Live Brine"

Following injection, sides were stacked into large tanks capable of holding several hundred pieces. These were sprinkled with salt, covered with an immersion brine, and held for several days. A characteristic feature of the process was that the immersion brine

Figure 18.1. Traditional Wiltshire curing as practiced 50 years ago involved the manual injection of a brine into pork sides, followed by immersion in a "live brine" and maturation for about 10 days. The process was slow and labor intensive.

was continually reused and consequently developed a unique microflora of salt-tolerant bacteria, typically $>10^6$/ml, which played an important role in converting nitrate to nitrite. Typically deep red in color, the brine also contained proteins in suspension. This so-called "live brine" was also believed to contribute to the characteristic flavor. The brine could be used almost indefinitely. To do so required regular checks on the composition, its temperature, and pH. Salt, nitrate, and nitrite were replenished when necessary. It was necessary to keep the brine chilled, 2–5°C, and the pH within strict limits. An unstable brine was associated with a high pH. Aeration helped maintain stability if the brine was unused for several days.

Maturation

Following immersion, the sides were removed, stacked, and allowed to mature for 10 to 14 days before further processing. The maturation period was believed to be important for flavor development and to improve sliceability.

Modern Wiltshire Cured Bacon

Bacon is still produced using a Wiltshire-style process, albeit adapted for modern production. Thus, manual injection of brine using a single needle has given way to multineedle injection, which is less labour intensive and achieves a better brine distribution. Although some whole sides are still cured, it is more common to use boneless loin pieces or bellies, which can be processed more rapidly (Fig. 18.2). The period of maturation, 2 to 4 days, is considerably shorter. As before, careful handling of the live brine is necessary to ensure stability.

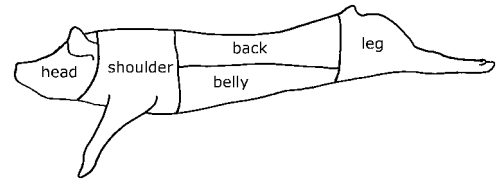

Figure 18.2. Whole sides were cured in traditional Wiltshire curing, but most modern bacon derives from the loin and belly, which produce back bacon and streaky bacon, respectively. Often the shoulders are used for sausages and the hindlimb for ham.

Modern Bacon Production

Although some Wiltshire-style bacon is still produced using a live brine, generally, this has been superseded by curing techniques that employ freshly prepared brines. Two techniques predominate: immersion curing and bag curing. Both methods are more rapid than traditional Wiltshire curing, due mainly to the use of smaller, boneless pieces. Back bacon prepared from the loin is the most common, accounting for 84% of the market, with streaky and middle bacon accounting for the rest (Fisher 2006). The production of dry-cured bacon takes longer, but even this can be achieved within about 10 days. An overview of the main stages involved in modern bacon manufacture is given in Figure 18.3.

Pig Production and Slaughter

Most bacon is made from pigs, of various genetics, reared in intensive indoor systems and fed a commercial concentrated diet. The pigs have a fast growth rate and are slaughtered at 90–110 kg when they are 5 to 6 months old. Such pigs are relatively lean, the average backfat thickness of UK pigs being about 11 mm (BPEX 2008). Bacon from organically produced pigs—reared less intensively and using organic feed—inevitably commands a premium price.

Slaughter and Chilling

Plants that have their own slaughtering facility will normally source pigs from farms located close to the factory, within a few hours' drive. A lairage area within the abattoir is provided to allow a period for the animals to rest prior to slaughter. A fine mist is sometimes used to exert a calming effect, except in very cold weather. Any animals kept overnight must have access to feed and water. Good stockmanship during unloading, in the lairage, and in moving animals to the

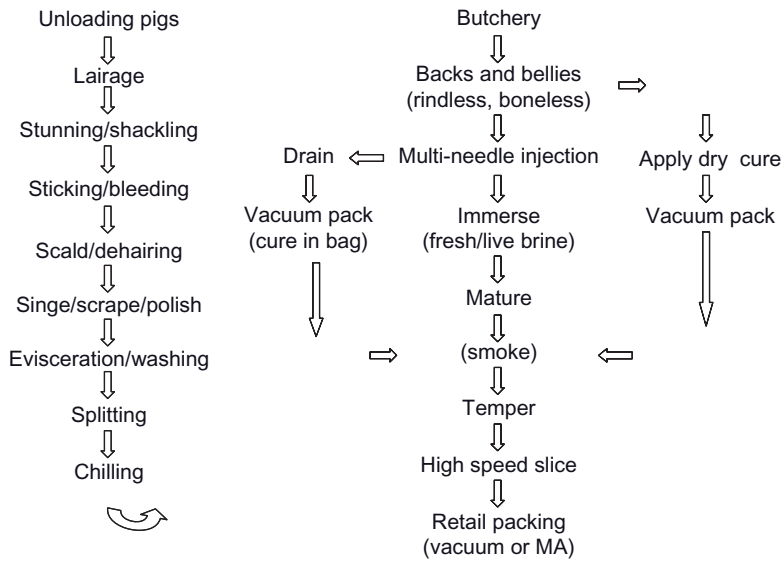

Figure 18.3. Diagram illustrating the major operations in bacon processing. After unloading, the pigs are killed, dressed, and processed in modern, large throughput abattoirs. Most bacon is produced from boneless, rindless backs (loins) and bellies that are immersion or bag cured, tempered, high-speed sliced, and packaged in a modified atmosphere (MA) or vacuum packed.

point of slaughter is important to reduce pre-slaughter stress that might otherwise cause a high incidence of PSE. The poor water-holding capacity of the latter can adversely affect brine uptake and retention (Kauffman et al. 1978; Fisher et al. 2000).

The pigs are killed humanely and dressed hygienically. Electrical stunning, followed by prompt sticking, has been the usual method, although this is being superseded by carbon dioxide stunning throughout Europe. Singeing the carcass improves the sliceability of rind-on product.

Toughness is not a problem in bacon, so interventions to improve tenderness (e.g., hip suspension, electrical stimulation, and long aging periods) are unnecessary. Butchery normally commences immediately after overnight chilling. The other parts of the carcass are sold fresh or used for processing (shoulders for sausages and the hindlimb for ham). Most plants employ a traceability system that can trace product back to the day of slaughter.

Immersion Curing (Tank Curing)

The pieces to be cured, whether boneless loins or bellies, are first injected mechanically with a brine containing salt and preservative (sodium nitrite alone or in combination with nitrate). Sodium polyphosphate, where included, will improve the water-holding capacity but its use is less common than formerly, due to consumer concerns. Sodium ascorbate may be added to promote development of the cured meat pigment and improve the color shelf life (Ranken 1981). Sugar is used in the production of sweet-cured bacon, which helps to mask the salty flavor. The target weight gain, typically 10%, is based on the total weight of the piece(s) to be injected, although it will be appreciated that proportionally more brine is taken up by muscle rather than adipose tissue.

Following injection, the pieces are stacked in small tanks, with a capacity for 30 to >50 pieces, covered with fresh immersion brine, and held for up to 3 days. The cured pieces are then removed, stacked, and matured just long enough to dry the surface. They are then ready for slicing.

Bag Curing

This process differs in that it has no immersion stage. Instead, the boneless pieces are injected and, after a short drainage period, placed in a moisture- and oxygen-impermeable bag, vacuum sealed, and held for a minimum of two days to allow time for equilibration and the development of the characteristic cured color. As there is no immersion stage, all the necessary salt, preservative, and other ingredients have to be introduced into the injection brine. The final salt content is typically 3%.

Dry Curing

Some bacon is dry cured, but the process is quite different and much shorter than that used in the dry curing of hams (e.g., Parma or Iberian), which can take up to two years to develop the characteristic aroma and flavor (Toldrá 2002). Often the process takes no more than a couple of weeks, which does not allow sufficient time for the proteolytic and lipolytic changes that occur in the production of dry-cured hams. The major contributors to flavor and odor in dry-cured bacon, therefore, are those derived from the curing ingredients and generated during cooking.

Production involves rubbing the dry-curing ingredients manually into the surfaces of exposed lean tissue. The treated pork is vacuum packed and stored under chill conditions for about 10 to 14 days (depending on the thickness of the product), after which it is ready to slice. A sweet cure can be obtained by rubbing in sugar after 7 days before repacking and leaving the product for another 7 days.

Dry-cured bacon is drier and has a higher meat content, about 97%, than that produced using a brine, but the flavor is similar in both.

Smoking

Traditional methods of smoking use natural wood smoke generated under controlled temperature and humidity conditions from hardwoods such as oak, beech, and hickory. The center of the product never rises above 30°C, so the product remains uncooked, albeit with an altered flavor, odor and color due to the action of the smoking process. Smoked bacon accounts for approximately 25%–30% of the UK market (Fisher 2006).

Tempering and High-Speed Slicing

Most bacon is sold pre-sliced. This is achieved using high-speed slicers operating at 800–1400 revolutions per minute (i.e., ~10–20 revolutions per s). This requires bacon to be sliced in a tempered (partly frozen) condition to maximize the yield of high-quality slices. The optimal conditions for slicing depend on both the bacon (its temperature, salt content, and the amount and composition of the adipose tissue) and the slicer (its design and slicing speed) (James and Bailey 1987; Brown et al. 2003). High-speed photography has demonstrated the importance of correct slicing temperature. If the temperature is too high, the bacon is too soft and distorts when presented to the slicing blade, resulting in a low yield of high-quality (well-defined) slices. If the temperature is too low, the bacon is more brittle and tends to shatter, again reducing the slicing yield. The optimal temperature varies with salt content. For bacon containing 3% salt, the optimal slicing temperature is about −7°C (Brown et al. 2003). The optimal temperature varies because salt lowers the initial freezing point (ifp), which, in turn, affects the ice content and the resulting mechanical properties of the semifrozen product. The temper temperature can be achieved using a one-stage system by placing the bacon in a cold room operating at the target temperature, or, more commonly, by a two-stage system employing a blast freezer and a separate cold room to achieve equilibration (Brown et al. 2003).

The amount and composition of the adipose tissue can also affect the firmness of the bacon to be sliced (Enser et al. 1984; Shackelford et al. 1990; Rentfrow et al. 2003; Teye et al. 2006), depending on the degree of unsaturation of the component fatty acids, which can vary widely. A high proportion of saturated fatty acids results in adipose tissue that is relatively firm, which, in turn, affects sliceability.

Packaging

Two systems are used for packing pre-sliced bacon: vacuum packing and modified atmosphere packing. Various studies have shown that the cured meat pigment nitric oxide myoglobin (NOMb) is unstable when exposed to light and air. A major concern, therefore, in both pack types is to exclude oxygen, which is detrimental to the stability of the cured meat pigment. This is quite different from fresh meats, where the predominant pigment, oxymyoglobin, is favored by high oxygen concentrations.

Vacuum Packing

Twenty years ago, the majority of pre-sliced bacon in the UK was vacuum packed. Vacuum packing's use continues but less commonly than formerly. Packing under vacuum extracts the air, and the package collapses around the meat. Any residual oxygen is depleted by tissue respiration, and carbon dioxide is produced. The resulting pattern of microbial growth is quite different from that which occurs in air. The growth of pseudomonads is inhibited, while lactic acid bacteria dominate and can reach high numbers without causing objectionable spoilage.

Modified Atmosphere Packing

Most retail-packed bacon employs a modified atmosphere having an initial composition of 70%–75% nitrogen and 25%–30% carbon dioxide. The residual oxygen level must be less than 1% to ensure a long color shelf life. Carbon dioxide, employed as a preservative, is highly soluble in meat (Jakobsen and Bertelsen 2004), forming carbonic acid. The acid is able to pass through bacterial cell walls into the cell, where it dissociates and interferes with normal cell metabolism. Lactic acid bacteria are less susceptible to the action of carbon dioxide and, therefore, constitute the predominant microflora. The other main gas, nitrogen, is inert and prevents pack collapse.

Storage Instructions

Once opened, the bacon should be kept refrigerated and consumed within three or four days. Although the microbiological quality cannot be guaranteed beyond about four days, anecdotal evidence suggests that some consumers may use bacon for up to a week after opening. Microbiological deterioration after opening may also be accompanied by color fading, especially on the surface of slices exposed to oxygen.

Industry Standards

Most bacon producers in the UK are members of the Charter Quality British Bacon scheme run by the British Meat Processors Association (BMPA). The BMPA standard (BMPA 2006), available from the association's website, lays down various standards and specifications that must be adhered to by the scheme's members.

Issues Facing the Industry

Low-Salt Bacon

In an effort to reduce the incidence of hypertension in the UK, the Food Standards Agency has set voluntary targets for the food industry aimed at reducing the average salt intake to 6 g/day from the current level of about 9 g/day (Matthews and Strong 2005). Meat and meat products currently contribute about 25% to the total dietary salt intake; this derives mainly from salt added to meat products (Henderson et al. 2003). Cured meats (bacon and ham) are a significant contributor to the total because of their high salt content and their popularity. However, reducing the salt content in cured meats is difficult to achieve without altering the shelf life. For example, Applegate (1989) demonstrated that reducing the salt content from 3% to 2% in vacuum-packed bacon resulted in unacceptably high levels of *Enterobacteriaceae* during the six-week storage period. The microbial flora was dominated by lactic acid bacteria, which were present at similar levels of about 10^8 in the low-salt bacon as well as the 3%-salt bacon. Reducing salt from 3% to 2% also influenced the amount and appearance of drip, the color of the product, and meat pH, as well as flavor. The study provides a good example of the multiple properties that can be affected in products where salt content is reduced.

Some low-salt bacon is available commercially. This can be achieved by replacing up to a third of the sodium chloride with potassium chloride, or by incorporating potassium lactate as a shelf-life extender. Potassium chloride has similar properties to sodium chloride in terms of water holding (Hamm 1960) and antimicrobial efficacy (Bidlas and Lambert 2008), but higher replacement levels are associated with a bitter flavor (Gou et al. 1996). The use of either additive would, of course, require a declaration in the list of ingredients.

Lower Nitrite Levels

In addition to reducing salt levels, the UK industry is faced with reducing nitrite levels in certain types of bacon to comply with new

EU legislation aimed at minimizing the formation of potentially carcinogenic nitrosamines while maintaining microbiological safety. Concern about the use of nitrite and nitrate in cured meats stems from research in the 1970s showing that nitrosamines were generated during the frying of bacon (e.g., Patterson et al. 1976) and could also be formed in vivo in the acidic conditions present in the stomach. Despite attempts to find safer alternatives (see Pegg and Shahidi 2000), nitrite continues to be used in curing, albeit at much lower levels than those employed 30 to 40 years ago when nitrite levels in bacon could be as high as 1000 mg/kg (Ingram 1971), well above current permitted levels (Table 18.1).

In North America, a demand for natural and organically cured meats has led to the use of natural sources of nitrate (e.g., sea salt, raw sugar, and celery) rather than using conventional curing ingredients. This approach can deliver the typical quality characteristics expected of cured meats, provided that sufficient nitrite is formed from the nitrate source, although, in practice, residual nitrite levels are often less than in conventionally cured product (Sebranek and Bacus 2007).

White Exudate in Cooked Bacon

Though bacon remains a popular product, one of the frequent complaints heard about modern bacon concerns the unsightly white liquor that sometimes exudes from bacon during cooking. There is no evidence that this affects eating quality, but the exudate nonetheless adversely affects appearance during cooking. Studies at the University of Bristol demonstrated that the exudate had a similar composition to drip (Sheard et al. 2001). It consists mainly of water, proteins derived from the sarcoplasm, and a relatively high level of salt. The amount of exudate was assessed subjectively by ranking photographs following "dry-frying" or objectively by collecting exudate in an ice-cooled tray during grilling. It was demonstrated that dry-cured bacon produced less exudate than that produced by Wiltshire cured bacon; the greatest amount of exudate was produced by rapidly cured bacon. Tempering increased the amount of exudate. It was also noticed that higher amounts of exudate resulted from pigs with the lowest ultimate pH, with least exudates at the highest pHs. Although interventions to reduce the amount of exudate were not investigated, it seems likely that improving the water-holding capacity (WHC)—by using phosphate, for example—would lead to less exudate.

"Tiger Stripe"

Modern bacon sometimes exhibits a regular alternating pattern of light and dark bands, which was described as "tiger stripe" when it was first reported (Voyle et al. 1986). The alternating pattern had a regular periodicity that seemed to coincide with the injection

Table 18.1. Changes in maximum permitted levels of nitrate and nitrite in bacon

Legislation	Ingoing amount (mg/kg)	Residual amount (mg/kg)
Preservatives Regs 1979[1]		200 nitrite, 500 nitrite + nitrate
Food Additives Regs 1995[2]	300 nitrate	175 nitrite, 250 nitrate
Food Additives Regs 2007[3]	150 nitrite and 150 nitrate*	

[1] Preservatives in Food Regulations 1979 (amended 1982)
[2] Miscellaneous Food Additives Regulations 1995 (implementing Directive 95/2/EC)
[3] Miscellaneous Food Additives & Sweeteners (Amendment) (England) Regulations 2007 (implementing Directive 2006/52/EC)
* exemptions for immersion and dry cured bacon where maximum residual amounts of 175 mg/kg nitrite and 250 mg/kg nitrate apply

marks caused by the needles of a mechanical injector during the curing process. Microscopic investigations revealed that the dark bands showed ordered myofibrillar structure (i.e., myofibrils with overlapping thick and thin filaments and well-defined Z lines), while the light bands exhibited a disordered structure in which the usual structural features were obscured by amorphous material which the authors attributed to denatured sarcoplasmic protein or denatured myofibrillar material. The phenomenon was attributed to localized variations in brine concentration around the injection sites. At the time, some affected batches of bacon were returned to the manufacturer, but the condition now appears to be accepted as a normal feature of modern bacon production. A similar phenomenon can also occur in moisture-enhanced pork (Gooding et al. 2009), suggesting a common mechanism in both types of product.

Boar Taint

The compounds responsible for boar taint, androstenone and skatole, were identified many years ago, but boar taint remains a persistent problem for the pork industry. However, quantifying the occurrence of off-odors or off-flavors in cooked bacon is difficult, partly because individuals are highly variable in their sensitivity, particularly in their response to the pheromone, androstenone (Annor-Frempong et al. 1997). Castration of entire male pigs is a common method of preventing boar taint, but, even here, taint could arise due to high levels of skatole. The latter is produced by fermentation of the amino acid tryptophan in the hind gut.

Most of the studies investigating boar taint in bacon were carried out more than 25 years ago (Rhodes 1971; Lesser et al. 1977; Mottram et al. 1982; Smith et al. 1983; Lundstrom et al. 1983, cited by Malmfors and Lundstrom 1983), when slaughter weights were lower and pig genetics were quite different from those used today and at a time when less was known about boar taint. A French study on bacon lardons (bacon cubes) prepared from gilts and boars at three different combinations of skatole (S) and androstenone (A) (low S/low A, low S/high A and high S/high A) and assessed by 96 consumers demonstrated that the combination of high skatole and high androstenone was significantly worse for several odor descriptors (Beague et al. 1997). This is to be expected, since androstenone and skatole are both volatile, particularly at the high temperatures attained during frying (a common method of cooking). Off-flavors during consumption may be less marked due to the partial volatilization of the taint compounds during cooking (Bonneau et al. 1992) and any abnormal flavors partially masked by the curing ingredients, especially salt.

Immuno-castration appears to be an effective way to reduce the incidence of boar taint in fresh pork (Prunier et al. 2006; Pearce et al. 2008), and its adoption could have a marked effect in reducing the volume of tainted bacon. This reduces both taint compounds because high levels of androstenone act naturally to increase skatole in the liver. The use of different fiber sources in the diet before slaughter (e.g., chicory) is effective in reducing skatole.

PSE and DFD

The incidence of the PSE condition in the UK pork industry has risen from 6% in the 1970s, to 13% in the 1980s, and 15% in the 1990s (Table 18.2). This is probably due to changes in abattoir operation (fewer plants with higher throughputs and greater pre-slaughter stress) and breeding programs that have resulted in pork with a higher incidence of white fibers and a greater tendency to PSE. The adverse effects of using PSE meat in cured meats are well documented (Kauffman et al. 1978; Honkavaara 1988; Clarke 1998; Fisher et al. 2000; O'Neill et al. 2003). Using

Table 18.2. Incidence of the PSE condition in pork *longissimus* muscle: reports from three large UK-based surveys over the last 30 years

	1970s[1]	1980s[2]	1990s[3]
N	6015	5383	5598
Mean pH_{45}	6.55	6.38	6.39
% PSE ($pH_{45} < 6.0$)	5.7	12.8	15.1

[1] Kempster & Cuthbertson, 1975
[2] Chadwick & Kempster, 1983
[3] Homer & Matthews, 1998

halothane-positive pigs, Fisher et al. (2000) demonstrated that brine take-up by PSE pork is almost half that of normal pork (halothane-negative pigs), resulting in a net increase in yield of just 3% compared with 10% for normal pork. The use of PSE in curing also results in increased drip at raw material intake and may result in more white exudate during cooking.

If the ultimate pH of muscle remains high (≥6.0) because of glycogen depletion through stress before slaughter, DFD muscle results. This has increased water retention, but the high pH results in a shorter microbial shelf life. However, if the ultimate pH of the loin muscle remains slightly above the "normal" value of 5.5 but is not high enough to cause the problems associated with DFD, the muscle retains more water during processing, resulting in more tender and juicy bacon. Some plants use very rapid chilling to achieve these "high" pH values.

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Chapter 19

Canned Products and Pâté

Isabel Guerrero Legarreta

Introduction

Canning is probably the most efficient meat preservation method. It ensures the destruction of pathogens and spoilage microorganisms and allows foods to be easily handled and transported. In addition, canning provides food for various distribution types: retail in small can sizes (250 to 500 ml) for domestic purposes; wholesale (750 ml to 1 L) for industrial restaurants and central facilities; and for hospitals, schools, and other institutions (946 ml, 1.89 L, and 3.89 L). Canned products' processing is calculated according to the expected shelf life: from semi-preserves requiring further preservation methods, to full and tropical preserves, with a shelf life of 4 years at 25°C and up to 1 year at 40°C, respectively. Heat treatments are also applied to develop specific sensory characteristics or physical properties, such as gels in luncheon meats or spreadability in pâté. This chapter discusses the theoretical principles of thermal treatment and the meat-canning process. Finally, pâté fabrication and quality characteristics are also described.

Effect of Intrinsic and Extrinsic Meat Characteristics on Meat Microbial Populations

Microbial communities in foods are not stable; heterotrophic populations change with time and the presence of specific chemicals. Meat is a very rich substrate, containing almost all nutrients necessary to support a wide variety of microbial populations. Raw materials for canned meats can be previously processed or refrigerated. The specific microbial associations in refrigerated raw meats are nonfermenting Gram psychrotrophs such as *Pseudomonas, Alcaligens, Flavobacterium, Shewella,* and *Moraxella.* When the meat is cured, such as with sausages or other previously processed meats treated with nitrates, nitrites, salt, and phosphates, the dominant bacterial populations change to Gram-positives, such as *Micrococcus, Lactobacillus, Carnobacterium,* and *Brochothrix*. However, meat sanitation depends on the destruction of specific microorganisms, particularly pathogens; shelf-life extension depends on intrinsic food characteristics, such as chemical composition and external conditions, especially temperature, time, and nutrient availability.

Among spoilage microorganisms in canned meats, *C. thermosacarolyticum* is of the first importance; its spores can survive and develop at very high temperatures. Other spoilage microorganisms in meats are the psychrophiles *Pseudomonas* sp. and *Achromobacter*; the mesophiles *E.* coli and *Bacillus subtilis*; the facultative thermophiles *Streptococcus thermophilus* and the already mentioned *Clostridium perfringens*; and the strict thermophiles *Cl.thermosaccharolyticum* and *Bacillus stearothermophilus* (Zamudio 2006).

Not all preservation methods are completely efficient; generally, more than one is applied, according to the well-known

"hurdle" theory (Leistner 1985). In the case of canning, although various treatment severities can be applied depending on the expected shelf life, alternative preservation methods in addition to heat are often used. For instance, canned sausages also include the previous addition of curing salts or the inclusion of antioxidants such as phenols in smoked sausages. According to the hurdle theory, it is not necessary to apply more hurdles than necessary to obtain microbiologically safe products with a considerable long shelf life. The net effect of the applied hurdles is an interaction of individual effects; therefore, if the ecology of a given food requires it, a more severe heat treatment must be applied due to intrinsic and extrinsic characteristics, such as high pH and low conductivity components (fat, carbohydrates, etc.), among others.

However, product susceptibility to deterioration of complex systems, such as that of canned meat products, depends on a wide variety of extrinsic and intrinsic characteristics. The most important are the following:

- pH of most canned meats is relatively high (pH > 6.5); a more severe heat treatment is necessary to destroy potentially present pathogens. A pH of 4.5 is the growth limit for *Clostridium botulinum*, a strict anaerobe widely present in nature and seldom found in canned foods. At this pH, *C. botulinum* can grow and produce toxins and heat-resistant spores; processing conditions to destroy this microorganism is the calculation basis for thermal processing of many foods.
- Water activity (a_w) is also a criterion for processing calculations; the limit value for *Cl. botulinum* is 0.97 for psychrotrophic species, and 0.95 for mesophile species. Unfortunately, a_w in most canned meats is above this value.
- Redox potential: there is a correlation between redox potential, heating, and the presence of additives (nitrates, phosphates, salt). Raw meat redox potential is −50 mV; this value is reduced when the meat is heated: in sausages, it decreases from +20 to −100 mV, depending on grinding conditions and additives (Zamudio 2006).
- Oxygen tension is decisive for the proliferation of certain strains. This is the case with strict anaerobes such as *Clostridium* spp. or microaerophiles such as lactic acid bacteria, which are present in very low numbers or even absent in the presence of oxygen. However, canned meat is a system totally void of oxygen.
- Food composition and structure also determine microbial colonization. Whereas meat cuts are mainly contaminated on the surface, microorganisms find their way to the inner part of the muscle, following the muscle structure, mainly through the perimysial layers. Ground products or those added with other food components allow microorganisms to colonize the inner as well as the surface food structures. Carbohydrates, fats, and proteins act as protectors against microbial destruction by heat.

In the striated muscle, caloric flux is also affected by the muscle fiber orientation. Conductivity, if this flux is perpendicular to the muscle fibers, is 1.72 kJ/h m °C at 78% relative humidity, 0°C; at the same conditions parallel to the muscle fibers, conductivity is 1.76 kJ/h m °C (Pérez and Calvelo 1984). Several additives act as antimicrobial agents: several medium-chain fatty acids, essential oils (cinnamon, clove, garlic, onion, and oregano), or proteins such as conalbumin.

Toxins

The pathogenicity of several microorganisms present in foods depends on their infection or intoxication ability. Infection is due to microbial colonization on the human organism; intoxication is a condition caused by the intake of a toxin, produced by the secondary

metabolism of certain microorganisms. Several of these toxins are thermostable, whereas others can be destroyed by heat treatments. The toxin *Aeromonas hydrophila* is heat sensitive*; Escherichia coli* 0157:H7 and *C. botulinum* (proteolytic types A, B, F, and nonproteolytic types B, E, F) toxins are medium heat resistant; *Vibrio* sp. (*V. cholera* and *V. parahaemolyticus)* and *Staphyloccocus aureus* toxins are highly heat resistant.

Microbial and Enzyme Destruction in Canned Foods

As stated before, heat treatment's first aim is to destroy pathogens, spoilage microorganisms, and enzymes. Theoretical considerations for microbial destruction are also valid for enzyme inactivation (Dziezak 1991). The main criteria for thermal destruction are: (1) all spores and viable cells able to grow and produce toxins must be eliminated, taking as a calculation basis *C. botulinum*, the most dangerous microorganism from the public health point of view; (2) spoilage microorganisms must be reduced to a limit that ensures food quality for a given time. From a commercial point of view, a food can be considered sterile if it is free from *Bacillus stearothermophilus* or *Clostridium perfringens*.

In general, the strict anaerobe *C. botulinum* is taken as the target microorganism due to its pathogenicity; however, other target microorganisms are *B. stearothermophilus, B. thermoacidurans*, *B. macerans,* and *B. polymyxa* (Guerrero Legarreta 2001), in addition to specific pathogens most likely to colonize a specific food. In the case of raw poultry meat and poultry products, these are *C. prefringens*, *Salmonella* spp., *Staphylococcus* spp., and *Campylobacter* spp. Microbial inactivation calculations are based on how long the food shelf life must be extended.

Sporulated thermophiles must also be considered if the food will be stored at high temperatures; this is the case with tropical preserves (Manev 1983). Heat treatment conditions destroying *C. botulinum* and *Clostridium sporogenes* result in a thermostable food, with considerably long shelf life and without the need of other preservation processing. Inactivation of either pathogen or spoilage-causing microorganisms is calculated by the heat penetration rate. Vegetative cells are destroyed at temperatures slightly higher than optimum growth temperatures, whereas spores can survive at higher temperatures (Zamudio 2006). Heat treatments depend on a time-temperature relationship.

Traditionally, process calculations consider that, since heat application involves the destruction of at least one microbial enzyme necessary to the bacterial metabolism, vegetative cells and spores are inhibited according to a first-order reaction rate equation (Baranyi and Roberts 1995), even though Peleg (2006) stated that there is evidence bacterial spore inactivation, including *C. botulinum* spores, does not follow first-order kinetics. The author states that the exponential inactivation rate depends on the spores' previous thermal history, which is not considered in the exponential inactivation rate equations that follow a log-linear Arrhenius model. However, the author concluded canning operations are generally a safe procedure due to over-processing.

When microbial populations are treated with humid heat at a temperature slightly higher than the maximum for growth, vegetative cells and spores are destroyed according to the equation:

$$-\frac{dc}{dt} = kc \qquad (19.1)$$

This means that cell concentration (dc) decreases (hence, the negative sign) with time (dt) in a direct proportion to viable cell concentration (c). This is a logarithmic cell destruction rate (for example: 10^3 to 10^2), but time increases linearly.

As mentioned before, another heat treatment objective is enzyme inactivation; meat enzymes (endogenous and exogenous) play an important role in meat spoilage, and it is, therefore, necessary to inactivate them. Enzyme inactivation depends on several factors and practically the same affecting microorganisms. However, in mixed-food products, several heat-resistant isoenzymes can be present, especially if plant material is added to the formulation, as peroxidases may be present; the process parameters are calculated taking into account the most heat-resistant enzyme (Braun et al. 1999).

Heat Transfer Mechanisms

In all thermal operations, the amount of transferred heat is necessary for calculations. Thermal processing is basically an operation in which heat flows from a hot element—the heating medium—to a cold element—the food. As it is a dynamic process, the heat flux is proportional to the driving force and inverse to the flow resistance. Heat transfer obeys one of the following mechanisms: conduction, convection, or radiation. In canning operations, only conduction and convection mechanisms take place. Radiation occurs in heating systems such as microwaves and infrared heating.

Conduction

In conduction, heat is transmitted by vibrations of adjacent molecules; this mechanism occurs in solids. This is the heating mechanism that takes place in canned products such as brines containing solid chunks (e.g., in soups containing meat pieces, canned sausage in brine, and products that gel during heating, such as luncheon meats and paté; Mittal and Blaisdell 1984). Conduction follows the Fourier law:

$$q = k\,(A\Delta T/L) \qquad (19.2)$$

where:

A = area of transference
ΔT = temperature difference
L = thickness of the material
k = thermal conductivity coefficient

The coefficient k depends on the food-intrinsic properties; it actually defines how heating is facilitated or prevented by the treated material. Comparing k for stainless steel versus k for meats makes clear the low conduction rate in foods. In stainless steel, k = 45.872 kg cal/h m^2 °C (Green and Maloney 1997), whereas on the average, k = 1.464 kg cal/h m^2 for meat (Mittal and Usborne 1985). Conduction may differ even in different cuts of the same animal, due to the particular chemical composition. Siripon et al. (2007) reported different average thermal conductivity for white and dark poultry meat.

Convection

Convection is carried out in fluids, due to density differences. It is the mechanism occurring in homogenous soups, brines, sauces, and syrups. This mechanism is based on Newtons's law:

$$q = hA\Delta T \qquad (19.3)$$

where:

A = area of transference
ΔT = temperature difference
h = heat transfer coefficient

The heat diffusion rate is higher if an external force is applied; can rotation decreases the temperature difference (ΔT) to a minimum (Welti-Chanes et al. 2003). The coefficient h describes the heating potential of a given medium; it depends on flow properties, surface type, and flow velocity of the heating medium. For instance, for boiling water, h = 1.464 kg cal/h m^2 °C; for condensing steam, h = 2.928 to 19.520 kg cal/h m^2 °C (Green and Maloney 1997). Therefore, to obtain the maximum heating rate during

canning, condensed steam is the most efficient heating medium through a barrier (the can) to the cold fluid inside the can.

Heating mechanisms may change in products that modify their physical characteristics during processing. For instance, in canned emulsions, such as luncheon meats and pâtés, where the product changes from semifluid to solid, the heating mechanisms change from convection to conduction. Process calculations must be carried out accordingly.

Finally, in products such as soups containing meat pieces or in sausages in brine, a combined heat and mass transfer mechanism takes place: heat transfer between fluids within the meat and between the product and the heating medium, and mass transfer as water and nutrients diffuse within the can.

Thermal Inactivation Parameters

In order to calculate the time-temperature relationship for a given heat process, several parameters have been developed that accurately describe the necessary time at a given temperature to achieve a given microbial destruction. Severe heat treatment can destroy all viable cells, and possibly all spores, although other food characteristics such as physicochemical (texture, color, water retention) and sensory quality can also be altered. Therefore, there must be a compromise between destruction of most undesirable microorganisms (pathogens and spoilage-related) and food quality.

D-Value

According to Equation 19.1, 90% of the microbial population is destroyed (1 log cycle) at a given time interval, provided a constant temperature is applied. This time interval differs from one microbial strain to another and is called decimal reduction time (D). It is given by the time in minutes necessary to destroy 90% of a given microbial population at a constant temperature. For instance, 90% of a *C. progenies* population is destroyed if heating at 110°C is maintained for 10 minutes; D value in this case is $D_{110°C} = 10$ min. At 115°C, the necessary time for the same microbial reduction is 3 minutes (i.e., $D_{115°C} = 3$ min); at 120°C, 1 min is necessary to obtain the same population reduction ($D_{120°C} = 1$ min). On the other hand, at different heating times, the number of destroyed microorganisms increases with time of exposure. For example, in order to destroy 6 log cycles (6D) of a given microbial population, the required heating times are: 120°C for 6 minutes ($D_{120°C} = 6$), or 115°C for 18 minutes ($D_{115°C} = 18$), or 110°C for 60 minutes ($D_{110°C} = 60$) (ICMSF 1996). D-values allow comparisons of the necessary heat treatment severity among different microorganisms or among temperatures.

z-Value

Heat resistance of a given microbial population is given by a z-value, which indicates the required temperature to reduce D-values by 1/10. The thermal death-time curve gives the heating time (x-axis) versus log number of survivors per ml or per g (y-axis); in this curve, the slope is the z-value, showing the temperature (in °C) required to reduce a given microbial population by 1 log cycle. For instance, if z = 10°C, then $D_{100°C} = 50$ minutes, therefore $D_{110°C} = 5$ minutes, and $D_{120°C} = 0.5$ minutes. This is shown in Figure 19.1.

F-value

F-value represents the thermal death extent of a given microbial strain, as well as treatment severity. It allows predictions concerning the product shelf life, as well as comparisons between different heat treatment conditions. As it is impossible to instantaneously reach the processing temperature in every part of a container or a can, the F-value is the sum of heat treatments in every

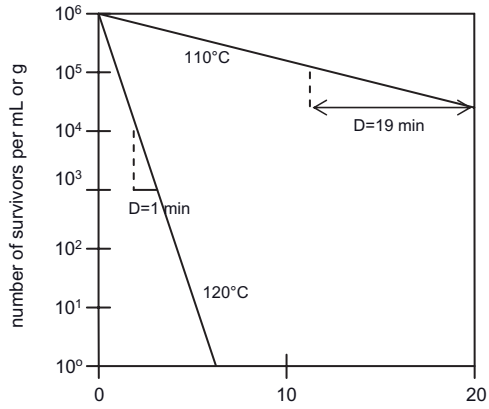

Figure 19.1. Thermal death time curve. (Adapted from Stiebing 1992.)

moment of the process: heating up, temperature holding, and cooling down. A hypothetical situation occurs assuming F = 1; this is the lethality effect when heating at 120°C for 1 minute. F_s is the sum of all F values in each point of the container

Heating in not homogenous in a food material, even less in canned meats or meat products, where fat, connective tissue, and other compounds such as carbohydrates and other additives are present, each with a wide range of heat-transfer capacities. Calculations, therefore, are carried out in reference to the point where heating occurs at the slowest rate or the "cold point"; at this point, the sum of all lethal effects is denoted as F_c. The cold-point position depends, therefore, on the food composition and the leading heating mechanisms. If convection is the main mechanism, the cold point is located along the vertical can axis. Can agitation, for instance when cans are rotated, increases the heat-transfer rate; in this case, the cold point is located approximately one-third up from the can bottom. This is the case with meat chunks in brine or canned sausages. If conduction is the main mechanism, the cold point is in the geometric can center. F_c is lower than F_s, as heating in the center is always lower than in the rest of the container (Guerrero Legarreta 2001). D and F are related through the equation:

$$F = D(\log a - \log b) \qquad (19.4)$$

where:

a = initial viable cell load
b = final viable cell load

In extreme cases, when the presence of *C. botulinum* is presumed or complete spore destruction is necessary, a "botulinum cook" is applied. This is a process, generally applied only to low-acid foods such as meats, fish, or dairy products, where cell number is reduced from 10^1 to 10^0, a 12 log-cycle reduction or 12D. Heat treatment ensures the probability of finding 1 spore in 10^{12} cans. Processing parameters in botulinum cook are (for *C. botulinum*): $D_{120°C}$ = 2.52 minutes and z = 10°C.

Process Lethality Calculations

When a new thermal process is designed or applied for the first time to a food, F-values are analyzed using thermocouples at various positions on the container, mainly at the cold point. Recently, the use of thermocouples has been substituted by radiotelemetry.

The rate at which a microbial population is destroyed and the total processed severity can be calculated by several methods; the easiest ones use the area under a curve that represents lethality versus time. Lethality is calculated by the equation:

$$\log(t/F) = (250 - T)/z \qquad (19.5)$$

where:

t = time at any given minute
T = temperature in the process

Another method for calculating the overall process lethality is by adding the F-value at every moment during the heating and cooling phases. Manev (1983) describes in detail the process for a tropical preserve expected to have a shelf life up to 1 year at 40°C; in this

case, F must be between 12 and 15. This type of preserves is intended for regions with extreme heat and humid weather, such as the humid tropics, with no other preservation facility. Total F-value for the heating phase is 9.4589, and for the cooling is 5.1602; the overall value is F = 14.6191.

Commercial Sterilization

The general principles underlying heat-transfer mechanisms and the response of foods to the caloric flow are the same for all processes. However, specific thermal processes have particular objectives. The objective of cooking (roasting, grilling, boiling, and steaming) and frying is mostly to destroy heat-sensitive organisms and toxins, to improve sensory characteristics, and to make the product more digestable; it is carried out at around 85°C, although frying can be done at 160° to 190°C. Scalding is applied in order to inactivate enzymes, remove gas trapped in the tissues, and clean the food material; it is carried out at around 65°C. Pasteurization destroys only part of the vegetative cell populations; therefore, further preservation methods must be applied. In most cases, the objective of pasteurization is to destroy pathogens, as is the case with milk. The high-temperature, short-time (HTST) pasteurization method involves temperatures around 70° to 73°C for 15 to 20 seconds, or 140° to 150°C for 1 to 45 seconds for fluid milk, whereas in the low-temperature, long-time method, heating is at 62°C for 30 minutes (for fluid milk).

Aseptic Processing

Sterilization is a process where severe treatments are applied to completely destroy *C. botulinum* or *C. perfringens*; although it results in a stable food, sensory characteristics such as texture and flavor are considerably altered. A sterile food is that where no viable microorganisms are present. However, treatments at temperatures above the maximum for bacterial growth destroy all viable cells; however, spores may survive in this condition.

In food-processing operations, the term "sterilization" is incorrect, as sterility is not fully achieved. This means that, although all pathogens are destroyed, some nonpathogens can survive, although environmental conditions are such that they cannot proliferate. Under this situation, it is said that foods are "commercially sterile," "microbiologically inactive," or "partially sterile."

There are two methods of commercial sterilization: placing the food in a container, with further heating; or heating and cooling the foods, then placing it in a container. The first method is the conventional canning operation, developed by Appert in the eighteenth century (therefore, it is also called "appertization"). The second method is called aseptic processing.

In aseptic processing, the food is commercially sterilized before packaging; it is based on the same principles as pasteurization, although the process is more severe. As the temperature applied is between 132° and 175°C, this process is similar to HTST. It is mainly based on enzyme inactivation, rather than microbial destruction. Once the container is filled with the food, a hot fluid such as brine is injected and the container sealed. This method is efficient only if further refrigeration is applied (Thumel 1995).

Canning

Canning processing consists of five stages: (1) food preparation (cleaning, cutting, selection, etc); (2) can, pouch, or jar filling; (3) air exhaustion; (4) sealing; and (5) thermal processing (heating and cooling).

Food Preparation

In the case of meat and meat products, the earlier operations depend on the type of product. Canned sausages are fabricated

according to certain formulations (curing, cooking, smoking, etc.). In the case of luncheon meat, an emulsion is prepared and the can filled with the raw meat batter.

Can Filling

Heat penetration depends on the solid-liquid ratio and the food distribution inside the can. In canned sausage distributed along the vertical axis, a convection-conduction mechanism takes place; if the solid material is loosely packed, the heating rate will be faster. In general, 30% of the can volume should be filled with a liquid, such as brine, to provide a high heat-transference rate. The brine is always added after the solids; in the case of pastes, the filling operations are generally carried out with automatic filling equipments; care must be taken not to incorporate bubbles. The headspace must be 0.5% of total can volume; efficiency during the exhausting phase depends on the headspace.

Exhausting

Oxygen is a very reactive substance that acts on food components. It modifies color, flavor, and overall quality. Air removal from the headspace is necessary to prevent these changes, as well as to improve the heat penetration rate and reduce the growth of aerobes. Vacuum formation in the can also prevents the risks of an increase in the pressure inside the can during heating, and possible can blowing or deformation.

Exhausting is carried out at normal atmosphere or under vacuum. If large meat pieces are put into the can, exhausting during filling and sealing is enough to evacuate the air; however, if pastes are canned, air can be incorporated if this operation is not carried out under vacuum. Cans are heated at 75° to 95°C immediately before filling and sealing; alternatively, the cans are placed in a conveyor where they are heated at 85° to 95°C, removing approximately 90% or more from the headspace (Mathlouthi 1986). Exhaustion is also carried out by heating, mechanical air removal, or by steam injection; if the last is applied, cans are immediately sealed. During can cooling, a vacuum is generated due to steam condensation, but if the headspace is excessive, a vacuum is not formed. Also, if air is not completely removed from the can, bacteria such as *Bacillus subtilis* and *B. mycoides* can grow.

Thermal Treatment

This includes three cycles: heating, temperature holding, and cooling. Time-temperature relationships during the heating phase are calculated according to microbial destruction and enzyme inactivation criteria, as described earlier, whereas cooling is applied for practical reasons, such as handling.

Commercial sterilization can be carried out by several methods, all based on the theoretical principles already discussed. The main methods are batch still retorts or continuous operations.

The batch method is still used in large industrial operations handling cans and glass jars, and all types of containers in small operations. However, Crang and others (2006) describe a process for bottling and canning sausage meat with domestic equipment. The authors concluded that some of the products in jars of thicker glass require a longer processing time to give the same level of safety. The method basically consists of loading the retort, closing it, and filling it with steam.

The second cycle, temperature holding, depends on the time-temperature relationship to satisfy the processing parameters (D, z, F).

Cooling is carried out by closing the steam valves and injecting cold water into the retorts. Care must be taken to gradually reduce the retort pressure in order to avoid deformation or breaking of containers.

Continuous retorts are generally used in large operations. The retort pressure difference is controlled to avoid deformation of

large-format cans or lid blowing in glass jars. In general, there are four systems, all using steam as the heating medium: Sterilmatic™, Orbitort™, Hydrostatic™, and Hydrolock™. Sterilmatic™ are Orbitort™ are similar; the cans are fed into the system through a pressure seal, moving through the retort in a helix conveyor that takes the cans through the heating-cooling zones. Heat transfer is increased by rolling the cans on rails.

After completing the thermal processing, cans are cooled by cold-water spray or immersion. This allows easier handling and reduces the pressure inside the can. However, water used for cooling must be sanitary, since seam failures or microscopic holes (pinholes) in the can allow water to enter into the food; microorganisms present in the water can grow and proliferate inside, causing sanitary failures as well as other alterations such as blowing. If cooling after heating is insufficient, thermophiles can grow. As a general rule, canned meat products are cooled down to 35°C. At this temperature, the can's outer surface cools rapidly (Guerrero Legarreta 2001).

Cans must be stored in small blocks in well-ventilated areas to allow them to reach room temperature at a fast rate. Recontamination after heat treatment is a very common problem, causing can blowing; it indicates seam failure. The main microorganisms responsible for this alteration are cocci and bacilli (Ray 1996).

Canned Meat Products

Canned meat products include meat stews, luncheon meat, sausages, sauces with meat pieces, and paste products. These products can be fully cooked before canning, as in the case of sausages, or can be homogenous products cooked inside the can, as in the case of luncheon meat. Products such as "chili con carne" ("peppers with meat"), sausages, and sauces with meat pieces have solid and fluid ingredients in the can. The heat process is then a combination of convection and conduction, as explained before. Chili con carne or "chili" is a spicy stew-like dish. The essential ingredients are, in addition to meat, chili peppers, garlic, and cumin. It often includes tomatoes, onions, beans, and other ingredients, as well.

Other products, such as luncheon meat and pâté, change their heating mechanisms during processing, as they turn from semifluid to solid. Pâté is a product in which an emulsion system turns into a gel.

Paste Products: Liver Pâté and Pâté de Foie Gras

Meat paste products, such as pâté, are similar to emulsions. A true emulsion is defined as two immiscible liquids, one of them being droplets or fat globules (the disperse or internal phase) dispersed into another (the continuous phase); the droplet or fat globule diameter in a true emulsion is between 0.1 and 100 μm (McClemens 1999). Meat emulsions, however, also contain in the disperse phase muscle fibers, small connective tissue fractions and carbohydrates, and the fat droplet diameter of the disperse phase is larger than 100 μm; therefore, meat emulsions are not considered true emulsions, but a "paste" or "batter." When heated, the proteins in the continuous phase gel, due to unfolding and interlinking. This phenomenon stabilizes the product. However, the high fat content provides a smooth texture and spreadability.

On the other hand, foie gras, a French term for "fat liver," is a traditional product fabricated only from the hypertrophied liver of goose or duck; this hypertrophy is obtained by supplying special diets to the animals. The practice of force-feeding geese to enlarge their livers dates back to at least 400 B.C. Egyptian hieroglyphics depict slaves force-feeding geese to enlarge their livers and obtain a primitive foie gras. To obtain foie-gras livers, animals are immobilized on farms and fed with diets high in calories; the liver

obtained has no pathology but is merely an organ with excessive fat content. Unlike other pâté, in pâté de foie gras fabrication, the liver is not mixed with meat or any other ingredients; it is only heat treated to obtain a product fulfilling sanitary regulations. French law requires that at least 80% of pâté de foie gras must be liver; this makes the product very expensive. There are three commercial types of pâté de foie gras: frais (fresh), mi-cut (semicooked), and bloc (restructured block). Mousse or purée de foie gras, a cheaper version, contains 55% liver.

An even cheaper product, or one for people not agreeing with animal force-feeding, is the chopped liver pâté made of other animals' livers. Pâté is French for "pie"; it is traditionally served baked in a crust (croûte) or molded as a terrine. The terms pâté and terrine are often used interchangeably. Originally, the crust was intended to hold the pâté together, not to be eaten. Terrines are not surrounded by a crust but laced in a mold and cooked in a water bath. Terrines are made of different components and have various structures; they can include meat chunks, such as terrine de campagne, or have the structure of a purée such as terrine de foie de volaille (poultry liver).

The basic ingredients of pâté may vary, but they are in general made from the liver of beef, pork, poultry, and duck; or from seafood, wild game, and even vegetables. However, liver and other viscera are usually included as part of the main ingredients. In addition, a number of other components are necessary for pâté fabrication, such as herbs, spices, milk, and starches. A smooth and creamy texture is obtained after grinding all the ingredients, although in some cases, a chunky product is desired. Although this type of products is generally called "pâté," the correct name is "paste" or "liver paste," preceded by the name of the animal used for its fabrication (i.e., chicken liver pâté). The most commonly merchandised are pork liver pâtés, followed by duck liver pâtés. Both products, liver pâté and pâté de foie gras, are high in calories, saturated fats, cholesterol, and sodium.

Liver Pâté

Processing

The main ingredient in liver pâté is chopped liver, which must be cleaned and freed from any connective tissue residue. Meat ingredients (viscera and lean meat) are first coarsely ground or chopped, and then cooked at approximately 80°C. Fat (45%–50% total formulation), previously scalded at around 65°C, is then added. Alternatively, fat substitutes are included in the formulation, as will be explained later. The meat block is then homogenized, adding hot broth, though this operation is done in order to obtain the desired texture (Totosaus and Pérez-Chabela 2005). In some cases, nonmeat proteins, such as skim-milk powder or soy protein concentrates, are included in the formulation to stabilize the emulsion. Once this paste is homogenized, the homogenized liver is added, followed by spices, herbs, and other additives, such as almonds and truffles.

Homogenization in the chopper forms an emulsion, where the main emulsifying agents are the liver proteins. However, in commercial operations, other emulsifiers are added in order to stabilize the product, as well as plasticizers such as sorbitol and glycerol to improve spreadability. The batter is then stuffed into casings or canned. As it is a semifluid material, it is important to avoid trapping bubbles during stuffing or canning, since air reduces heat transference, resulting in an underprocessed food; air can also promote lipid and pigment oxidation, reducing the final product quality. Time-temperature relationship depends on casing or can diameter, in addition to processing parameters. In small casings (50 mm diameter), heating must be at 70° to 74°C for 41 to

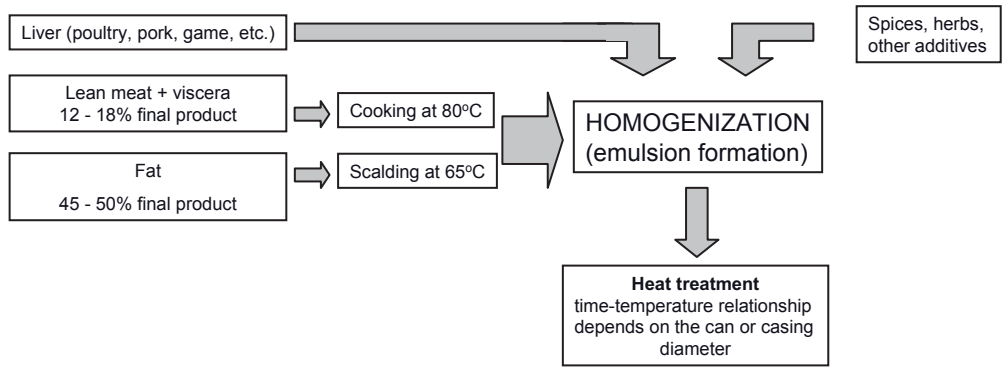

Figure 19.2. Flow diagram of liver pâté fabrication.

58 minutes at the product center; in large formats (150 mm diameter), 68° to 70°C for 314 to 360 minutes are necessary for a fully processed material (Totosaus and Pérez-Chabela 2005). Figure 19.2 depicts the general process to obtain liver pâté. In addition to ensuring product microbial safety, heating develops sensory characteristics such as flavor and texture; the emulsion turns into a gel, stabilizing the product, although the high fat content also contributes to the desirable spreadability characteristic of this meat product.

In the case of pâté foie gras, the high fat content (44%, Table 19.1) makes this product melt too easily, so it is served chilled; liver pâtés can be served warm or hot. Chefs recommend that canned pâté age for three months before opening, to develop flavor.

Physicochemical Characteristics

Since pâté is a semisolid food that is expected to be consumed as a spread, texture in general and spreadability in particular are the most important physicochemical characteristics.

Spreadability, a subjective texture characteristic of semisolid foods, is related to the material yield stress, the minimum shear stress required to initiate flow (σo); it is inversely proportional to σo. Kryscio and others (2008) developed a method to measure spreadability of pharmaceutical topical formulations, based on a torque exerted by a vane. It involves the immersion of vane blades into a sample, followed by slow rotation at a constant speed until the torque exerted on the vane reaches a maximum value and the sample begins to flow. Torque versus time curves are used to determine yield stress, and the maximum torque exerted on the vane by the fluid is measured. Daubert and others (2007) reported a method for rapid and quantitative measurement of spreadability based on the yield point of food items. The authors concluded that this textural property has been linked to the yield stress of a material, but observations

Table 19.1. Nutritional content of pork liver pâté and pâté de foie-gras (100 g)

	energy (kcal)	protein (g)	fat(g)	Cholesterol (mg)	sodium (mg)	Iron (mg)
pork liver pâté	305	10	28	96	660	3.5
pâté de foie-gras	448	10	44	380	740	6.4

http://www.consumer.es/web/es/alimentacion/aprender_a_comer_bien/curiosidades/2008/01/19/145977.php

support that strain at yielding may also be important.

Low-Fat Pâté

In emulsified low-fat meat, the disperse phase is partially or totally replaced by other materials that contribute to the formation of a similar two-phase physical system. Fat substitutes are substances such as starch, hydrocolloids, nonfat dry milk, gums (pectin, carrageenan, gellan, xanthan, locust bean, etc.), and plant proteins. Other carbohydrates, such as starches (preferably pregelatized starch), develop instant and stable viscosity; konjac flour also acts as a substitute for fat particles. These ingredients also provide gelling and texturizing properties; they bind juice and brine, control syneresis, improve sliceability, and increase product yield. Protein-polysaccharide co-gelling allows a reduction in lipid content. Kaack and others (2006) studied the effect of fiber with high cellulose content in reduced-fat pig liver paté, and found that this product had better flavor and texture than the original product.

One of the main problems with low-fat pastes is developing good spreading characteristics. Patel and Gupta (2006) describe a low-fat spread based on soy. The formulation also included skim-milk powder; sodium citrate to increase flowability and decreased oiling-off of the finished product; carrageenan and guar gum to effectively enhance spreadability; plasticizers (sorbitol and glycerol) that favored viscosity, texture, and flavor characteristics; and annatto and β-carotene as colorants. The spread had distinctly superior spreadability properties, keeping for about three months at refrigeration temperature.

A U.S. patent (5693350) describes a process for the preparation of a meat pâté having low-fat content. In the patent description, the expression "pâté having a low-fat content" designates pâtés having a total fat content of less than 10%. The formulation includes 40% to 80% ground lean meats (3%–8% fat), up to 15% fat substitute, 15%–50% added water, 1.2%–2.4% nitrite salts, and up to 0.3% phosphates. The authors reported that the meat emulsion undergoes proteolytic digestion and cooking, and when later subjected to hydrostatic pressure (>400,000 kPa) for enough time, obtains <4.5 protease units/g pâté. The product spreads better than traditional pâtés.

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Chapter 20

Dry-Cured Ham

Fidel Toldrá and M. Concepción Aristoy

Introduction

Dry-cured ham constitutes a typical meat product with high consumption in many countries, especially in the Mediterranean area. For centuries, the traditional processing of hams consisted of rearing pigs at home and slaughtering them by the beginning of wintertime (i.e., the end of November). This practice was more common in old times, but today it is restricted to rural areas located near the mountains where cool, dry winds blow most of the year. Hams were hand-salted by rubbing salt on the surface and left for a couple of weeks in the cold. Hams were then hung in big rooms at the top of the house for the drying process (during spring and summer). The drying was controlled through manual opening of windows, depending on the weather conditions. The end of the process was based on subjective evaluation of the hams, and manufacturers transmitted the experience from generation to generation (Toldrá et al. 1997a). Today, the processing technology has changed and substantially improved, thanks to the scientific knowledge developed in the last 30 years. This mainly affects the knowledge of the proteolysis and lipolysis phenomena during the ripening period, which have a decisive contribution to flavor and texture development (Parolari 1996; Toldrá 1998, 2004a; Toldrá and Flores 1998; Toldrá et al. 2004). The processing technology for the production of dry-cured ham is discussed in this chapter.

Types of Dry-Cured Hams

There are many varieties of dry-cured hams, depending on the genetics, type of feed, rearing conditions of the pigs, the type of processing conditions (i.e., additional smoking), and the region or country of origin. As a result, many different types of hams are produced throughout the world. The European Union (EU) gives different labels to protect these hams, such as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), or Traditional Speciality Guaranteed (TSG). Hams are then controlled by consortiums (i.e., the Parma Consortium or the Serrano Foundation) that inspect the compliance with all the specific requirements (Toldrá 2006a, 2007a). Mediterranean hams are characterized by a long drying period and eaten without further smoking or cooking, while those produced in northern areas are typically short-ripened and smoked (Toldrá 2004b).

Hams in the Mediterranean Area

The Spanish Iberian hams have four PDO. These hams are produced in southwestern areas of Spain, from free-range reared pigs, through a very long process that may reach up to 2 years or longer. The genetics comes from autochthonous Iberian heavy pigs, pure or crossed with Duroc, that are bred extensively and fattened with acorn and grass essential for the final characteristic and

intense flavor of the product (Toldrá et al. 1996; Carrapiso et al. 2003; Cava et al. 2004). The four PDOs of Iberian ham in Spain are Dehesa de Extremadura, Guijuelo, los Pedroches, and Jabugo. An Iberian ham ready for consumption is shown in Figure 20.1. Spanish Serrano hams are produced throughout Spain from standard light pigs, mostly intensively reared. These hams are produced in one-half to 2 years, depending on the final quality of the ham, which has three levels: reserva, gran reserva, and bodega. Other good-quality hams are produced under the PDO Teruel, from rustic pigs and under strict processing conditions of the Consortium, or the TSG of Trévelez.

Corsican hams are produced in Corsica (France) from autochthonous heavy pigs bred in an extensive system and fattened with chestnuts. The processing may take up to 18 months, but the total production is very short. Bayonne hams hold a PGI and are produced in up to 12 months.

Parma hams are produced in the northwest of Italy from specific crossbreeds of white pigs (basically Landrace and Large White), slaughtered at about 160 kg live weight. The raw materials and processing conditions are carefully controlled under the Consortium specifications, since these hams have a PDO. Processing time may take 12 months or longer. Italian San Daniele hams also have a PDO and are produced in the northeast of Italy in a minimum of 12 months.

Hams in Northern Europe

Hams in northern Europe are usually processed for shorter times than Mediterranean hams and are smoked and cooked before consumption. Some examples are the Fenalâr ham, which is produced from lamb or mutton in Norway. Another Norwegian ham is the Spekeskinke ham, which is ripened for 12 or more months (Haseth et al. 2007). Other traditional European hams are the German Westphalian ham, the Katenschinken (cold smoked ham), and the Finnish "sauna" hams.

Hams in America

American hams, known as country-style hams or country hams, are salted and post-salted and then aged for about 1 month and then smoked. These hams are typically con-

Figure 20.1. A typical Spanish Iberian ham as an entire piece.

sumed after cooking (pan frying, baking in the oven, or roasting). Some of the most well known are produced in Kentucky and Virginia, but they are also produced in Tennessee, North Carolina, Pennsylvania, and Missouri (Stalder et al. 2007).

Hams in China

Ham production in other areas of the world has also grown significantly in recent years. Good examples of typical hams produced in China are the Yinghua hams, traditionally produced in the Jinhua district, a mountainous region in China with four distinct seasons (Du and Ahn 2001; Zhou and Zhao 2007), Xuanwei hams, produced in the Yunnan province in the southwest of China where there are high altitudes (Yang et al. 2005), and Rugao hams in the Jiangsu province.

Raw Materials

Raw Hams

It is important to discriminate DFD (dark, firm, and dry) hams because their high pH can prompt the development of undesirable microorganisms. This type of ham may be used for other types of products. On the other hand, PSE (pale, soft, and exudative) hams create some difficulties to processors because of an excessive rapid water loss due to their lower water-binding capacity (Arnau et al. 1995) and an unpleasant salty taste due to an excess of salt intake. This type of ham requires a specific control during processing. Frozen/thawed hams must be carefully controlled when received at the factory and must be thawed before processing. Selection of hams by weight facilitates the control of time during the salting stage, as will be described later. The content and composition of fat is important for correct flavor development. Fat composition in fatty acids depends on the feed given to pigs (Toldrá et al. 1997b; Jiménez-Colmenero et al. 2006) and the crossbreed used (Armero et al. 2002). The detection of any undesirable oxidation and/or development of rancid off-flavors is essential at this stage. Easy controls for unsaturation and freshness consist of the measurement of the iodine index and the acid index, respectively (Toldrá 2002).

In general, hams from heavy pigs give better quality (reddish color, higher content of intramuscular fat, better marbling, etc.) than industrial-standard pigs, mainly due to the age but also to the type of crossbreeding. Main reasons for differences due to genetics are related to chemical and biochemical characteristics of respective muscles (i.e., different enzyme profile) (Toldrá et al. 1996).

Other Ingredients

Marine salt is the main ingredient for dry-cured ham. Particular flavors depend on the salt's mineral and trace element composition. Salt mainly contributes to a reduction in water activity, partial solubilization of myofibrillar proteins, and typical salty taste (Toldrá 2002).

Nitrate and nitrite are added to the curing salt in order to achieve levels of 120–150 mg/kg in the ham just at the beginning of the process. Nitrite contributes to the preservation effect against pathogens, as well as to the typical red color formation in ham by forming nitrosomyoglobin, but it also contributes to certain antioxidant activity (Pegg and Shahidi 2000).

Processing Stages

The processing of dry-cured hams appears simple, but complex chemical and biochemical reactions take place inside hams. There is a minimum length of time for the muscle enzymes to develop the characteristic flavor. Main processing stages are the reception of hams, salting, post-salting, ripening, and drying, as shown in Figure 20.2.

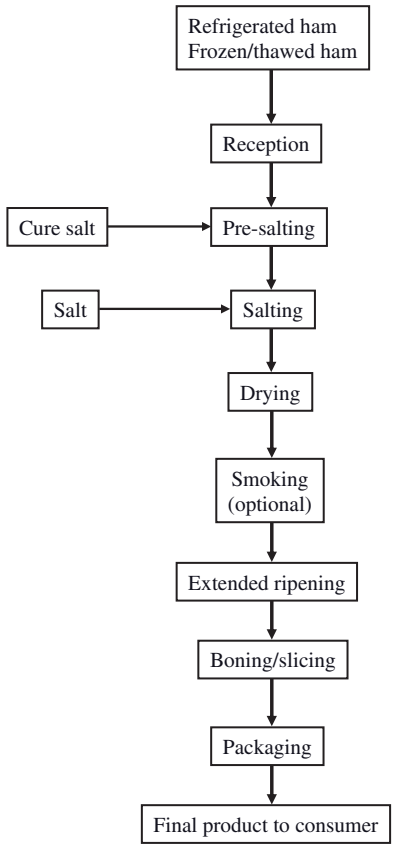

Figure 20.2. Process flow diagram for the processing of dry-cured hams. Reproduced from F. Toldrá, Ham, in *Food Product Manufacturing Handbook of Food Product Manufacturing*, vol. 2, edited by Y. H. Hui, R. Chandan, S. Clark, N. Cross, J. Dobbs, W. J. Hurst, L. M. L. Nollet, E. Shimoni, N. Sinha, E. B. Smith, S. Surapat, A. Titchenal, F. Toldrá (New York: John Wiley Interscience, 2007).

Reception

This step is crucial because the entire process depends on the quality of raw hams.

Refrigerated hams are stored for 1–2 days at 2–4°C to reach a uniform temperature. Frozen hams are allowed to thaw till they also reach an internal temperature of about 2–4°C. Hams are registered on their surfaces to facilitate traceability and are subjected to pressing rollers for bleeding. Just before salting, part of the skin is removed, in order to allow salt penetration and water evaporation. The size of this area depends on the different types of hams. Hams are classified by pH and weight, as the amount of salt is proportional to each individual weight.

Pre-Salting

The main goal of this stage is the incorporation of nitrate on the surface of the ham. Hams are weighed and then rubbed on their external surface with the curing salt (a mixture of sodium chloride and potassium nitrate) to get a final nitrate concentration of 150 mg kg^{-1} inside the ham. Some nitrite may also be added. In some cases, the curing salt may be directly applied in the salting stage (i.e., for French and country-style hams). Nitrate is not a preservative but is slowly reduced to nitrite by the enzyme nitrate reductase, a bacterial enzyme present in the natural flora (i.e., Micrococcaceae) of ham, and thus serves as a slow source for the generation of nitrite inside the ham. Nitrite is very effective as a protective agent against botulism (Cassens 1995). The European Union allows a maximum addition of 150 ppm potassium nitrate or 300 ppm for the combination of potassium nitrate + sodium nitrite, while the United States allows 156 ppm sodium nitrite (1/4 ounce per 100 pounds of meat).

Salting

This stage is carried out to assure the penetration of salt into the ham. Salt exerts important functions in the ham, such as an initial reduction of a_w and inhibition of the growth of spoilage microorganisms; it facilitates the partial solubilization of myofibrillar proteins and gives a characteristic salty taste to hams.

The incorporation of salt depends on the type of ham and the country of origin. For instance, in the case of Parma hams, the amount of salt to be added is proportional to the weight of the ham. Salt is applied on the external surface of the hams, spread evenly

or hand-rubbed, and left for 2–3 weeks. Typical amounts may be 20–30 g medium-grain salt per kg on the lean surface and 10–20 g of wet salt per kg on the skin (Parolari 1996). In other cases, as in Spain, hams are placed in large containers, fat side down, and surrounded by dry salt (usually rough sea salt), and time of salting is strictly controlled to 1.1 day per kg (Toldrá 2002). Temperature is kept at about 2–4°C and relative humidity at 90–95%. Salting is shorter for thawed hams in order to avoid an excess of salt intake.

Hams experience a slight weight reduction due to moisture loss, about 3–4%, in this stage. It is important to remove the excess salt by rinsing and brushing the hams at the end of this stage.

Post-Salting

The main goal is to equilibrate the salt and nitrate content inside the hams. This stage is also known as equalization. Salt and nitrate, once they have penetrated into the ham, have to diffuse to the inner area. Diffusion rate is very slow and usually takes around 40 to 60 days, depending on the size of the ham, pH, amount of intramuscular fat, and temperature. The temperature is kept below 6°C and the relative humidity within the range of 80–90%; temperature may slightly increase toward the last days of this stage. There are some additional weight losses in this stage, around 4–6%.

Smoking

The use of smoke is optional and depends on the typical traditions and location (i.e., northern countries). As a result, smoking is used in short processes like American country-style ham or German Westphalia ham. These hams have a particular smoky flavor. In addition, smoke compounds acted as a kind of preservative due to their bactericidal effect (Ellis 2001).

Ripening-Drying

There are two main objectives for this stage: (1) to dry the hams till they reach about 32% of weight loss, and (2) to provide enough ripening time for enzymes to react and contribute to flavor. This stage, therefore, is very important for the final quality of dry-cured ham, and the conditions in the drying chamber must be controlled and verified. A typical view of hams in a drying chamber is shown in Figure 20.3. Air speed, temperature, and relative humidity are usually computer-controlled in modern drying chambers, but they must be checked periodically. Water has to diffuse from the inner part of the ham to the surface and then it is evaporated to the chamber environment. Both rates, diffusion and evaporation, should proceed in a similar fashion. Thus, an excess of evaporation, when the relative humidity has a lower value than normal, may cause excessive evaporation on the surface of the ham and produce dehydration. A prolonged dehydration, known as hardening, gives a dry, hard texture and dark color to the external area of the ham (Toldrá 2006b). Once the ham has been dehydrated in this way, it is very difficult to get further diffusion of water to the surface.

Ripening and drying conditions are very different for each type of ham and country, and may also vary depending on the salt content (Andrés et al. 2005). Drying temperatures normally range from 16° to 25°C, depending on the processing time; longer times require lower temperatures, with the relative humidity between 65% and 80%. The length of the process has large variations, from 3 to 36 months. Usually, at least 6 to 9 months of process are necessary to get a ham of an acceptable quality. However, those hams with better quality may have several months of extended ripening. They are covered with a layer of lard to prevent further dehydration and allow a longer enzyme action and more intense flavor development (see Fig. 20.4). The reduction in

Figure 20.3. Hams in a dry-curing chamber.

water activity experienced during drying affects enzymatic hydrolysis reactions, like proteolysis, slowing its rate. On the other hand, the pH range is quite limited for the full process, starting at a pH of about 5.6–5.8 at the beginning and reaching values near 6.4 for the final product. PSE hams have a pH evolution similar to normal ones, after the initial few days, even though they take more salt due to their initial lower pH and excessive moisture (Arnau et al. 1995).

The quality of hams (texture, appearance, color, and flavor) is monitored and evaluated by experts. There is a traditional sniffing test, still in use, for the rapid evaluation of the flavor quality of the ham and/or the detection of any spoilage inside the ham. This technique consists of the insertion of a small

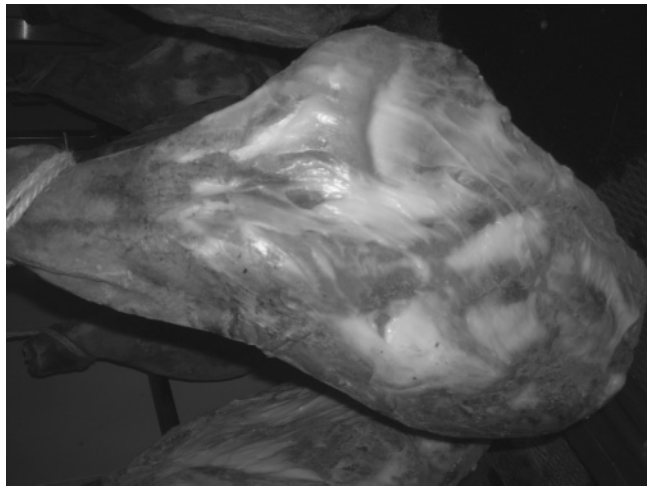

Figure 20.4. Dry-cured ham at the end of drying and before submission to the cellar. Ham is completely covered by a fat layer.

probe to the bones' junction and then a rapid sniffing detects the flavor or any off-flavor inside the ham (Parolari 1996).

Extended Ripening

In some cases, as in the case of Iberian hams, hams are submitted to ripening cellars at mild temperatures for long periods of time, up to 24 months at temperatures 10–20°C and relative humidity of 65% to 82% (Estévez et al. 2007). This long ripening time allows the development of exquisite and intense flavors generated through further chemical and enzymatic reactions. The proteolysis phenomena is very intense and large amounts of free amino acids are generated; in most cases, tyrosine crystals, which are quite insoluble, may be observed as white spots on the cut surface. These crystals, as well as typical marbling, can be observed in Figure 20.5 where a cut section of dry-cured ham is shown.

Final Product

Hams may be commercialized in several ways. The traditional distribution was as an entire piece (including the bone and foot). More recently, hams have been distributed as boned pieces, where the bones have been excised and removed and the piece compressed in molds. Boned hams can be sliced at the retailer shop or directly by consumers at home. The commercial distribution, which is growing very rapidly in the market, consists of slices packaged under vacuum or modified atmosphere (Toldrá et al. 2004).

Quality of the Product

Color

The typical bright red cured color is due to the formation of nitrosomyoglobin, which is generated by the reaction of nitric oxide with myoglobin. Of course, the intensity of color increases with the concentration of myoglobin, which is larger in muscles with an oxidative pattern (Aristoy and Toldrá 1998), and also tends to be larger in muscles from older animals (Rosell and Toldrá 1998). Those hams without added nitrate or nitrite present a pinky-red color, which is assumed to be due to a Zn protoporphyrin IX complex that constitutes a major chromophore in dry-cured ham (Moller et al., 2007). The polymerization of this pigment is suggested to be due

Figure 20.5. Cut section of dry-cured ham. Tyrosine crystals can be observed as a small white spots.

to initial protein denaturation or partial degradation of myoglobin, which associates through noncovalent binding to zinc porphyrin (Adamsen et al. 2006a). In fact, the use of nitrite as a curing agent has been reported to inhibit completely the formation of Zn-porphyrin in hams (Adamsen et al. 2006b). When the product is smoked, some surface dark colors may appear as a consequence of the pyrolytic decomposition of wood.

Texture

Texture of the product depends not only on the extent of myofibrillar protein breakdown but also on other factors, such as the extent of drying, the degradation of the connective tissue, and the content in intramuscular fat, which also exerts a positive influence on some texture and appearance traits. Proteolysis of key myofibrillar and associated proteins is responsible for tenderization. An intense degradation of the myofibrillar structure is observed during dry curing. Major structural proteins, such as titin, nebulin, and troponin T, as well as heavy and light chains of myosin and α-actinin, are severely proteolyzed (Toldrá et al. 1993). Two clear fragments corresponding to 150 and 85 KDa appear during processing. A large number of peptides resulting from actin and titin breakdown have been recently identified in dry-cured ham (Sentandreu et al. 2007; Mora et al. 2009). Hams produced from PSE meats show an absence of these fragments when compared with normal hams, and there is a trend toward softer hams. In fact, the application of a texture analysis shows that PSE hams have lower hardness, springiness, cohesiveness, and chewiness (Tabilo et al. 1999).

Flavor

The term flavor represents the overall perception of taste and aroma. Taste is mainly associated with nonvolatile compounds, such as free amino acids, and small peptides that are accumulated by the end of the process, while aroma is linked to the generation of volatile compounds with important aromatic characteristics.

Protein and lipid hydrolysis, also known respectively as proteolysis and lipolysis, constitute two of the most important enzymatic phenomena, responsible for the generation of compounds with direct influence on taste and aroma (Toldrá 1992, 2006c). Peptides and free amino acids are generated in large amounts from the progressive enzymatic degradation of major sarcoplasmic and myofibrillar proteins (Toldrá and Etherington 1988; Toldrá 2007b). Most of the muscle proteases show very good stability in long dry-cured ham processes (Toldrá 2004b), and the generation rate for free amino acids is very high, especially up to 9 months (Toldrá et al. 2000). Some free amino acids, such as glutamic acid, glycine, alanine, valine, proline, histidine, and leucine, have been found in savory fractions of ham. More specifically, lysine and tyrosine have been correlated with aged taste, and glutamic acid, aspartic acid, methionine, phenylalanine, tryptophan, lysine, leucine, and isoleucine have been correlated with the length of the drying and the fully ripened ham taste. Bitter tastes are found in hams with excessive amounts of tryptophan, tyrosine, and phenylalanine (Toldrá 2002).

Free fatty acids are generated from the progressive enzymatic breakdown of triacylglycerols and phospholipids (Motilva et al. 1992; Buscailhon et al. 1994a). Such lipolytic enzymes are located in muscle and adipose tissue and also show very good stability (Motilva et al. 1993a, b; Toldrá 1998, 2007b). The released free fatty acids are then partly oxidized, generating a large number of volatile compounds with particular aroma characteristics (Berdagué et al. 1991; Buscailhon et al. 1993; Coutron-Gambetti and Gandemer 1999). These volatile compounds, nearly two hundred of them,

are representative of most classes of organic compounds, such as aldehydes, alcohols, hydrocarbons, pyrazines, ketones, esters, lactones, furans, sulfur, chloride compounds, and carboxylic acids (Buscailhon et al. 1994b; Flores et al. 1998). Some volatile compounds with important aroma characteristics, such as pyrazines, sulfide compounds, and branched-chain aldehydes, may also originate from amino acids degradation reactions, but their generation rates may depend on the processing conditions (Flores et al. 1997). The final flavor of the ham depends on the specific aroma and odor thresholds for each particular volatile compound.

Safety Aspects

Dry-cured hams usually present low bacterial counts due to limiting factors, such as its high salt content; the use of nitrate, which is reduced to nitrite inside the ham; and the progressive reduction in water activity. Nitrite is a powerful inhibitor of the growth of *Clostridium botulinum*, but sometimes it may not reach all the areas inside the ham. Main spoilage or putrefaction is detected with a sniffing test, usually performed by the end of the process. This test consists of the insertion of a small probe to the bones' junction. Any off-flavor is rapidly sniffed and detected, indicating some type of spoilage inside the ham, and thus it must be rejected for consumption (Parolari 1996).

The natural flora of ham is composed of certain lactic acid bacteria, such as *L. sakei*, *L. curvatus*, and *P. pentosaceus*, but the counts are below 10^4. These bacteria have good exo-proteolytic activity, but their contribution to proteolysis is minimal due to their low counts. Other bacteria, such as *S. xylosus*, have nitrate reductase activity, which is an important enzyme for the reduction of nitrate to nitrite. Amines levels are usually low or even negligible in normal dry-cured hams, but these levels could rise in case of spoilage with microorganisms with decarboxylase activity. Some molds (usually *Penicillium*) and yeasts (mainly *Candida zeylanoides* and *Debaryomices hansenii*) may grow and develop on the outer surface of the ham if it is stored in high humidity and high temperatures (Toldrá 2004, 2006b).

Recent and Future Trends

Dry-curing of hams is a very long and slow process, and for this reason there have been many proposals to accelerate it (Marriott et al. 1987, 1992). Initial strategies were based on boning and skinning of hams for better penetration and diffusion of salt into them (Montgomery et al. 1976; Kemp et al. 1980; Marriott et al. 1983); in other cases, better diffusion was achieved by tumbling of hams in rotating drums, even though some physical damage did occur (Leak et al. 1984). Freezing and thawing of hams produces a membrane disruption that can also facilitate salt diffusion (Kemp et al. 1982), and an accelerated protein and lipid hydrolysis during the initial months has been reported, even though differences tend to minimize with time (Motilva et al. 1994).

Other recent proposals have been based on the simultaneous vacuum brine impregnation method that can be applied with fresh hams or while frozen hams are thawed (Flores et al. 2009). This process gives a substantial reduction in the time needed for thawing and salting, without affecting the biochemical reactions taking place during the processing (Barat et al. 2006) or the sensory quality of the final product (Flores et al. 2006). The control of proteolysis in dry-cured ham is an important development that has been recently proposed (Toldrá 2006b). This uses the process parameters (pH, salt content, water activity, etc.) to control the muscle endoproteases, mainly involved in texture degradation, and exoproteases, directly involved in the generation of small peptides and free amino acids related to flavor.

Another relevant trend is salt reduction in dry-cured ham, especially since high salt content raises blood pressure in salt-sensitive hypertensive consumers. Most strategies consist of the partial substitution of sodium chloride with mixtures of other chloride salts (Armenteros et al. 2009) or alternative salts such as potassium lactate (Costa-Corredor et al. 2009).

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Chapter 21

Mold-Ripened Sausages

Kálmán Incze

Introduction

Mold-ripened sausages are raw fermented products with usually longer ripening and drying time. Earlier, this meant a technology without any kind of additives that reduced ripening-drying time (starter culture, chemical acidulant), but in the last half century, these sausages are manufactured more and more with the help of starter cultures, or sometimes with chemical acidulants. Traditionally produced sausages are no longer dominant.

Due to the optimal nutrient composition of meat, an important feature equally favored by human beings, other carnivora, and microorganisms, it has always been the target of microbes that break down and transform this most valuable source of protein, fat, and vitamins for the sake of their own growth.

The result of their metabolic activity—spoilage and/or poisoning—must have been realized by early humanity, especially with this type of food, which was a tasty source of essential nutrients and energy; therefore, humans felt forced to find ways to keep these hard-to-get meats available to them for a longer time.

It is probably a fairly acceptable guess that our ancestors arrived at the idea of drying meat as a way to keep meat longer without spoilage. In early times, meat drying took place in caves over fires or in the sun, later in nomads' tents and on open fires; this also allowed for the advantageous effects of smoke (Incze 2007).

The advantages of mincing meat and fat, mixing with salt, using the intestines of game or domesticated animals, and drying evidently came later. Some think that dry sausage making has been known for two to four thousand years, and that Roman legions had a supply of dried sausages that did not spoil for a long time (Bacus 1984).

Raw sausage ripening and drying used meat, fat, salt, and spice, with no substances added to reduce ripening time because nothing was known about microorganisms and their role in this process. Still, early sausage makers must most likely have had positive experiences with the addition of a small amount of successfully produced sausage to the new sausage raw material (back slopping), similar to the leavening given to bread. Thanks also to the pioneering works of Pasteur in fermentation and to the excellent scientists Niinivaara and Niven, a determinant change came through in this technology: with their contribution, we learned about the important role of microorganisms, and we are able to exploit their abilities in manufacturing raw sausages, thus ensuring consistency in quality and food safety, along with reduced ripening-drying time.

Interestingly enough, the conquest of useful microbes, starter cultures as they are called, was overwhelmingly successful in sausage technology, while it was not so successful in the production of traditional raw ham (Iberian and Parma ham). Some dry sausage technologies also insist on traditional

technology without application of any kind of starter culture (Hungarian salami, for example, where staphylococci, lactobacilli, yeasts, and molds are not applied; Incze 1987).

Classification of Mold-Ripened Sausages

Mold-ripened sausages can be classified similarly to fermented raw sausages without mold, with only a few differences. Mold growth needs time and a supporting environment with adequate temperature and relative humidity; mold needs to be present either in the form of native house mycoflora or more often as artificial inoculation with a mold starter culture. These parameters in ripening rooms are different from those in systems for sausages without mold, but this difference applies only to a few days or weeks, depending on the speed of the molds' growth and on the diameter of the sausage. Mold growth and changes in ripening parameters and other measurable features are the basic differences between sausages with and without mold (Table 21.1).

Mold-ripened sausages can be manufactured entirely traditionally (no starter culture in and on the sausage), semitraditionally (either no bacterial or no mold starter culture), or with bacterial and mold starter culture simultaneously; this latter technology is becoming the most widespread. Applying staphylococci and/or lactobacilli and yeasts can contribute to aroma formation as well as to the reduction of ripening-drying time. This time reduction can be achieved with the help of acidification as a consequence of carbohydrate breakdown and lactic acid production by lactobacilli. The reason for time reduction is the well-known fact that muscle protein moisture is loosely bound and can be given off more easily when it is close to the isoelectric point. A clear distinction can generally be made between traditional and starter culture fermented sausages (low-acid and high-acid sausages), yet this is not so unequivocal when the original low pH of starter fermented sausage increases by the end of the process because of a long drying time. Usually no difference in terms of pH can be detected between the two types of sausages if other starters are added instead of lactobacilli. It has to be emphasized that low a_w-value as a consequence of a longer drying time is of vital importance in order to avoid or at least to inhibit rapid breakdown of lactic acid by molds when a_w is high, in this way losing the inhibitory activity against pathogenic bacteria (e.g., staphylococci).

Technology of Mold-Ripened Sausages: Raw Materials

Since dry-sausage manufacture, unlike the dairy industry, does not have the advantage of pasteurization for reducing undesired

Table 21.1. Classification of mold ripened sausages and suggested parameters

Measured feature	Traditional (no starterculture)	With mold starter only	With bacterial and/or yeast starter only	Bacterial and mold starter
Drying time	long*	long*	shorter with lactic starter**	shorter with lactic starter**
pH	5.6–6.2	5.6–6.2	≤5.3 at the beginning of ripening with lactic starter culture	≤5.3 at the beginning of ripening
a_w	<0.90	<0.90	≤0.93***	≤0.93***

* some weeks to several months depending on diameter
** cca 3 weeks to 1–2 months depending on diameter but never some days (!) only, as common with spreadable items
*** for safety reason (in order to prevent lactic acid breakdown) it is advisable to reduce it to lower than 0.95, an acceptable value in sausages without mold

microbial load in raw materials, it requires raw materials of high hygienic quality before adding the starter culture; otherwise, health and/or spoilage risk occurs. It is generally accepted that the total viable count of raw materials should not reach 10^6/g, and this should not involve pathogenic microorganisms at or above the given limit, which varies from country to country. This requirement is sometimes hard to meet, a fact that causes problems mainly with those pathogenic microbes that are rather resistant to lowered a_w- and pH-value (*Listeria*, enterohemorrhagic *E. coli*).

High technological, hygienic, and sensory quality of raw materials, ingredients, and additives, as well as proper and thoroughly controlled processes, are the preconditions for high-quality dry sausage.

Main Technological Steps During Mold-Ripened Sausage Production

The selected ingredients (meat, fat) have to be chilled (meat) and frozen (fat) to 1° to 2°C and −5° to −7°C, respectively. During comminution and blending spices, carbohydrates and starter culture (if used) is added with the other ingredients and with curing salt. Stuffing is made under vacuum, sausages are washed, and the surface is dried afterward, so that undesired microorganisms do not grow. After the core temperature reaches ambient temperature, incubation (if applicable) and smoking (if applicable) is applied. During ripening, the surface is inoculated with mold starter (if applicable), and temperature and relative humidity parameters are adjusted to promote the molds' growth. Major critical points in dry sausage technology differ as to whether traditional or starter culture technology is applied (Incze 2004a).

In a high-quality product, properly pigmented meat from possibly heavy-weight pigs is used with hard fat (lower unsaturated: saturated fatty acid ratio, PUFA : TFA ≤ 12%–16%). Fat from animals fed with feed containing higher levels of unsaturated fatty acid is not suitable because of its softness and because of its tendency to oxidate rapidly (i.e., become rancid). When products are manufactured by means of technology that produces extended shelf life (dry sausage), this kind of effort (use of meat and fat with high PUFA ratio) to make health food cannot be justified anyway, because of the rapid inactivation of the "good fat."

Mostly pork and/or beef, lamb, poultry, goat, camel, horse, and game are used in these sausages, depending on historical, ethnic, and religious traditions (Santchurn and Collignan 2007; Vural and Özvural 2007). Pork meat and fat is not used in sausages that meet Islamic and Kosher rules. It is generally accepted that the drying speed of pork is lower than that of beef or lamb, partly because of its higher marbling properties, which make the drying of dry sausage made from pork less risky (case hardening) than that of sausage made entirely from beef. The microbial quality of poultry and game has to be controlled very thoroughly (mainly for salmonellae, listeriae, EHEC, and parasites), in order to keep the safety risk as low as possible. Low-temperature freezing (–18°C and below) for several days to several weeks, depending on the temperature, can be a useful weapon against parasites.

Ingredients Other Than Meat and Fat: Additives

Spices and curing salts are the most important substances to be added to comminuted meat and fat when manufacturing dry sausage. The combination of spices, salt, and metabolites from tissue and bacterial enzyme activities taking place during ripening results in a high variety of aroma-rich raw dry sausages, which no other technology is capable of reaching. For better safety, better sensoric value (color and taste), and for its antioxidative effect, curing salt, $NaNO_2$ or KNO_3, is usually added to the sausage ($NaNO_2$ in the form of nitrite salt: 99.5% NaCl, 0.5%

$NaNO_2$). As a result of the reaction between meat pigment and curing salt ($NaNO_2$), a stable red color is formed that becomes deeper because of drying. Details on the chemistry of color formation are discussed by Møller and Skibsted (2007). Because of the suspicion of nitrosamine formation from nitrate and nitrite added to meat products, curing salts ran into cross fire from critics; since the 1970s, a good amount of research has been aimed at elucidating the conditions for formation and inhibition of nitrosamines. Since nitrite is an efficient substance for the inhibition of botulotoxin formation, it is still widely used but usually in combination with ascorbate, a proven inhibitor of nitrosamine formation. Other, mainly natural antioxidants can be used, too, in order to retard oxidative changes causing organoleptic faults and color and flavor deficiencies. With a much more complicated system, the advantageous effects of curing salt could theoretically be replaced (Demeyer et al. 2008) by applying natural (extracted) or artificial (industrially synthesized) colors in combination with antioxidants and hurdle technology (Leistner 2000) that would work with cooked items; still, its efficiency and safety, not to mention taste, may be questioned in the case of raw dried sausages. Initial salt concentration amounts to 2.5–3.5%. Extreme care has to be taken with concentrations lower than 2.5% in order to avoid safety risks.

Carbohydrates must be added to sausages with lactic starters, usually in a concentration of 0.3%–0.7%, depending on the type of carbohydrate and the amount of spices with high carbohydrate content. Commonly used carbohydrates are glucose, sucrose, and sometimes lactose (all with different breakdown times; glucose has the fastest).

Depending on quality requirements, pork rind and plant proteins are also used for dry sausage production in different concentrations. Pork rind is usually prepared by acidification, making homogenization more successful, while plant proteins are added, usually as powders, sometimes as a separate emulsion.

In order to inhibit the growth of undesired, mainly pathogenic microorganisms (e.g., *Listeria monocytogenes*), lactate and acetate can be used. An interesting possibility may be the application of *L. monocytogenes* specific bacteriophage.

Temperature of Meat and Fat

Adequate temperature control is important not only during storage of meat and fat but even more during comminution. Depending on the technology, meat and fat are ground (Italian) or chopped (German, Hungarian, etc.). When grinding, chilled meat and possibly frozen fat can be used, but in a bowl chopper, even, uniform comminution with clear cut fat particles can be attained only with frozen fat (–5—–7°C).

Should the temperature be higher, smearing of fat particles will occur during chopping and stuffing, which causes drying failure, leading to hygienic risk and sensoric problems (Incze 1992). During chopping or after grinding, spices, curing salt, additives, and starter culture, if used, are added.

Stuffing machines have to handle the comminuted and blended meat batter without smearing or oxygen bubbles if possible. Sausages are stuffed in natural or artificial casing with good vapor permeability. As for artificial casings, mostly fibrous cellulose and collagen casings are used. Detailed descriptions of various types are given in Savic and Savic (2002).

Use of Starter Cultures

Lactobacilli (lactic starters) metabolize carbohydrates added to lactic acid, thus reducing pH, as mentioned, and contributing to the characteristic aroma that is nevertheless not as acidic as with short-ripened products. Staphylococci and yeasts are used mainly for forming aroma substances and for better

color stability. For long-ripened mold-fermented sausages, the application of mixed cultures to the sausage is more common than the use of lactobacilli alone.

It is important to note that incubation temperatures of starter cultures differ in Europe versus North America, where the usual temperature goes above 30°C (32–37°C), while it is below 30°C (20–24°C) in Europe, but may be higher in some northern countries. At these temperatures, the use of starter cultures is also necessary for safety reasons, to suppress the growth potential of undesired microbes.

In fermented dry sausages, bacteria and yeasts are used as starter cultures in the sausage mix, and in mold-ripened sausages, the surface is inoculated by molds. In short-ripened and dried sausages, emphasis is put on the role of lactic starter cultures (*L. sakei, L. curvatus, L. plantarum, L. rhamnosus, Pediococcus acidilactici*, and *P. pentosaceus*) for rapid pH-reduction; if aroma richness is preferred, then lactic starters are used in combination with staphylococci and *Kocuria* (*Staph. xylosus, S. carnosus, S. equorum*, and *K. varians*, to name the most widely used ones; Cocconcelli 2007), or these latter bacteria are utilized alone, too, contributing basically to aroma and color formation and color stability. Some starter and other microbial cultures also produce, in addition to lactic acid, specific antibacterial substances, called bacteriocins, and these protective or bioprotective cultures can be effective against bacteria such as *Listeria monocytogenes, Cl. botulinum*, and *Staph. aureus* (Vignolo and Fadda 2007).

Since yeasts in great variety were earlier isolated from fermented sausages, it was supposed that they also contributed to aroma formation because of their intensive enzymic activity, including lipolytic and proteolytic activity producing volatiles (Olesen and Stahnke 2000). In 1977, Coretti found that *Debaryomyces hanseni* added to fermented sausage in combination with lactobacilli actually gave a better organoleptic characteristic. Even today, this yeast is widely used in starter cultures (Selgas and Garcia 2007). (See Chapter 10 on starter cultures in this book for more details.) Species for possible mold starters are less numerous than bacterial starters, and not only species but also strains of the same species have to be controlled for nontoxicity. Even if this requirement is fulfilled, as with *Penicillium nalgiovense, P. camemberti, P. chrysogenum*, and *P. gladioli*, great care has to be taken in favor of growth support of the starter cultures in order that they are not outgrown by undesired and invasive wild types. Spotti and Berni (2007) give a good summary on mold starters, including such factors as their growth requirements, speed, and enzymic activity.

Smoking

In the case of traditional mold-ripened sausages, the adjustment of a significantly lower temperature range is necessary during smoking in the first phase of ripening-drying (10°–15°C), since there is no pH-drop that would inhibit the growth of undesired microbes, and low temperature is the only efficient inhibitory factor against the growth of spoilage and pathogenic bacteria. This difference in temperatures (compared with starter culture fermentation technology) is maintained at the beginning of ripening until mold growth is initiated, since molds used as starter cultures require higher temperatures.

In northern and eastern parts of Europe, more or less intensive smoking is applied, while in Mediterranean regions, smoking is generally not applied.

In the case of mold-fermented sausages, smoking is a delicate matter: mold starters are usually not adapted to intensive smoking, causing uneven, spotted growth on the surface; intensive smoking on the other hand has the valuable effect of antioxidative and good sensoric nature. It is easier to form

mold cover on sausages that are not smoked; light smoking as an alternative can be a compromise. In those cases where native house mycoflora grows on the surface of sausages, they have been adapted to intensive smoking, too (e.g., Hungarian salami).

Prerequisites of efficient smoking are a dry sausage surface, sufficiently intense smoke concentration, and lower relative humidity (<80%). Smoking lasts from several hours to several days to even several weeks. For more details on smoking, see Chapter 12.

Ripening and Drying

Ripening is a rather complicated process, controlled to some extent by technicians and completed mostly by tissue enzymes and the metabolic activity of inoculated and/or indigenous microorganisms (Incze 2004b). Drying is a not less complicated process because the behavior of proteinaceus material determines the possible rate of moisture evaporation, which is entirely different from that of wood, for instance. Uniform air distribution is a precondition for uniform and consistent sausage quality.

During smoking, ripening, and drying, air parameters in the drying rooms have to be controlled precisely, in order for weight loss to take place as required, thus ensuring optimal drying and mold growth, and avoiding case hardening with all its sensoric, hygienic, and spoilage problems.

Temperature requirements differ, depending not only on whether the technology involves starter cultures but also on the speciality of the technology typical to a region or country. Accordingly:

- starter culture may require high (>30°C) or low (20°–24°C) temperature during incubation,
- temperature reduction after necessary pH-drop may take place stepwise or drastically,
- low-acid sausages are ripened-dried at lower temperatures, but temperature and tenure may be different: 12–13°C for the first 2 weeks and gradually increasing later (Hungarian salami); 10°C for 50 days in the case of an Italian-type sausage (Spaziani et al. 2009); or continuous 14°–16°C, etc.

Similarly, relative humidity values also differ from technology to technology, where not only starter versus traditional technology can be distinguished, but actual values are different in terms of air velocity and diameter, as well.

These parameters—temperature, relative humidity, air velocity, and air distribution—change, depending on chemical composition (fat and moisture content, type of meat), pH (traditional or lactic starter product), and diameter of the sausage, but total expected weight loss also has some influence (Figs. 21.1 and 21.2).

Keeping this in mind, it is easy to accept that there are great differences in the total lengths of mold-ripened dry sausage production time. While about 3 weeks are sufficient for ripening and drying of a ca 36-mm sausage, 12 to 15 weeks are necessary with a 70-mm sausage and even more for a traditional mold-ripened sausage (salami) of larger caliber (see Fig. 21.2). As pointed out earlier, sausages with lactic starter show a lower pH-range; consequently, they can be dried faster without case hardening problems.

It is important to mention that pH-drop as a consequence of the breakdown of added carbohydrate from lactic starters to basically lactic acid has several other advantageous technological, sensoric, economic, and safety effects. Due to the coagulating effect of lactic acid, the protein solution around meat and fat particles changes to a firm structure, which stabilizes the matrix and makes the sausage sliceable at a much higher moisture content than with traditional dry sausages, where coagulation of this protein matrix comes

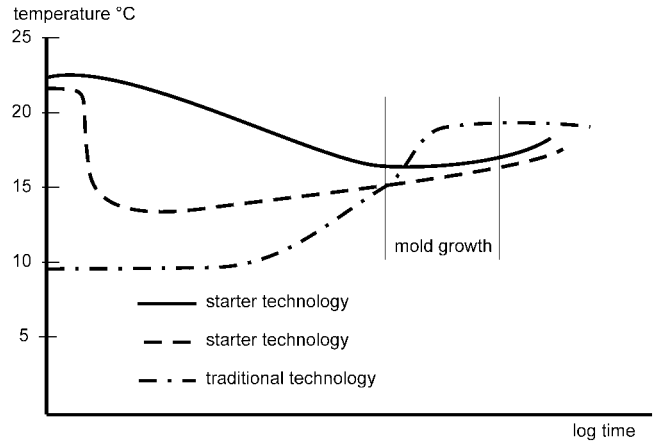

Figure 21.1. Characteristic curves for temperature pattern during ripening-drying of sausages manufacturated traditionally or with starter cultures.

through at a much later stage because of the saline concentration. The early sliceability of lactic starter fermented sausages and the special taste are characteristic features of these commodities, while shorter ripening-drying time is a further benefit. The reason for the shorter ripening-drying time is not only the more rapid drying but also the fact that relatively higher moisture content (and thus higher a_w-value) in combination with lowered pH-value ensure safety in terms of hygiene and spoilage prevention. Although intensive metabolic activity of microflora and tissue enzymic activity take place during the first part of ripening-drying, these processes slow down to a minimum later on, with the a_w-value reduction to around 0.85. For a detailed discussion of the drying of sausages see Chapter 11.

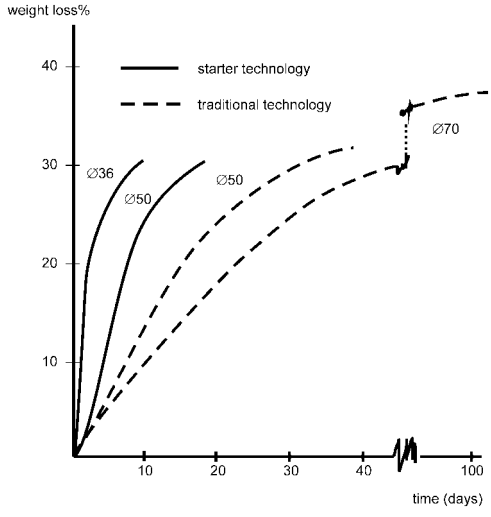

Figure 21.2. Weight loss of sausages during ripening-drying as a function of technology and diameter in millimeters.

Formation of Mold Cover

Mold growth on food, a phenomenon known for ages, evolved by chance, was rejected in most cases (with all types of foods except some dairy and meat foods), and yet was advantageous in some traditional, Old World–style sausages and hams. In fact, artificial inoculations on the surface of sausages have been used for decades. Many more types are produced in larger volumes and are favored in Europe; in America, they are also known and manufactured as salami and country-cured ham.

For a better control of growth and a uniform mold layer, the utilization of mold starters has become more and more popular in recent years.

Regardless of the form of "inoculation"—be it indigenous house mycoflora or artificial inoculation with mold starter cultures—successful growth on and adhesion of molds to the surface have several prerequisites:

- The surface of the sausage casing has to be less contaminated, so after stuffing, washing is expedient.
- Since different strains of *Kocuria* and yeasts are common contaminants and they prefer wet surfaces, drying of the sausage should take place as soon as possible and efficiently in order to inhibit contaminant growth (drying has to last as long as it takes for core temperature to reach ambient temperature, thus avoiding the dew-point effect); inoculation can also take place after stuffing, if no smoking is applied in the given technology.
- If the sausage is to be smoked, an efficient means against growth of the undesired microorganisms mentioned above is to start the smoking immediately after the surface dries, and the sausage's inoculation should take place afterward.
- If no smoking is applied, the sausage is inoculated by dipping or spraying.
- If spore deposit of house mycoflora is relied on (no mold starter is applied), our task is the same as after artificial inoculation: to ensure, if possible, optimal conditions for the growth of molds (i.e., favorable humidity and growth temperature).

Since optimum relative humidity and temperature for mold growth might also favor the growth of undesired microbes, adjustment and control of these parameters have to be done carefully and compromise is necessary; humidity and temperature parameters should be suboptimal for spoilage microflora.

It is worth mentioning that in addition to the control of relative humidity and temperature, air velocity also plays an important role; high velocity does not support growth, and relative humidity value is also more critical in this case.

Mold growth is not always optimal; no uniform uninterrupted cover is the result, which can be corrected by wet or dry brushing. Brushing is actually a very efficient way of secondary inoculation, because it makes use of a very high inoculum encouraging growth of molds well adapted to the given circumstances, which results in a rapid and uniform mold growth covering the salami surface entirely.

Advantages of mold cover that are usually mentioned include (Incze 2004b):

- It gives some equilibrating control of moisture evaporation, supporting in this way a more uniform drying with less chance for case hardening.
- To some extent it is an oxygen barrier that slows down oxidative changes (rancidity).
- As a light barrier, it slows down oxidative changes.
- It contributes to the specific organoleptic characteristic of these types of sausages.
- Through metabolizing peroxides, it slows down rancidity (Spotti and Berni 2007).

Microbiological and Chemical Changes in Mold-Ripened Sausages

Initial microflora of dry sausages depend on the ingredients, additives, and the surfaces (cutting and deboning table, utensils, machines, employees) the raw materials touch. Numerous different species may originate from such contamination: members of *Enterobacteriaceae, Lactobacilli, Staphylococci, Enterococci, Bacilli, Clostridia, Listeriae*, pseudomonads, yeasts, and so on. It is important to mention that total viable count per se does not give reliable

information on hygienic status or spoilage tendency, but a too high initial count may indicate problems of a microbiological nature.

Depending on the initial microbial load, on the use of lactic starter, and on the technological parameters (temperature, relative humidity, and air velocity), undesired microorganisms are outgrown through inhibition by useful microorganisms, if necessary measures are taken during processing at the right time and to the right extent. As a result of a combination of low temperature, lowered pH, drying (lowered a_w), and competition with added starter cultures, if applicable, bacteria that are sensitive to the factors mentioned above change in their number and die off. Pseudomonads cannot survive longer than a few days; enteric bacteria do not survive either; *clostridia* and *staphylococci* are inhibited; and even *listeriae* and EHEC strains can be inhibited in their growth. Cocci and lactobacilli are more likely to grow, the latter being less sensitive to low pH. As drying proceeds, a lactobacilli-dominance can be observed, and lactobacilli account for the majority of microorganisms in the final product.

As a result of salt, soluble protein goes into solution and forms a net among meat and fat particles, which is gelified by lactic acid or by concentrating salt. This matrix ensures good adhesion between meat and fat particles and also good sliceability; binding and structure is formed this way (van't Hooft 1999; Incze 2007).

During ripening, tissue and bacterial enzymic activity causes the breakdown of protein (proteolysis) and lipids (lipolysis). Muscle tissue enzymes (endoenzymes) play a role in dry sausages during the first part of ripening, and enzymes of microorganisms are responsible mainly for proteolysis and lipolysis. As a result of this metabolism, myofibrillar and sarcoplasmic proteins are partly broken down and nonprotein nitrogen (NPN) compounds (e.g., (poly)peptides, amino-N, and ammonia-nitrogen) develop, contributing to taste formation and to a pH-shift in the alcalic direction (Nagy et al. 1988; Incze 2004a; Talon et al. 2004). Cocci play a more important role in proteolysis than lactic acid bacteria, but molds are also active in this process.

With similar reactions in tissue and microbial enzymes, lipids are also broken down, resulting in some flavor development, caused mainly by the short-chain fatty acids (Talon et al. 2004). Unsaturated fatty acids are oxidized easily; lipid oxidation is an autocatalytic process. Proteolytic and lipolytic compounds do not always render a pleasant flavor and aroma, depending on many intrinsic and extrinsic factors not easy to control. It is worth mentioning that volatile components coming from a variety of spices, their interaction with each other and with the meat and fat involved, and the multilateral effect of smoke substances do have more intensive aroma effect than can be expected from enzymic breakdown in sausage (Schmidt and Berger 1998). It is interesting to mention that depending on the sausage diameter, flavor differences can be observed: aroma is more intense in larger sausages, due possibly to more anaerobic fermentation. More details on microbiological and biochemical changes and aroma formation can be found in other publications (Talon et al. 2004; Tjener and Stahnke 2007) and in Chapter 9 of this book.

Safety of Mold-Ripened Sausages

Dividing these products basically into two categories depending on whether lactic starters are used or they are manufactured by traditional methods provides more information on technology, microbiology, and safety than do distinctions such as northern and southern types (Talon et al. 2004). Thus, their safety features can also be discussed differently.

Products Manufactured with Lactic Starter Cultures

Safety of the products that are manufactured with the help of lactic acid starter cultures relies on two bases: formation of lactic acid and eventually some other compounds with inhibitory effect (bacteriocins, peroxides, etc.) on the one side, and the gradually decreasing a_w-value on the other. This twofold effect, together with the possible outgrowth of undesired microorganisms from lactic acid bacteria, contribute to the safety of a product where no other bacteriostatic or bactericidal treatment, such as heating, high hydrostatic pressure, or irradiation, is applied. It is of vital importance that metabolic activity of lactic starter cultures (i.e., formation of lactic acid and pH-drop during the initial phase of ripening [incubation]) should take place as rapidly as possible so that pathogenic and spoilage microflora have no or very slight chance for growth; otherwise, at a relatively high temperature of incubation, they could grow. Although *Listeria monocytogenes* and enterohemorrhagic *E. coli* are relatively tolerant to lower pH, salmonellae and *Staphylococcus aureus* are not; consequently, pH has to be lowered in order to inhibit their growth. Since *Staphylococcus aureus* tolerate lower a_w, temperature has to be kept low until pH is lowered. According to the voluntary guidelines of the American Meat Institute, tenure of incubation above 15°C is considered critical, because above this temperature, growth of staphylococci occurs. On the other hand, high temperatures evidently affect the growth of *Staphylococcus aureus*; the higher the temperature, the shorter the time that can be tolerated in order to inhibit growth of staphylococci, but this helps to retard growth of other undesired microorganisms as well.

The process is correctly controlled if the time elapse is not more than 720 degrees × hours until pH drops to 5.3, when the highest fermentation temperature is below 32°C, or not more than 560 degrees × hours when the highest fermentation temperature is between 32°–40°C, or less than 500 degrees × hours when the highest fermentation temperature is higher than 40°C. In this calculation, degrees above 15°C are taken into account.

In the case of fermented sausages manufactured with the addition of lactic starter culture, it is generally accepted that the safety of these products is ensured by the combination of low pH (below 5.3) and low a_w (below 0.95). It has to be mentioned, though, that this pH-value remains as such mainly with short fermented sausages and increases with longer dried products (when, because of an a_w of lower than 0.95, safety is still guaranteed even with a higher pH-value), but a rapid pH drop below 5.3 during incubation period must not be omitted.

As for enterohemorrhagic *E. coli* and *Listeria monocytogenes*, lower pH and lower temperature have no such dramatic inhibitory effect as on staphylococci, salmonellae and other pathogenic bacteria. In 1994, an *E. coli* O157:H7(EHEC) outbreak was linked to (presliced) dry fermented salami in the United States (Centers for Disease Control and Prevention 1995a; Tilden et al. 1996), followed by an outbreak in Australia caused by a semidry fermented sausage (Centers for Disease Control and Prevention 1995b); since then, the good safety record of dry sausages has been questioned, and drastic treatments were worked out and required in the United States, mainly because of hamburger-borne EHEC infections, hemolytic uremic syndromes, and the resulting death toll (Incze 1998). This kind of reaction has not been typical in Europe, partly because EHEC-infection is not so frequent, partly because no dry sausage was involved and it has been proven that EHEC growth is not supported at an a_w typical for dry sausage. It has also been found that if sausages are stored at ambient temperature, *E. coli* die off through metabolic exhaustion (Leistner 2000).

Similarly, lowered pH and/or low a_w does not support the growth of *Listeria monocytogenes*; this is why it is accepted that in these types of products (belonging to RTE), a maximum of 10^2/g CFU is tolerated, although zero tolerance was the rule earlier.

In challenge tests, if starter culture was applied, it was found that the number of *Listeria monocytogenes* decreased by 2 to 3 logs in artificially inoculated dry sausages affected by fermentation and smoking; this change was observed to be less during storage in sausages without starter culture (Ingham et al. 2004; Farber et al. 2007).

Low-Acid, Traditional Dry Sausages

Unlike lactic starter-produced sausages, the higher pH of these traditional sausages (5.6–6.2) has no inhibitory effect whatsoever against undesirable microorganisms. In order to fulfil the safety requirement, low temperature is practically the only possibility for inhibiting the growth of pathogenic and spoilage microorganisms efficiently, because neither initial salt concentration (a_w) nor curing additive exerts a remarkable effect. This low temperature (definitely below 15°C, preferably 10°–12°C) has to be maintained as long as water activity value decreases during drying to a value of about 0.92–0.93, yet temperature should be raised moderately, which is necessary for mold growth (16°–18°C or somewhat higher). As a result of further drying, water activity decreases, and as is generally accepted, dry sausages (with or without mold) can be considered to be safe at an a_w-value of 0.89–0.90 and also below, at ambient (room) temperature, just as in sausages with a combination of low pH and relatively low a_w. This safety measure means that because of the low a_w-value, undesired microbes die off.

It is more common in the United States to use the moisture : protein (M : P) ratio rather than the water activity value (a_w) for distinguishing semidry and dry sausages and for characterizing shelf life and safety (Sebranek 2004; Incze 2007), although M : P-values are listed sometimes in recipes (Wahl 2004). As a matter of fact, a_w- or a_w- *and* pH-value are more informative, and critical values of M : P ratio do differ in Europe and in the United States (Incze 2007). It has to be mentioned that the importance of salt is missing from this M : P ratio; in other words, it gives fairly little information on the level of inhibition of microorganisms.

Molds and Mycotoxins

Due to the discovery of aflatoxins, research on mold toxins (mycotoxins) began and developed by leaps and bounds. The presence of molds on foods and feeds became more suspicious than before, and a wide range of experiments and analyses were started, testing molds for their capability of toxin production. As a result of this research work, several hundreds of mycotoxins were detected (Püssa 2008), and new ones are still to come. In the course of this testing of different types of commodities, foods of plant and animal origin were investigated equally. Mold-covered salami and country ham were examined in this way as well (Leistner et al. 1965; Bullerman et al. 1969; Burmeister and Leistner 1970; Ciegler et al. 1972; Wu et al. 1974). The aims of these experiments were manifold:

- to gather information on the most common molds growing on salami and cured meat products;
- to test them for growth and mycotoxin production on the foods in question (artificial inoculation, challenge tests in modern terminology);
- to test the effect of intrinsic and extrinsic factors on mycotoxin production, thus finding ways for inhibition.

As a result of these experiments, it has become clear that mycotoxin production can

be found in artificially inoculated meats; if mycotoxic mold was the test strain, toxin formation is influenced by several factors, out of which genetics, food composition, temperature, relative humidity (or a_w of medium), competitive inhibition, and eventual inhibitory substances are most important. In this respect, it was found that *Penicillium chrysogenum* as starter culture may sometimes produce toxin (Spotti and Berni 2007); in the absence of carbohydrate, toxin production does not take place, the presence of sulfhydril groups inactivates patulin (Hofmann et al. 1971), and a combination of suboptimal temperature, the presence of competing molds and intensive smoking inhibits mold growth and toxin production (Incze and Frank 1976a, b).

Due to concerns regarding a potential mycotoxin presence in mold-covered traditional meat products, research as a further step concentrated on selecting mold strains that are suitable from a technological point of view (good growth under given circumstances, good appearance), produce neither mycotoxin nor antibiotics, and have no cellulase activity that would cause detrimental changes in cellulose casing. These requirements are not easy to meet, for which reason only a few molds have been found suitable for use as mold starters, and these are widely utilized.

Packaging of Mold-Ripened Sausages

Although mold cover renders several advantages as mentioned, a basic disadvantage has to be calculated, too. While dry sausages without mold are ideal meat products for packaging, ensuring long shelf life, and sausages from good raw material that are smoked and thoroughly dried can be stored for a long time at ambient temperature in vacuum or modified atmosphere (MAP with CO_2 and N_2), no such packaging can be applied to sausages with mold on them—or only for a few days, maybe weeks—without organoleptic faults. The reason for this limitation is that the mold layer sticks sufficiently to the casing surface if the inside moisture can evaporate when it reaches the surface. Should this not be the case, as when the sausages are in packages impermeable to water vapor or even when they are tightly packaged in a box, the moisture coming out from inner layers makes the mold layer wet, loosening it so that it comes off, which gives an unattractive appearance. One more disadvantage of sensoric nature also has to be mentioned: in some cases *Kocuria* strains grow together with yeasts as the first microbes at the beginning of ripening, even if technological processes were controlled. This layer does not disappear, yet if it is covered by a sound layer of molds, it cannot be seen and does not smell, and if moisture can evaporate from the surface, no problems evolve. If, however, no evaporation can take place because of improper packaging, a characteristic unpleasant odor appears, caused by the wet layer of these microorganisms.

The usual compromise is to apply a perforated film pouch through which moisture can evaporate, which maintains the mold layer for a while. Another possibility is to wrap the rods in cellophane, through which moisture can also evaporate. Neither type of packaging gives the possibility for longer storage, however, and a further weight loss also has to be calculated.

Peeled, sliced sausage (either in vacuum or modified atmosphere) is a reliable method of packaging with a long shelf life. Although vacuum packaging ensures longer shelf life, slices stick together and are not easy to take apart, so MAP is generally the preferred choice.

Shelf Life

Unlike the case of cooked meat products, the shelf life of dry sausages has practically no limitation of a microbial nature, since low a_w

alone or a combination of relatively low a_w and low pH inhibits the growth of pathogenic and spoilage microorganisms at room temperature. Actual shelf life of dry sausages is determined by the time until organoleptic changes take place. Limiting factors of shelf life are as follows:

- excessive drying,
- melting of fat,
- temperature,
- light,
- discoloration,
- rancidity, and
- aroma loss.

As mentioned before, a mold layer retards drying but evidently does not inhibit it, and unfortunately, there is no packaging suitable for longer storage with sausages that are in rods. Because of excessive drying, sausages shrink, and this pressure makes the liquid part of the fat separate from its crystalline part, a process accelerated by elevated temperature. Elevated temperature combined with light speeds up discoloration and rancidity in the presence of oxygen; it is nevertheless true that a mold layer counteracts to some extent these processes, supported by the mold's metabolizing peroxides (Spotti and Berni 2007). Too low storage temperatures also cause adverse organoleptic changes in the form of phosphate crystals.

Partly because of oxidation, partly because of other reactions during excessive drying, aroma loss also takes place after longer storage, as a result of which odor intensity can decrease drastically.

Excessive drying, discoloration, rancidity, aroma loss—all these phenomena can be observed only in sausages stored in rods that are not vacuum packed, yet not in MAP sliced products if residual oxygen in the package is kept at a minimum and the film is a high CO_2- and O_2-barrier type.

Consequently, if mold-ripened sausage is peeled off, sliced, and vacuum packed, or if MAP is applied, a long shelf life can definitely be guaranteed without the limitations mentioned above or with those limitations at a much lower intensity. Use-by date gives information on the shelf life, which should be determined based on testing. Prepacked sliced mold-ripened sausages can be stored for several months.

Preconditions for high sensoric quality at the end of the use-by date are:

- high-quality product,
- packaging film with high CO_2 barrier,
- packages with good seal strength,
- low residual oxygen (<0.1%),
- storage temperature not higher than 18°C, and
- no excessive exposure to light.

Quality Defects: Cause and Prevention

If each step during processing and storage is carefully controlled, excellent sensoric value is the result of a mold-fermented sausage. This rigorous control is needed because with dry sausage technology, no preliminary reduction of undesired microorganisms can proceed as in heat-treatment technologies. Furthermore, drying of low-acid sausages requires more careful and different controls than with lactic starter-produced sausage. This is why failures may occur if any or some of the following faults are committed:

- hygienic failure with raw material,
- inappropriate raw material (color, flavor, odor defect),
- improper comminution (uneven particle size, smearing),
- failure with additives (actual concentration),
- improper stuffing (wrong temperature, wrong type of stuffing),
- wrong temperature pattern during ripening-drying,

- incorrect control of relative humidity,
- inadequate pH drop (in case of lactic starter sausage),
- inadequate a_w drop,
- inadequate packaging, and
- inadequate storage in the factory, in retail locations, or at home.

Most but not all of the defects caused by these faults can be detected by our sense organs. Some of the most common failures are (Incze 2004b):

- *Deformation* of the sausage is caused either by fat film or by too-rapid drying. Drying is too rapid if the difference between relative humidity and equilibrium relative humidity is too high and/or if air velocity is too high.
- *Incomplete* mold cover is caused either by scarce growth or by its coming off due to improper storage (too high humidity).
- *Discoloration* on the surface can be observed if undesirable molds grow on the sausage, causing yellow, black, etc., colored spots or larger fields. Discoloration in the sausage takes place if curing salt is not evenly distributed, if sausages are smoked when relative humidity is too high, or if sausages are stored in a humid place.
- Fat particles are *indistinct* on the cut surface of sausage if comminution is carried out improperly: if temperature is insufficiently low, if the wrong stuffing machine is used, or if soft fat is used (higher unsaturated : saturated fatty acid ratio).
- *Odor* problems can be observed if the raw material was not faultless (rancid fat, microbial activity), or if the growth of spoilage microorganisms was not inhibited during ripening-drying by low temperature, low pH, or by lowering of a_w.
- *Fattening-through* on the surface occurs if drying or storage temperature is too high or if cellulose casing is decomposed in spots by cellulase activity of molds.
- Unsatisfactory *sliceability* is caused if a protein matrix cannot be formed for lack of coagulation: if it is not coagulated by lactic acid or by saline concentration because of a fat film (temperature and/or machinery failure, soft fat). Other reasons may be: too high concentration of dry spices (>2.5%–3%), insufficient pH drop, late stuffing if GdL is used, and coagulation of protein before stuffing.
- Last but not least, if raw materials, ingredients, additives, or technological processes do not meet hygienic requirements (high initial microbial load, presence of pathogenic microorganisms, too high temperature during ripening-drying, insufficiently low pH and/or a_w), safety risks arise that may cause *food-borne disease*.

On the other hand, when dry sausage is produced according to the requirements of Good Hygienic and Good Manufacturing Practice while operating HACCP correctly, a product is born that not only has an excellent safety record but is superior to other products because of its unique richness of aroma.

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Chapter 22

Semidry and Dry Fermented Sausages

Graciela Vignolo, Cecilia Fontana, and Silvina Fadda

Introduction

Fermentation and drying can be considered to be the oldest way to preserve raw materials. Although the historical origin of fermented meat products remains unknown, fragmentary bibliographical research has traced it back more than 2500 years in China. Many of these products have been known in Europe since the thirteenth through the fourteenth centuries, after being introduced by Marco Polo. Preservative and palatability effects must have been experienced after mixing comminuted fresh meat with salt contaminated with nitrate, spices, or herbs; stuffing it into animal intestines; and then drying it. Early humans were certainly aware of the preservative value of salt and drying, and therefore, over the centuries, humans were able to develop cured products. Proof of sausage production was first documented in ancient Greece, where it may have been encouraged by the existing climate conditions (Liepe 1983). The Romans inherited this tradition, and from then on, fermented sausages spread to central, eastern, and northern European countries, as well as to America and Australia where fermented sausages were recognized as the heritage of European immigrants (Demeyer 2004; Fadda and Vignolo 2007). Despite the widespread production of fermented sausages, Europe is still the major producer and consumer of these meat products, production and per capita consumption figures being highest in Germany, Italy, Spain, and France (Lücke 1998; FICT 2002; Di Cagno et al. 2008). Production in the New World is much lower: in the United States, the annual production of dried fermented sausages is probably less than 5% of the total sausage production (Maddock 2007). The remarkable technological advances and significant improvements in meat hygiene that occurred about 50 years ago have been capitalized on for the development of a range of fermented meat products in which differences among countries and regions are the result of meat species availability, environmental conditions, and traditions. Nevertheless, the stability of fermented meat products is mainly determined by a combination of acidification brought about by lactic acid bacteria (LAB) and lowering of water activity (a_w) during curing and drying. In addition, biochemical and physicochemical changes occur as a result of the interactions among microorganisms, meat, fat, and processing technology, which is what produces the wide range of available fermented sausages. The main characteristics of semidry and dry fermented sausages and the most relevant products worldwide are analyzed here.

Fermented Sausage Classification

Since the manufacture of fermented sausages has been adapted mainly to raw material availability and environmental conditions of the production area, fermented sausage classification was carried out considering various criteria, such as moisture content, mois-

ture : protein ratio (M : P), weight loss, a_w, surface treatment, meat and fat comminution degree, and geographical region. Semidry and dry fermented sausages can be distinguished on the basis of a_w value (Incze 2004) or M : P ratio (Sebranek 2004). These parameters are mainly applied in Europe or in the United States, respectively. In terms of shelf life and safety, moisture content alone is not sufficiently informative compared with a_w and pH values. The combination of initial moisture/salt and moisture/protein contents, as well as the extent of drying, will determine final a_w and M : P ratio, respectively. More specifically, M : P ratio provides information about the extent of drying of the lean meat portion. Nevertheless, final values of 0.90 to 0.91 for a_w and 2.0 : 1 for M : P ratio can be considered as the borderline defining dry and semidry fermented sausages. Even when sausages with similar names are very different according to the region in which they are produced, a general classification based on final moisture content, a_w level, and M : P ratio was attempted by Ockerman and Basu (2007). In this chapter, a description based on two groups of fermented sausages, semidry or quickly fermented and dry or slowly fermented sausages, is reported.

Semidry Fermented Sausages

These sausages differ greatly from dry sausages because of their pronounced tangy flavor from forced fermentation, resulting in lactic acid accumulation and a bulk of other products from fermentation breakdown. The term "semidry" is unequivocal; these products are dryer than water-added cooked meat products but have a higher moisture content than dry sausages (Incze 2007). In the United States, semidry sausages are fermented and cooked but are not usually dried (Sebranek 2004), while in Europe they involve a broader range of products, most of them experiencing weight loss after fermentation because of cooking or hot smoking. They are usually stuffed in medium- and large-diameter natural casings; the length of fermentation and drying/smoking depends on their type but rarely exceeds several days. The final pH of semidry sausages is explicitly acid (4.7 to 5.2–5.4), with a lactic acid content of 0.5% to 1.3%; although they are often finely chopped and spreadable, many of them can be sliced, their moisture being 35% or higher. Semidry sausages are often smoked and slightly cooked by the heat used in the smokehouse, which occasionally reaches nearly 60°C for a strictly limited time. After smoking, the sausages may be air dried for a relatively short time. Compared with dry sausages, these products show higher a_w values (>0.90–0.91), so that a lower pH is needed for satisfactory protection against undesired microorganisms. However, due to their M : P ratio ranging from 2.3 : 1 to 3.7 : 1, semidry sausages require refrigeration. This category of sausages is popular in Northern European countries and in North America. The use of starter cultures to produce semidry fermented sausages has proved to be particularly successful to keep their stability.

Dry Fermented Sausages

In general, these products have a final pH ranging between 5.2 and 5.8, which is consistent with the lower lactic acid content (0.5%–1.0%), a moisture lower than 30%, and an M : P lower than 2.3 : 1. The main difference with semidry fermented sausages is the long ripening and drying process, during which biochemical and physical changes occur that strongly influence their stability and safety. Due to a_w, which ranges from 0.85 to 0.91, dry fermented sausages exhibit high shelf stability and can be kept without refrigeration. The typical lower a_w values of these products is achieved by air-drying in Mediterranean countries and by smoking in northern countries. The long ripening process of dry fermented sausages promotes the growth of starter cultures, which contributes

largely to their sensory quality, while safety is mainly ensured by drying and low a_w. Even when dry fermented sausages are mainly made with pork meat, the formulation, degree of grinding, level of fermentation, smoking intensity, temperature of ripening, and type and size of casing will determine final product characteristics.

Fermented Sausage Manufacture

Fermented sausages can be defined as a meat product made of a mixture of mainly beef and pork meat, and less often of poultry, mutton, lamb, goat, horse, ostrich, and game meat (Vural and Özvural 2007); pork fat, salt, curing agents, sugar, spices, and in many cases starter cultures are added. The mix, including as little oxygen as possible, is placed into steam-permeable casings and subjected to a fermentation and drying process.

Ingredients and Additives

Meat and Fat

Meat from adult, well-fed animals is preferred, owing to its higher myoglobin content, which favors stable color formation. Since meat and meat products featured prominently in recent food scandals, meat wholesomeness and safety must be guaranteed; this involves emerging pathogens, parasites, BSE, avian influenza, and chemical residues hazards (Skandamis and Nychas 2007; Nørrung and Buncic 2008). Although the meat used depends on eating habits, customs, and animal species availability in the geographical region, pork meat is mostly used, sometimes mixed with beef or mutton meat (Vural and Özvural 2007). Pork is used for top-grade raw sausages in Mediterranean countries, as the flavor and appearance of such products is preferred, while in Germany, the addition of beef is not considered to diminish quality. Moreover, due to beef availability (Argentina) and religious reasons (Muslim countries), beef and lamb in sausage formulation are used. The functional characteristics of meat, such as composition, pH, and binding properties, are major criteria when selecting meat for fermented sausages production. Meat and fat composition is variable, depending on the species used and the anatomical region of the animal. Although the percentage of fat may vary (10%–40%), it must be firm, white, and fresh, with a high melting point and a low content of polyunsaturated fatty acids to avoid rancidity and fat exudation and for a clear-cut surface of sausages (Demeyer 2004; Lebert et al. 2007). As a rule, meat with a pH above 5.9 contains low lactate and sugar levels; water is tightly retained, resulting in poor binding conditions and possible contamination. Selection of meat that has minimum microbial loads is critical; safety risks and unwanted flavors and texture may be introduced. For beef, optimal pH is 5.4 to 5.5, while pork meat usually acidifies faster, with a final pH of 5.7 to 5.8. PSE (pale, soft, and exudative) pork meat and DFD (dry, firm and dark) beef muscle with pH > 6.2 should be avoided. The meat : fat ratio is generally 2 : 1 in the mix of most industrial sausages, whereas in traditional sausage, this ratio is variable. Beef and pork are somewhat preferred for semidry sausages and northern-type products, while pork seems to be more suitable for dry sausage manufacture and for Mediterranean products (Demeyer 2004).

Additives

NaCl is normally added at levels ranging from 2% to 4%, depending on technology and market demands. Salt performs many functions, including microbial growth suppression, a_w reduction, salt-soluble protein release, and prooxidant effects. Nitrite and/or nitrate are added to the meat batter at a level of 150 to 250 ppm, depending on the meat

product and country regulations (Honikel 2008). Potassium nitrate (saltpeter) was the original curing agent and was added to meat unintentionally as a salt contaminant. Nitrate is very stable and must be converted to nitrite by nitrate-reducing bacteria (*Micrococcaceae*). Nitrites added to the meat or converted from nitrates undergo chemical reductions to NO at pH 5.4 to 5.5; this binding to meat myoglobin to form the heat stable NO-myoglobin is responsible for the typical cured red color of fermented sausages (Honikel 2008). The use of ascorbates has become a common practice, the main objective being the improvement of the stability of the red nitrosylated pigment and the prevention of lipid oxidation (Balev et al. 2005). Sugars are also commonly added to fermented sausages, among which the most often used are dextrose, glucose, sucrose, and lactose, as well as corn syrup and different starches (Rust 2007). The main role of sugars in fermented meat products is to act as substrates for LAB to produce lactic acid, the type of sugar influencing the rate of pH decline. Dextrose and glucose promote a more rapid acidification rate compared with the disaccharides lactose and sucrose (Demeyer 2004). Short-processed fermented products are usually supplemented with 0.5% to 0.7% glucose or sucrose or 1% lactose, while for long-ripening dry sausages, common levels are around 0.3% glucose or sucrose or 0.5% lactose (Ruiz 2007). However, some semidry products like Lebanon bologna, which is fermented to a very low pH, may actually require higher (2%–4%) sugar levels (Rust 2007). Spices are mostly what differentiate fermented sausages. Ground pepper (0.2%–0.3%) is usually present in all types of sausages, particularly in Mediterranean fermented sausages; they also may contain high levels (1%–3%) of paprika and/or garlic. Whole mustard seed, coriander, ginger, cardamom, nutmeg, and cloves, among other spices, are also used in semidry fermented sausage formulation (Chi and Wu 2007). Spices have also proved to act as effective antioxidants, to stimulate LAB activity by supplying Mn, and to inhibit undesirable organisms (Arora and Kaur 1999; Aguirrezábal et al. 2000; Hagen et al. 2000; Chi and Wu 2007). In industrially produced fermented sausages, a variable number of other additives are also included, among which natural (cochineal and paprika extracts) and artificial colorants are added to improve cured red pigment stability (Roncalés 2007). Variable amounts (0.5%–3%) of exogenous proteins are often used to assure protein gellation and phosphates to act as thickeners, humectants, and gelling agents. To achieve consumer demands for extremely savory products, flavor enhancers (glutamic and guanylic acids), and flavoring agents (protein hydrolysates, herbs, and smoke extracts) are also added (Roncalés 2007). For a rapid pH drop, chemical acidulants such as lactic or citric acid or glucono-delta lactone may be used, although different flavors in the final products may be obtained (Rust 2007).

Starter Cultures

The need for process standardization as well as quality assurance strategies has led to the use of starter cultures, thus overcoming the need to rely on the "in-house" flora or "back-sloping" for the fermentation process. The breakthrough in the use of starter cultures in the United States was achieved as a result of the work of Deibel and Niven (1957), while in Europe, micrococci were introduced as starters by Niinivaara (1955) to prevent color and flavor defects. After these first experiences, Nurmi (1966) developed a mixed culture composed of lactobacilli and micrococci. Studies on the ecology of fermented sausages showed that LAB, mainly *Lactobacillus* and coagulase-negative cocci (CNC) represented by *Micrococcaceae*, are the two main bacterial groups technologically important in the fermentation and

ripening of sausage. During the last decade, the diversity of LAB and CNC in traditional fermented sausages has been extensively investigated (Lebert et al. 2007). The most common LAB species identified are *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum*, with *L. sakei* prevailing. Among CNC, *Staphylococcus xylosus* and *Staphylococcus carnosum* are the most common species identified from traditional products. Pediococci and enterococci have also been often identified from fermented sausages. Fast acidification and lower pH values can be ensured by *Pediococcus* in semidry sausages in which they grow and metabolize carbohydrates at higher temperatures (Incze 2007), while *Lactobacillus* are mostly used in dry sausage production.

The earliest production of fermented sausages was based on spontaneous fermentation due to the development of the microbiota naturally present in the raw material. Indigenous LAB usually present in raw meat at low numbers (10^2–10^3 cfu/g) rapidly dominate fermentation, NaCl, nitrate/nitrite, and an anaerobic environment favoring LAB growth and establishment in the meat fermentation ecosystem. During this process, two basic microbiological reactions occur simultaneously and interdependently: a decrease in meat batter pH via glycolysis by LAB and nitric oxide production by CNC through nitrate/nitrite reduction. Due to the acid production by carbohydrates, LAB are responsible for the "tangy" flavor of sausages (Demeyer 2004). Acidification also induces meat proteins' denaturation and coagulation that, along with the drying process, favor sausage texture development (Barbut 2007). During ripening, degradation of meat proteins is carried out by endogenous and bacterial enzymes (Sanz et al. 2002). It has been demonstrated that *L. sakei* and *L. curvatus* isolated from meat possess proteolytic activity on muscle proteins and play an important role in amino acid generation (Sanz and Toldrá 2002; Talon et al. 2002). Indeed, the complete genome sequence of *L. sakei* revealed its competitiveness to grow on meat, resisting adverse environmental conditions during fermentation, such as high salt and low glucose levels and changing redox conditions (Chaillou et al. 2005). On the other hand, CNC organisms, in particular *Staphylococcus*, contribute to flavor by catabolizing amino acid and free fatty acids, and producing a range of volatile compounds that enhance cured meat aroma (Stahnke 2002; Beck 2005) and play a role in color formation through their nitrate reductase. Yeasts and molds also contribute to flavor through lipolytic and proteolytic activities and lactic acid degradation (Spotti and Berni 2007). From a safety point of view, the use of bacteriocinogenic LAB as bioprotective cultures for naturally controlling the shelf life and safety of fermented meat products has been extensively reported (Vignolo and Fadda 2007; Castellano et al. 2008). Starter cultures may be associated with potential risk factors, such as the production of biogenic amines, the presence of acquired genes for antimicrobial resistance, and enterotoxin production (Cocconcelli 2007; Vidal-Carou et al. 2007).

With a view to starter culture selection for semidry and dry fermented sausages, LAB and CNC strains with useful metabolic activities and benefits during sausage fermentation must be selected (Table 22.1). Although these requirements may be fulfilled, final product characteristics determining the uniqueness of the fermented sausage will be highly dependent on the particular strains involved. During the past few decades, the use of commercial starter cultures in meat fermentation has led to process stabilization and reduction in product variability, causing a loss of bacterial biodiversity. Pure cultures isolated from traditional fermented meats exhibit a diversity of metabolic activities that diverge strongly from industrial bulk starters. They are often more dependent on their own

Table 22.1. Requirements of LAB and CNC strains to be used as starter cultures for semidry and dry fermented sausages

Microbial group	Metabolic activity	Benefits during sausage fermentation
LAB	Acidification (rate and extent)	Modulation of acid/tangy flavor Inhibition of pathogen and contaminants Texture development Acceleration of color formation and drying
	Proteolytic (aminopeptidasic and peptidasic) activity	Flavor development (nonvolatile taste compounds)
	Antimicrobial (bacteriocins) activity	Inhibition of pathogen and contaminants Shelf-life extension
	Antioxidant activity (catalase production)	Protection of color
CNC	Nitrate-reductase activity	Formation of cured-red typical color Removal of excess nitrate
	Catabolism of branched-chain amino acids/free fatty acids	Flavor development (volatile aroma compounds)
Yeasts and Molds	Antioxidant activity	Prevents rancidity
	Proteolytic activity	Flavor development
	Antioxidant activity	Prevents rancidity Improvement of color

biosynthetic capacities, harboring more amino acid converting enzymes that play a key role in the flavor characteristics of traditional products (Leroy and De Vuyst 2004). A recent trend exists in the isolation of wild-type strains to be used as autochthonous starter cultures toward safety improvement and preservation of typical sensory qualities. The isolation and selection of these wild strains is of great interest to standardize quality and limit unsafe compound formation while preserving product specificity (Benito et al. 2007; Villani et al. 2007; Di Cagno et al. 2008; Talon et al. 2008). In addition, the selection of appropriate starter cultures and barrier microflora from the "in-house" flora of small-scale producers would be a way to improve safety without affecting their typicity (Chevallier et al. 2006).

Processing Technology

Since the basic principles for the main operations of the manufacturing process are amply described in the corresponding chapters elsewhere in this book, only a brief consideration on each stage will be made. Essential sequential steps for the manufacture of dry and semidry fermented sausage are shown in the flow chart (Fig. 22.1).

Meat Selection, Grinding, or Chopping

Meat from healthy animals, mostly pork and beef, is used in fermented sausage production. Animal species, anatomical region of the animal, composition, and microbiological quality have a great influence on the functional characteristics of meat. Bones should be removed, connective tissue membranes trimmed off, and soft intermuscular fatty tissue detached. Meat and fat are chilled (grinding technology) or frozen (bowl chopper technology) and comminuted to the desired particle size. The cutter (a rapid rotating set of knives in a slowly rotating bowl) has become the established means of chopping. Warming of the meat batter is prevented by using chilled meat and frozen fat, and chopping is usually carried out under vacuum to avoid oxygen interference with drying and color development. The relative rotation speeds of the bowl and knives determine the particle size of meat and fat, and are optimized to minimize fat tissue damage and batter temperature increase. Both lean and fat

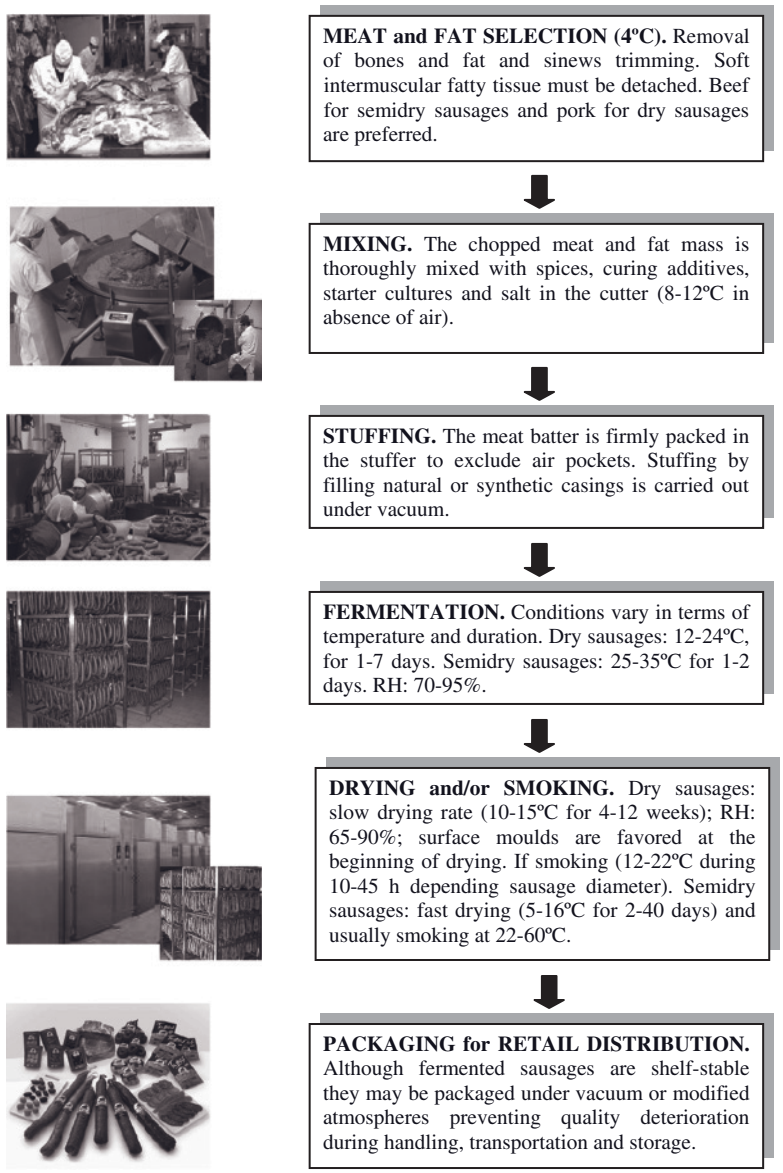

Figure 22.1. Flow diagram of the processing of dry and semidry fermented sausages

colors are important; a discrete red color for lean and white for fat, allowing a particle distinction, is important. In general, the finer the degree of grinding and chopping, the more complete the protein extraction, while the spreading or slicing properties of finished products are improved.

Mixing and Stuffing

Once meat and fat have been comminuted, curing salts (NaCl, nitrates/nitrites), additives (ascorbic acid, colorants), other ingredients (sugars, spices/aromatic herbs), and starter cultures (LAB and/or CNC bacteria)

are added to the meat batter and thoroughly mixed in the bowl chopper. Mixing should be sufficient to uniformly distribute ingredients; over-mixing must be avoided. After thorough mixing, the meat batter should be either immediately stuffed or kept under refrigeration and protected from air to enable optimal color development and microbiota stabilization. Stuffing of the meat batter into natural or synthetic casings is carried out under vacuum, thus preventing abnormal color or flavors. The casing diameters (and the sausages) vary considerably; small diameters (35–40 mm) are generally used for spreadable sausages, while sliceable sausages are usually marketed in large-diameter casings. For mold-fermented raw sausages, a small diameter is required to ensure a sufficient oxygen supply for the full development of aroma (Spotti and Berni 2007).

Fermentation

Stuffed sausages are placed in ripening chambers under controlled temperature, relative humidity (RH), and air speed conditions, depending on the sausage type to be produced. For traditional fermented sausages, natural and less controlled conditions are applied (Lebert et al. 2007). Conditions of fermentation vary in terms of temperature and duration (Tables 22.2 and 22.3). In general, the higher the fermentation temperature, the faster the lactic acid production. In Europe, fermentation may be carried out at relatively high temperatures (18–24°C) for 1 to 2 days for German and Italian dry fermented sausages, while fermentation for 7 days has been reported for Greek, Argentinean, and certain Italian dry sausages. Fermentation at lower temperatures (10–17°C) for approximately one week is usually used for traditional French, Spanish, and Portuguese dry fermented sausages. For semidry sausages, particularly in the United States, temperature is usually raised slowly to over 35°C to shorten fermentation time, which is frequently one day or less. Even when these products undergo a shorter ripening period than dry fermented sausages, fermentation of raw sliceable semidry sausages lasts longer if no heat treatment is applied (Incze 2007).

Air-Drying or Smoking

After the fermentation stage, sausages are either air dried or smoked. Drying is a key operation, especially for dry fermented sausage production; the drying rate should be as low as possible. A crucial aspect is avoiding the pronounced surface coagulation of proteins so that water diffusion from the center outward is hindered (Andrés et al. 2007). Drying kinetics and duration vary, depending on temperature and air velocity. These parameters are less important than for the fermentation stage and range from 10° to 15°C for 4 to 12 weeks for French, Italian, Spanish, Argentinean, and Greek dry fermented sausages. Higher drying temperatures (16–18°C) were reported for dry sausages produced in Greece and East Europe, accompanied by shorter ripening. The RH during fermentation varies from a minimum of 63% to 75%, to maximum values of 86% to 95% in dry fermented sausages, leading to variable a_w in traditional sausages at the end of drying (Lebert et al. 2007). At the beginning of drying, RH can be as high as 98%, which leads to a distinctive surface colonization by mold and yeasts. It was reported that yeasts are the predominant organisms on the surface (95%) during the first two weeks, and then molds and yeasts are present in equal amounts (Samelis and Sofos 2003). On the surface of dry fermented sausages, mold growth is desirable as it prevents excessive drying, protects from oxidative reactions, and contributes to flavor development (Spotti and Berni 2007). Selection of fungal starter cultures among the naturally occurring molds on the sausages'

surface enables control of molding at the first stages, making the starter presence predominant. In central and northern European countries, smoking is a common technology. Smoke contributes to the antimicrobial and antioxidant effect, besides generating specific flavor and color components. Smoking technology has experienced an important evolution in the last few years; smoking chambers in which a controlled combustion of wood (300–600°C) to minimize the production of polycyclic hydrocarbons have been developed. In American semidry fermented sausages, a brief drying stage after fermentation precedes smoking (Rust 2007).

Types of Semidry and Dry Fermented Sausages Worldwide

A description of types and distribution of semidry and dry fermented sausages worldwide is presented. Technological features are also shown in Tables 22.2 and 22.3.

American

Since fermented sausages' manufacturing practices in the United States and in many Latin American countries were introduced by European immigrants, many typical European fermented sausages can be found in the Americas. American semidry sausages are considered to be acidified processed meat products with an M : P ranging from 3.7 : 1 to 2.3 : 1 and a pH below 5.0. Summer sausages are a loosely defined variety of semidry sausages and are usually a mix of beef and pork, the predominant seasonings being black pepper, mustard, coriander, and garlic. Summer sausages stuffed in different casing types (40–120 mm) and smoked are very popular in the United States (Rust 2007). Among semidry fermented sausages, Lebanon bologna is a unique product originated by Pennsylvania German immigrants in the town of Lebanon, Pennsylvania. It is produced entirely from beef and is a moist, heavily smoked, fermented, ready-to-eat sausage with an M : P of 3.5 : 1 that is often not processed above 48.9°C (Chikthimmah et al. 2001). The starter culture involving *L. plantarum*, *P. acidilactici*, and micrococci was specifically developed to obtain a very low pH (4.4–4.7), and it ferments the meat batter at 35–38°C (Smith and Palumbo 1973). In traditional processes, black pepper is added as seasoning, and the coarse ground meat is pre-salted and aged for several days under refrigeration. Sweet bologna is produced with 10% to 12% of sugar instead of the 2% to 4% in Lebanon bologna. Snack sticks are shelf-stable semidry sausages that include hot seasonings and edible collagen casings. The production technology of dry sausages in the United States has borrowed heavily from European knowledge and experience. These products are small-diameter, moderately chopped, and cold smoked or not. American dry sausages tend to be milder and usually have less smoked flavor and salt than in Europe, and *P. acidilactici* or direct acidulation is used to reach a pH of 5.4 or less. Other popular dry sausages are salami made from pork and small amounts of beef, seasoned with garlic; pepperoni are also made of pork and beef, and are usually smoked; and chorizo is highly spiced and smoked. About 90% of the pepperoni produced (115,000 tons/year) is sold in stick form for pizza topping (Faith et al. 1997).

Although fermented sausages are produced in different Latin American countries, their manufacture has a long history in Argentina, Brazil, and Uruguay, mainly due to Iberian and Italian traditions, as well as to the quality and availability of meat. Many salami-style sausages made of beef and/or pork meat are produced in Argentina, among them *Salame de Milán*, which is produced in different regions, and *Salame Tandilero*, from the town of Tandil (Buenos Aires),

Table 22.2. Dry fermented sausages characteristics and examples

Process T (°C)	Process time	Moisture (%)	a_w	Final pH	M : P	Drying	Examples	References
24 to 15	5–6	35	0.82–0.90	5.8–6.7	1.1–2.1 : 1	Air	Southern Italy	Parente et al. (2001)
						Air	*Salciccia/Soppressata*	Villani et al. (2007)
							Naples-style salami	Bonomo et al. (2008)
30 to 12	5–6	35						Coppola et al. (2000)
22 to 12	4–17	40–46		5.2		Air	Nort East Italian salami	Comi et al. (2005)
								Rantsiou et al. (2005)
20 to 10	7–8	41–47	0.91–0.92	5.6–5.7	2.1 : 1	Air	Northen Italy *salami*	Di Cagno et al. (2008)
			0.87–0.89	5.9–6.6	1.1–1.8 : 1			
24 to 12	3–4	32–33	0.80–0.92	4.6–4.9		Smoke	Greek sausages	Papamanoli et al. (2003)
								Rantsiou et al. (2005)
24 to 16	4–5	28–34				Air		Kozačinski et al. (2008)
24 to 14	4	29	0–77–0.80	4.8–5.1		Air	Southern Greece salami	Drosinos et al. (2007)
				4.8				
12	9		0.83–0.93	6.2–6.5	2.0 : 1	Air	French *Saucisson sec*	Chevallier et al. (2006)
22 to 11	6–12			5.2–6.3		Air		Lebert et al. (2007)
24 to 12	3–12		0.85–0.86	5.0		Air	Central west Spain	García-Varona et al. (2000)
15 to 10	16		0.77–0.80	5.6–5.9		Air	*Salchichón/Chorizo/Fuet*	Aymerich et al. (2003)
20 to 10	8–12	44–55	0.92–0.94	5.5–5.6	1.1–1.3 : 1	Air	*Salchichón/Chorizo*	Benito et al. (2007)
				5.4–5.6		Smoke	*Androlla/Botillo*	Lorenzo et al. (2000)
15	14–24	29–41	0.83	5.3–5.4	1.0–1.1 : 1	Smoke	Potuguese	
	20			5.5		Smoke	*Chouriça/Salpicão*	Ferreira et al. (2007)
							Painho de Portalegre	Roseiro et al. (2008)
25 to 18	7–8	30	0.89–0.95	4.6–5.5		Air	Turkish *sucuks*	Erkmen and Bozkurt (2004)
20 to 15	4–5	20–23		5.3–5.7		Smoke	Hungarian salami	Kozačinski et al. (2008)
20 to 16	4	13–15	0.91–0.96	5.3–5.5		Smoke	Croatian sausages	Kozačinski et al. (2008)
36 to 13	2–3		0.90	4.7–4.8	1.6 : 1	Variable	US pepperoni	Faith et al. (1997)
23 to 15	2	28–30	0.9	5.2–5.4	1.4 : 1	Air	Argentinean sausages	Fontana et al. (2005)

Process time in weeks; M/P: Moisture/Protein ratio

Table 22.3. Semidry fermented sausages characteristics and examples

Process T (°C)	Process time	Moisture (%)	a_w	pH	M:P	Drying	Examples	References
	2–8	52		5.1	4.7:1	S	Portuguese *Alheiras* sausage	Ferreira et al. (2006)
25 to 16	9		0.95	4.5		S	Dutch sausages	Houben and vant'Hooft (2005)
24 to 15	35	45–49	<0.91	5.1		S	Ireland sausages	Hughes et al. (2002)
35 to 5	40	60–65		4.4–4.7	3.5:1	V	US *Lebanon Bologna*	Chikthimmah et al. (2001)
30	7	70		4.4–4.8	3.5:1		Thai *Nham* sausage	Visessanguan et al. (2006)

Process time in days; M/P: Moisture/Protein ratio

using local artisanal techniques (Fontana et al. 2005; Fadda and Vignolo 2007).

Mediterranean

The Mediterranean region has a wide variety of fermented meat products due to the variation in the use of raw materials, formulations, and manufacturing processes that originate in the habits and customs of the different countries and regions. Two technologies can be clearly distinguished: dry-curing, southern, or Mediterranean; and wet/pickled curing or northern (Talon et al. 2004). A brief description of the main fermented sausages produced in South European countries is presented.

Italy

Unsmoked dry fermented sausages made mostly of pork are by far the most popular products in Italy. In Southern Italy, there is a wide variety of typical dry fermented sausages prepared according to traditional methods, *Salciccia* and *Soppressata* among the most appreciated ones. In particular, *Soppressata* of *Vallo di Diano* and *Molisana* are pork meat sausages produced in the Campania region in a large number of small artisanal plants (Parente et al. 2001; Villani et al. 2007; Bonomo et al. 2008). Naples-type salami is also a popular Southern Italian dry fermented sausage made of coarsely minced pork meat (Coppola et al. 2000). In Northeast Italy, traditional dry fermented sausages made of fresh pork display unique organoleptic sensory profiles characterized by accented acidity, slight sourness, and elastic semihard consistency (Comi et al. 2005; Rantsiou et al. 2005; Spaziani et al. 2008), while in Northern Italy, Protected Designation of Origin (PDO) fermented dry sausages are produced exclusively from local pig breeds (Di Cagno et al. 2008).

Spain and Portugal

In Spain, around one-fifth of the total meat manufactured products are dry-cured sausages. Spanish chorizo, *Salchichón*, and *Fuet* are dry fermented sausages produced in the central-west region of Extremadura, with wide acceptance among consumers. Chorizo, with a total annual production of more than 80,000 tons, is made of minced pork meat, cayenne pepper, paprika, and garlic, stuffed in natural or artificial casings, and ripened at low temperatures (García-Varona et al. 2000), while *Salchichón* and *Fuet*, to which black and white pepper is added, undergo ripening for four months (Aymerich et al.

2003; Benito et al. 2007). Other Spanish traditional dry-cured sausages are *Androlla* and *Botillo*, produced in the Galicia region using low-quality pork meat seasoned with paprika, garlic, and sometimes onion; these sausages are subjected to a smoking-heating process followed by drying-ripening and are consumed after cooking (Lorenzo et al. 2000). In Portugal, traditional fermented sausages are mostly made of pork meat from autochthonous pig breeds. Since the eighteenth century, in the northern part of the country, traditional *Salpicão de Vinhais* and *Chouriça de Vinhais* are produced and consumed without further cooking. Both smoked products are made from raw pork meat to which wine and spices are added; in the production of *Chouriça*, horseshoe-shaped small pieces of meat and fat are used, while in *Salpicão*, bigger lean meat pieces are used (Ferreira et al. 2007). *Alheiras*, a traditional, smoked, semidry fermented sausage produced from pork and other types of meat whose origin dates back to the fifteenth century, is an important economic resource with a production of more than 500 tons/year (Ferreira et al. 2006). *Painho de Portalegre* is a smoked dry sausage containing paprika and garlic produced using pork meat from the Alentejano pig breed (Roseiro et al. 2008). Most of these products have been entered successfully into the register of Protected Geographic Indication (PGI).

Greece

Dry fermented sausages (*salami aeros*) are typical Greek products, with a production of more than 10,000 tons/year (Samelis et al. 1998). Most of them are produced using pork and beef meat, and they are smoked before they are ripened (Papamanoli et al. 2003; Rantsiou et al. 2005; Drosinos et al. 2007).

France

In France, semidry fermented sausages are only moderately dried, smoked, and eaten either raw or heat treated. Some well-known semidry products are *saucisse de Montbeliard* and *Morteau*, both from the Franche-Compté Eastern France region. *Saucisson sec*, typical pork dry fermented sausages produced in central and southern France, are important products in the meat industry, with a production of 10,0000 tons in 2002 (Lebert et al. 2007). There is a wide variety of *saucisson sec* produced in small-scale processing units without starter cultures (Chevallier et al. 2006). Due to their great economic significance, safety improvement with preservation of typical qualities of these traditional sausages were recently carried out, and autochthonous starter cultures have been developed (Lebert et al. 2007; Talon et al. 2008).

Central European

Germany

Sausage quality is characterized by the use of valuable parts of the carcass, so that the drying stage is not an obligately important feature for quality. Although the manufacture of fermented sausages began only 160 years ago, Germany is a major producer of fermented meat products that accounts for 40% of European production. Most semidry fermented sausages are produced from pork and beef meat, the wide range of products depending on the extent of drying and regional traditions (Schwing and Neidhardt 2007). In the northwestern region, sausages are strongly smoked, soft, sliceable, or spreadable with a mild acid flavor, typical products being *Bregenwurst*, a semidry spreadable pork sausage originally from Lower Saxony, and *Frankfurter Rindswurt*, a smoked sausage made of pure pork. Westphalian salami, made with fast technology from pork meat, pepper, garlic, and sometimes mustard seeds, is a smoked, firm, sliceable product with a distinct fermentation/sour flavor. The sausages are stuffed into large-diameter casings and ripened by lowering the temperature

from 24°C to 12–14°C until a water loss of 25% is obtained. In the central region of Turingia, a wide variety of fermented sausages has been produced for hundreds of years, among them *Brätwurst*, which is made using finely minced pork, beef, or veal to which caraway, marjoram, and garlic are added. *Teewurst*, which originated during the nineteenth century in the Pomerania region, is a high-quality, spreadable sausage from pork meat with a high fat content (30%–40%) that is smoked and ripened. *Feldkieker* sausage, based on freshly slaughtered pork meat with the addition of syrup or honey and red wine, is ripened for 8 to 12 months and either air dried or smoked. In Southern Germany, mildly smoked and acid, well-dried sausages are produced. *Landjäger*, typical of the Black Forest region, is a dry fermented sausage made of roughly equal amounts of pork and beef meat, spiced with pepper and cumin, and usually produced as links 15 to 20 cm in length and pressed before drying (14–16°C; weight loss of 35%) to give a rectangular cross section. *Weisswurst* (white sausage) is a typical Bavarian sausage made of finely minced veal meat. Most of these traditional German fermented meat products have acquired PGI status. Although they have several regional differences (meat type and seasonings), other popular sausage varieties within Germany include *Schlackwurst*, *Metwurst*, and *Cervelatwurst*. *Metwurst* are strongly flavored sausages made from minced pork, cured and smoked, soft in the south and firmer in the north (*Holsteiner* sausage) due to longer smoking. *Cervelatwurst*, which has its origin in the Italian Milan-type sausage, is made of beef and pork meat, and has a fine particle size.

Austria and Switzerland

In these countries, fermented sausages are very similar to German salamis. However, a typical Austrian product is the square-shaped *Kantwurst* that is cured for 7 weeks, reaching a weight loss of about 35%. *Cervelat*, made of beef and pork, is the typical Switzerland sausage.

The Netherlands

Dutch semidry sausages are manufactured from pork and/or beef, and, in some products, cooked pork rind. The most popular Dutch products are finely chopped salami, *Cervelat*, *Snijworst* (with high fat content and rind added), *Farmersmetworst* (which is coarsely chopped), and chorizo (which is less spicy than the Spanish product).

Eastern European

Hungary

Hungarian salami is one of the world's two trade names for salami, Milano salami being the other. These fermented sausages combine smoke and mold application. The traditional technology is based on the Italian pre-drying technique developed during the nineteenth century, but sausages are smoked and pH does not drop below 5.5, so the final flavor of the product does not contain acidic notes (Incze 1986). Many PDO dry fermented sausages are produced, among them *Szegedi téliszalámi* (winter salami), which is made of mangalitsa pork breed, with horse large intestine traditionally used as a casing. It acquires a grey mold cover and has a firm texture and excellent keeping quality after a 30% weight loss reached in 3 to 4 months. Hot *Kolbász* is also a very popular smoked, dry fermented sausage seasoned with hot paprika and not mold covered (Schwing and Neidhardt 2007).

Bulgaria

Due to naturally good climatic conditions, Bulgaria has a long tradition of fermented sausage making. *Loukanka* is one of the outstanding Bulgarian products, with a particu-

lar flat shape, very dry texture, extremely mild flavor, and a typical final pH not below 5.3. It is a finely grained pork sausage with a typical cumin-pepper and garlic flavor whose flat shape is obtained by pressing during the fermentation process.

Slovakia and Czech Republic

Fermented sausages in this region are similar to German-type sausages, fermented to a mild acidity and having a typical rounded flavor profile. Slovakian sausages are lightly smoked, while traditional Czech fermented sausages are heavily smoked and produced with relatively fatty meat batters. A speciality is Lovecky salami, which has a characteristic rectangular shape due to its particular pressing.

Poland

A typical Polish sausage (*kielbasa*) is *Polska*, which is made from pork meat, stuffed in natural casings, ripened at a low temperature (6°C), and smoked. This semidry fermented sausage has a soft texture due to limited drying (total weight loss of 12%–17%) and is eaten cooked (Pisula 2004).

Russia

Russian fermented sausage must lose 40% of its weight, and final pH must reach 5.0. Typical products are Moscow-type and Russian-type salamis made from pork and beef meat. A particular feature of Moscow-type salami is the large size of fat particles (7–8 mm) that give the sausage a rough surface.

Scandinavian

Denmark

Dansk spegepølse is a typical Danish semidry fermented sausage characterized by added color (cochineal) and finely minced meat. Produced from pork, it is smoked and has a final pH of 4.7 to 4.9. It can be produced by direct curing or tank curing, wherein sausages are put in brine, causing salt enrichment and moisture removal. The high fat (49%), high salt (4.5%), low moisture (36%), and moderate drying loss (13%), together with a low pH, ensure a safe final product. *Sønderjysk spegepølse*, produced in South Jutland, is made from pork and beef meat, and starter cultures are generally used.

Sweden

Swedish *Medwurst* is made from pork meat and contains boiled potatoes, in addition to spices and seasonings. High fermentation temperatures (30–35°C) and smoke are applied. The sausages are often heat-treated after fermentation.

Norway and Finland

Typical Norwegian fermented sausages are made using "unusual" meats, among them mutton, lamb, horse, and reindeer. The use of wild animal meat is a common practice all over Scandinavia. A large range of so-called *Morr* sausages based on old traditional methods is produced, including *Fårepølse* and *Stabbur*. In these products, the presence of meat from free-ranging animals and sometimes blood gives a special strong flavor to the final product. Two typical Finnish fermented sausages are *Kotimainen* and *Venäläinen*, which are often quite sour and acidic (pH 4.6–4.9) with a strong smoky taste and low fat content.

Asian

Middle Eastern

Due to religious concerns, pork meat is not used in Middle Eastern countries, but a variety of meats (mutton, beef, goat, camel, and horse) is used for fermented sausage pro-

duction. Turkish style sausages, *Soudjouk* or *sucuk*, are the most popular meat product in Turkey, and they are mostly produced using traditional methods in small-scale facilities with air-drying. These sausages are made from mutton and/or beef meat, fat (18%), garlic, spices (black and red pepper, cumin, and cinnamon), and vegetable oil (Erkmen and Bozkurt 2004). Traditional *sucuk* are widely produced in Turkey; more than 60,000 tons are manufactured yearly. In Lebanon, fermented beef sausages that are strongly smoked are produced (El Magoli and Abd-Allach 2004). Sausages often contain rice, wheat, and corn flour, and different flavors are obtained depending on the addition of olive oil, garlic, onion, paprika, and black pepper.

East Asian

Lap Cheong is a general term for Chinese sausages, which are traditionally made during the winter months. The ingredients used vary among regions, but basically pork meat and fat are used, and spices, soy sauce, and alcoholic beverages are often added. Other products made in a way similar to *Lap Cheong* but replacing pork meat with duck, chicken, or pig liver are Cantonese speciality products. *Nham* is a typical Thai fermented pork sausage with a NaCl content of 2% to 3%, normally made of minced pork, shredded cooked pork rind, cooked rice, garlic, and nitrite, which is tightly wrapped in banana leaves after mixing. Fermentation of *Nham* usually takes 3 to 5 days at 30°C; a final pH of 4.4 to 4.8 is achieved, after which it is cooked and consumed (Visessanguan et al. 2006). *Goon Chiang* is also a Thai sausage in which pork is marinated with nitrite at low temperatures, followed by grinding and mixing with sugar before stuffing in pork casings and dried at 60°C. In the Philippines, *Longamisa* is a pork sweet-sour sausage containing vinegar, soy sauce, and sugar that can be smoked after stuffing. *Urutan*, a Balinese traditional dry fermented sausage prepared from lean pork and various kinds of spices, is fermented under warm conditions at temperatures from 25° to 50°C during sun drying (Antara et al. 2004). Korean popular products, *Sundae* and *Soonday*, are stuffed sausages made with pig or beef blood, rice, and a wide variety of seasonings and spices that are steamed before consumption.

African

Boerewors are traditional, small-caliber, fresh sausages from South Africa made of game and beef, usually mixed with pork or lamb and spices (usually coriander seeds, black pepper, and nutmeg). When these sausages undergo a warm drying process after being flattened, they become *Dröewors*, a typical snack food. Other fermented products from Northeastern Africa (*Miriss*, *Mussram*) are made from fat, goat meat, and offal.

Safety of Semidry and Dry Fermented Sausages

Semidry and dry fermented sausages are considered ready-to-eat products, referring to those products that do not undergo thermal treatments before consumption. These products become stable and safe through a sequence of hurdles, some of which are specifically included (NaCl, $NaNO_2$/$NaNO_3$, ascorbate), while others are indirectly created in the stuffed mix (low Eh, antagonistic substances, low a_w). By means of these hurdles, spoilage and food-poisoning bacteria are inhibited, whereas the desirable organisms, especially LAB, are hardly affected. Apart from LAB, GCC, molds, and yeasts involved in sausage fermentation, beef and pork meat as the major components of cured sausages regularly contain pathogenic bacteria and are often implicated in the spread of food-borne diseases. Raw materials (meat and casings) are the main vehicles for food-borne pathogens and contaminants. Chilling inhibits the

growth of a selection of pathogens, from mesophilic to psychrotrophic organisms, and since most pathogens are mesophiles, meat obtained in good hygienic conditions would presumably not be implicated as sanitary risks. Still, the growth of pathogenic bacteria, overcoming the existent natural hurdles, can occur. Food poisoning from *Staphylococcus aureus*, *Salmonellae*, and *Clostridium* has been traditionally implicated in fermented dry sausage (Mataragas et al. 2008). On the other hand, emergent pathogens within the genera *Campylobacter*, *Yersinia*, *Listeria monocytogenes*, and enterohemorrhagic *E. coli* (EHEC) have also been involved in outbreaks caused by fermented sausage (CDC 1995; Sofos 2008). Since there is no epidemiological evidence for the involvement of fermented sausages in recent outbreaks of listeriosis, up to 100 cells of *L. monocytogens* per gram can be tolerated (ICMSF 2002). Fermented sausage conditions, curing additives, and the presence of LAB starter cultures may act as significant hurdles for the control of these pathogens (Table 22.4). However, they are not sufficient to prevent the survival of *L. monocytogenes* or EHEC during the manufacturing process; prevalence, survival, and growth in traditional meat products have recently been reported by Skandamis and Nychas (2007). An additional hurdle to reduce the risk of *L. monocytogenes* would be the use of competitive bacteriocin-producing starter cultures or bioprotective cultures (Vignolo and Fadda 2007; Castellano et al. 2008). Differences in the production technology of dry and semidry fermented sausages highly influence their safety, the degree of drying and the ripening time being the most important features. Hurdles that combine a pH/a_w drop, pH/a_w drop and heat treatment, and a_w drop and heat treatment will ensure safety of both dry and semidry products. Nevertheless, these conditions are seldom found in either dry (relatively higher pH and lower a_w) or semidry (relatively lower pH and higher a_w) fermented sausages. Since the effect of hurdles works well at pH values of ≤5.3 and parallel a_w values at ≤0.95, there is a rather limited opportunity to meet food safety requirements with short- or medium-time ripened semidry sausages, while traditionally long-time ripened dry sausages are in a much better position. Experimental data suggest that heating semidry sausages is the only effective method for a 5-log reduction of EHEC and *L. monocytogenes*, and is a further safety-improving solution (Chikthimmah et al. 2001). During fermented sausages' production and storage, meat undergoes major chemical changes, leading to the formation of harmful biological compounds, such as polycyclic aromatic hydrocarbons and lipid oxidation products, nitrosamines being hardly ever formed, since high temperatures and secondary amines necessary to react with nitrite are not present (Honikel 2008). On the other hand, the risk for biogenic amines and micotoxin production, either by starter organisms (LAB, CNC, and molds) or spoilage microbiota, is higher for dry fermented sausages due to the intense aminogenesis that occurs during fermentation (Vidal-Carou et al. 2007) and surface molding.

Table 22.4. Main hurdles inhibitory to pathogens present in dry and semidry fermented sausages

Pathogen	Hurdles
Staphylococcus aureus	pH < 5.1; a_w < 0.86; bacteriocins
Salmonella	pH < 5.0; a_w < 0.95; NaCl/$NaNO_2$
Clostridium perfringes	LAB (acid and bacteriocins)
Yersinia enterocolitica	LAB (acid)
Campylobacter jejuni	LAB (acid)
Listeria monocytogenes	a_w < 0.90; bacteriocins
Escherichia coli (ECEH)	LAB (acid)

Trends in Fermented Sausage Production

The history of meat products during the last twenty-five years can be divided in terms of

realizations, threats, and opportunities into three consecutive and complementary periods in which quality, food safety, and nutrition/health were successively emphasized (Vandendriessche 2008). The "nutrition and health" period has only just started. Answers to the meat industry's questions as to how to develop new healthier meat and meat products will undoubtedly come through functional gene- and protein-expression studies in the different meat ecosystems; hence the implementation of "omics" technologies within integrated programs of environmental microbiology (Nelson et al. 2007). The concept of health products includes what is known as "functional foods," defined as foods that are used to prevent and treat certain disorders in addition to their nutritional value per se (Jiménez–Colmenero et al. 2001). Regrettably, meat has an unfortunate image related to fat, saturated fatty acids, cholesterol, salt, and nitrate/nitrite content; these are associated with cardiovascular diseases, some types of cancer, and obesity. However, such a view disregards the fact that meat plays a critical role in the maintenance of human health as a source of proteins, vitamins, and minerals. Different strategies for the development of healthier meat products have been suggested. These include reduction of sodium, nitrites, fat, and cholesterol content, as well as incorporation of functional ingredients (Fernández-Ginés et al. 2005). On the other hand, the performance of commercial starter cultures has been questioned, since their behavior is different when applied to different types of fermented meat products. It is crucial, therefore, to provide traditional producers with the means to produce safe and standardized products while preserving their typical sensory quality. As a response to these needs and to the demands for health products, the use of a new generation of starter cultures has already been suggested (Leroy and De Vuyst 2004). The so-called "functional starter cultures" contribute to food safety by producing antimicrobial compounds and also provide sensorial, technological, nutritional, and/or health advantages. Recently, new starter cultures of LAB and CNC bacteria with important functionalities have been developed. The control over proteolytic and lipolytic activities of starter cultures' bacteria during meat fermentation has led to improved aroma and flavor characteristics, as well as improved physiological functions of the generated peptides. Emphasizing bioactive metabolites' production (vitamins, bioactive peptides, and organic and fatty acids) in meat is a step toward improving its health image and developing functional meat products (Arihara 2006). Since most fermented sausages are usually not heated, they are adequate for the carriage of probiotic strains, either selected among naturally present bacteria or from existing probiotic strains (De Vuyst and Leroy 2008). In addition, functional starters have also been used for technological advantages, such as the acceleration of fermented sausage processing by means of high temperatures and enzyme addition (Fernandez et al. 2000).

The consumer's behavior toward typical dry fermented sausages in a recent survey in Italy indicated that these sausages are part of consumption habits, reinforcing the impression that food consumption is neither an isolated phenomenon nor exclusively focused on food products per se, but is part of a wider social context (Conter et al. 2008). Traditional fermented sausages constitute a highly appreciated specialty with gastronomic value and are a rich source of bacterial biodiversity, the deliberate use of which in industrial processes could help to enhance the quality of the final product and offer health, marketing, and technological advantages.

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Chapter 23

Restructured Whole-Tissue Meats

Mustafa M. Farouk

Introduction

The trends toward increased consumption of steaks (Sloan 2000) and ready meals (Datamonitor 2007), as well as the demand for convenience and visual impact (RTS 2006), coupled with the push for adding value by meat processors, all point to a higher demand for restructured meats in the future. Excellent literature is available on restructuring meats, the methods involved, and the type of products produced (Pearson and Dutson 1987; Pearson and Gillett 1999; Sheard 2002). However, not much has been written in these sources on restructuring whole-tissue meats. The common objective of restructuring whole-tissue meat is to achieve a product that not only imitates but also possesses the attributes of a whole-tissue product. In other words, a restructured steak from cheaper muscles in the fore- or hindquarter of a carcass should, when served to a consumer, look and eat like steaks from the more expensive middle cuts, such as the rib-eye roll, striploin, or tenderloin (Farouk et al. 2005c). The fundamentals of restructuring whole-tissue meats are now well understood; however, the technology for automating some of the processes involved in the manufacture are still lagging behind and thus limiting the realization of the full market potential of these products.

This chapter discusses some of the recent developments in the restructuring of meats, with particular emphasis on the restructuring of whole-tissue meats, including intact muscles and/or whole boneless cuts from some of the larger common animal species, such as cattle, sheep, deer, and pigs.

Restructured Whole-Tissue Meats

Definitions and Importance

Restructuring refers to a group of procedures that partially or completely disassemble meat and then bind together the meat pieces to form a cohesive mass that resembles an intact muscle (Pearson and Gillett 1999). This definition encompasses a variety of meat products, including fine and coarse emulsion type products such as sausages. For this reason, restructured meats are classified according to the degree of comminution and the process involved in their preparation. For instance, Pearson and Gillett (1999) referred to meat products manufactured using muscles that were ground, chopped, emulsified, sliced, or flaked as restructured meats, and products manufactured from intact muscles or sections of muscles as sectioned-and-formed meat products. In this chapter, the term restructured whole-tissue meat is used to define restructured products manufactured from whole boneless cuts and comprising a number of muscles, intact, or sections of muscles that are bound together using hot- or cold-set binding systems. Hot-set binding systems require heat to "set" the bind, which produces a cooked product, while no heat is required in a cold-set system; the bind is achieved on the raw meat.

Data on the current production of whole-tissue restructured meat products is difficult to obtain or estimate. This is because most of the products are not identified as restructured during merchandizing. The difficulty of obtaining data on restructured meats was raised earlier by Secrist (1987) and more than a decade later by Pearson and Gillett (1999). Some of the terminology used to describe restructured meats includes boneless beef fillet (heat-and-serve restructured microwaveable steak), sandwich steaks, joysteak, ribsteak, grillsteak, sandwich meat, reformed steaks, reformed roast/joints, lamb medallion, and meat cutlets (Pork McRibs). Restructured meats are also included as ingredients in ready meals and are categorized as processed meats, too. For example, the Roast Lamb and Roast Beef Dinners marketed by Unilever's Birds Eye (2008)—a major UK ready meals manufacturer—contained 15% restructured beef or lamb. Therefore, considering that the global ready-meals market reached a value of USD 46.9 billion, one can gauge the importance of restructured meats by the volume and value of ready meals and processed meats in the market today (Datamonitor 2007). The total West European processed meat market alone, which includes delicatessen, frozen convenience meat, canned meat, cured meat, and bacon and ham, was worth Euros 116.5 billion (FFT 2007).

Advantages and Disadvantages

The advantages of restructured meat products include ease of slicing; more accurate portion control; lower cooking losses; uniformity of color, texture, and fat distribution; minimum waste to consumer and processor; accurate prediction of yield; and programming for nutritive value (Secrist 1987). According to Pearson and Gillett (1999), sectioned and formed products have the added advantage in that cheaper cuts can be utilized in producing attractive bonded products, they can be readily molded or shaped to meet a particular demand, and they can be manufactured to resemble higher-priced cuts. Another advantage of restructuring is that hot-boned pre-rigor meat can be cold-set restructured without the need to wait for the meat to go into rigor to be firm enough to be sliced, resulting in cost savings in time and space to the processor (Farouk et al. 2005b).

The disadvantages of whole-tissue restructuring relative to intact meat is that major investment is required to produce certain products, processing requires a high input of both energy and labor, fiber alignment is still done manually as no equipment is available to automate the process, and the final products may have poorer color, higher levels of oxidation, and excessive connective tissue.

Raw Materials for Restructuring Whole-Tissue Meats

When producing restructured whole-tissue meats, a wide selection of meat cuts and processing aids are available to the processor; which material is used would depend on the method used in the restructuring, the end use or market outlet of the product, and the processing cost. The selection of raw material of high quality and functionality is of primary importance in the manufacture of any type of processed meat product, including restructured meats. Meat that is microbiologically safe and free from flavor, odor, color and other aesthetic defects should be used in producing whole-tissue restructured meats. Depending on the method of restructuring and the binders to be employed, pre- or post-rigor, hot- or cold-boned, and chilled-never-frozen or frozen-thawed meat are suitable for use.

Coarsely Diced/Sliced Muscle Strips

For the manufacture of whole-tissue restructured meats from coarsely diced or sliced muscle strips, lean skeletal muscles from the

fore- or hindquarter of the carcass can be used. Some of the muscles and cuts from beef and lamb carcasses that were successfully restructured into whole-tissue steaks or free-flow individually quick-frozen cubes (meat cubes with individual cubes separated, which allows the needed quantity of cubes to be poured out of the package, leaving the remaining cubes in the package until needed) are shown in Figure 23.1. Beef middle cuts such as the tenderloin, strip loin, and cube roll (rib-eye roll) are considered premium

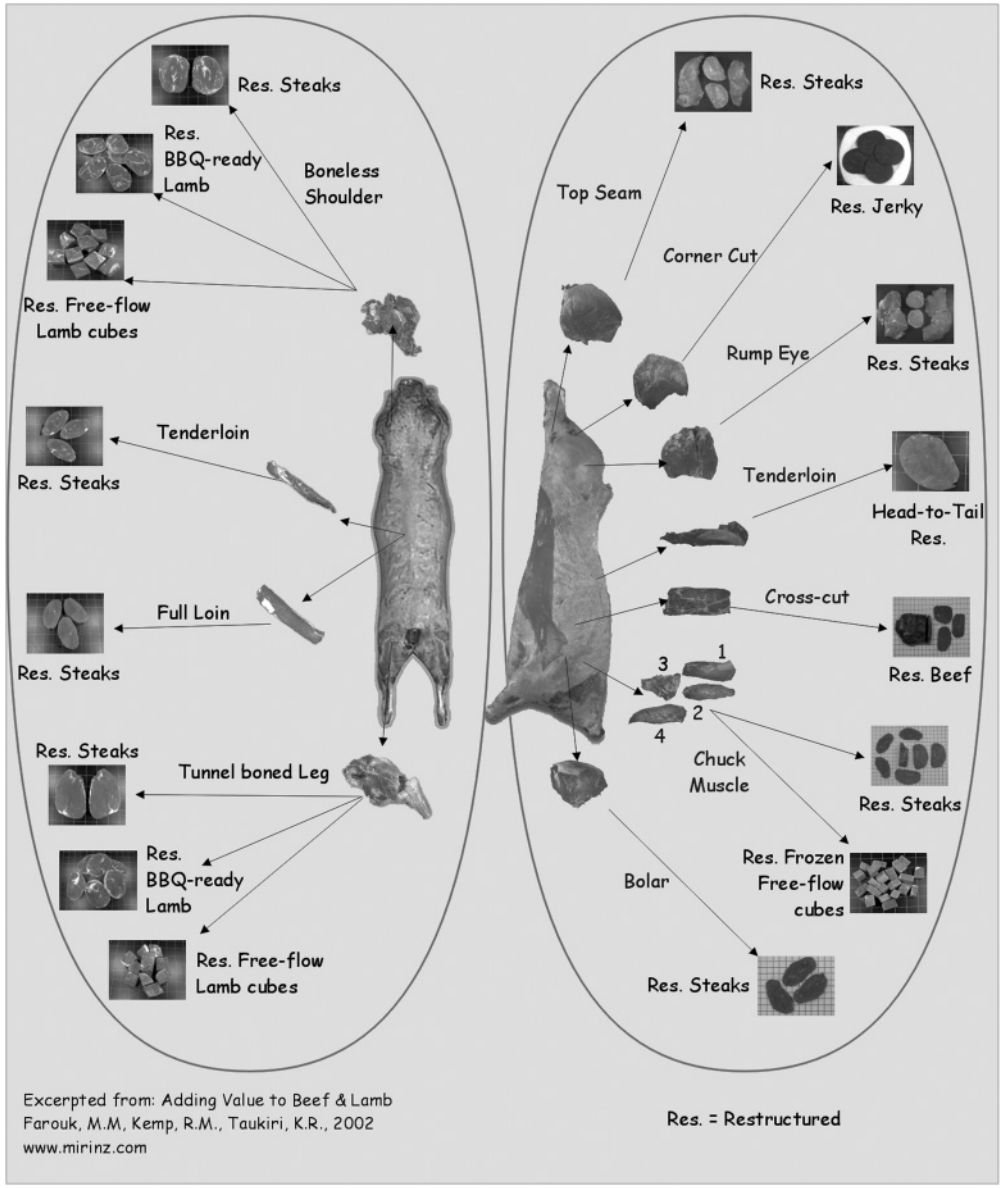

Figure 23.1. Whole-tissue restructured products from the intact muscles/cuts of beef and lamb carcasses.

Table 23.1. Mean scores of the consumer sensory evaluation of restructured steaks from whole muscle tissues. Venison (Farouk, 2001b); Beef (Farouk, 2000; 2001b); Lamb (Farouk 2002)

Species	Muscle restructured	Method of restructuring	Overall acceptability
Venison			
	Denver leg muscles	Cold-set	6.4
	Denver leg muscles	Hot-set	6.0
	Clods	Cold-set	6.7
	Chuck	Cold-set	6.4
Beef	*Infraspinatus*	Cold-set	5.7
	Triceps brachii	Cold-set	5.9
	Gluteus medius	Cold-set	7.2
	Vastus lateralis	Cold-set	6.4
Lamb	Strip loin	Cold-set	7.2
	Tenderloin	Cold-set	7.2
Venison Controls	Intact strip loin	Not restructured	5.9
	Intact tenderloin	Not restructured	7.1

Sensory scale: 1 = dislike intensely and 9 = like extremely

cuts and are rarely restructured. Off-cuts from premium cuts and lean trimmings are suitable raw materials for restructuring into whole-tissue meat products. We have previously restructured steaks (hot- and cold-set) using muscle strips from the fore- and hindquarter of beef (Farouk 2000, 2001a), lamb (Farouk 2002), and venison (2001b) that were rated by consumers as similar in their eating quality to intact strip loin steaks from the same carcasses from which the restructured steaks were produced (Table 23.1 and Fig. 23.2).

Intact Muscles/Cuts

Intact whole muscles and primal or sub-primal cuts from beef, veal, lamb/mutton, venison, pork, and chevon can be restructured into whole-tissue products. Whole tenderloins from lamb/mutton, entire boneless lamb shoulder, and tunnel-boned or whole

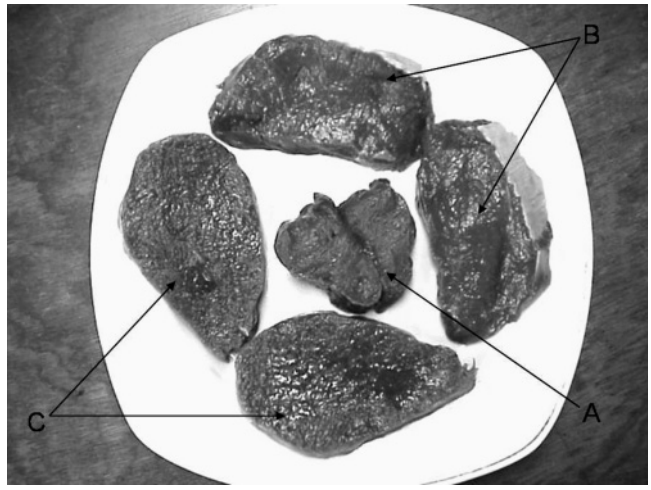

Figure 23.2. A photograph of venison products. Intact tenderloin steaks (A); intact strip loin steaks (B); and whole-tissue restructured steaks from Denver leg muscles (C).

boneless lamb legs (Fig. 23.1; Farouk et al. 2002) or boneless hams (Huang et al. 1997) have been restructured to look like intact muscles.

Hot- and Cold-Set Binders

In hot-set restructuring, myofibrillar proteins are extracted by the combined effect of salt, phosphate, and mechanical action to form a surface protein matrix; heat is then applied to set the proteins (Trout and Schmidt 1987). The surface-binding matrix can be created by the use of various starches and proteins, such as milk proteins, soy protein, blood plasma protein, tapioca, and potato starch and carrageenan (Fraser et al. 1993). Other hot-set binders include whey proteins; wheat gluten and surimi; egg white powder; raw egg white; bovine, porcine, lamb, and broiler plasma powders; and gelatine (Chen and Trout 1991; Lu and Chen 1999). The major drawback of the hot-set method is that the product must be marketed pre-cooked and/or frozen.

Several cold-set techniques have been developed to meet the demand for restructured meats that can be sold in the raw chilled state (Clarke et al. 1988; Esguerra 1994; Nielsen et al. 1996). Alginate, a polysaccharide extracted from brown seaweed, can be used for cold-set binding comminuted or diced pieces of meat (Clarke et al. 1988; Al-Joher and Clarke 1993; Schaake et al. 1993; Boles and Shand 1998). Ingredients commonly used in alginate binding systems include alginate salt, a calcium source, an acidulant, and a sequestrant (FMC Biopolymer 2001). Sodium alginate, calcium carbonate, and GDL (glucon-delta-lactone) are the forms of alginate, calcium, and acidulant sources mostly used in meat applications. A thermo-irreversible gel is formed when calcium ions are introduced into an alginate solution. Gelation using alginate-binding system is time and concentration dependent.

Both mammalian and microbial transglutaminase (Tgase, protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) have been used to bind muscle pieces (Akamittath and Ball 1992; Kuraishi et al. 1997). Transglutaminase catalyzes an acyl transfer reaction between the γ-carboxylamide group of a peptide-bound residue and a primary amine (Dickinson 1997). ACTIVA™ (Ajinomoto 2004) TG-S is a transglutaminase preparation for meat processing that is used in many countries for restructuring meat. Activa TG-S is derived from microorganisms and does not require Ca^{2+} for binding activity (Ajinomoto 2004). The optimum temperature and pH for Activa™ transglutaminase are 55°C and 6 to 7, respectively (Ajinomoto 2004). Reviews of transglutaminase catalyzed reactions and use for food processing have been published (Motoki and Seguro 1998; De Jong and Koppelman 2002).

Fibrimex (Harimex Inc., Alberta, Canada) is a blood-based cold binding system that binds meat pieces based on the blood clotting action between fibrinogen and thrombin, resulting in the conversion of fibrinogen to fibrin which cross-link with collagen and gel, thereby binding the meat pieces being restructured (Boles and Shand 1998).

Pearl Meat cold-set binders are a carbohydrate, protein, and bone ash mix. Pearl F is a fine white powder manufactured by Chiba Flour Milling Co. Ltd, Japan, and used to bind seam-boned muscle and large meat pieces (Esguerra 1994), and Pearl E is a protein-active meat binder developed by Earlee Products Qld, Australia, and used in binding odd-sized pieces of raw meat (http://www.earlee.com.au/contact.htm).

Various types of hot- and cold-set binding systems were compared in previous studies using meat from different species of animals. Fraser et al. (1993) compared several hot-set binders, including salt and phosphate, dairy protein, tapioca starch, soy protein, potato starch, and carrageenan, for restructuring whole-tissue lamb products. Alginate, Fibrimex, and Pearl F binding systems were

compared in the whole-tissue restructuring of beef (Esguerra 1994). Gutzke and Tobin (1998) evaluated the use of commercially available cold-set binders, including Pearl F, Alginate, ACTIVA, and Protein Activated Meat Binder in restructuring venison. Farouk (2005b) compared Alginate and Activa in restructuring beef; and Activa and Fibrimex were compared in the restructuring of pork (Flores et al. 2007). Outcomes of these and other studies indicate that the choice of binder depends on the end use of the product and on the processing cost.

Processing of Whole-Tissue Restructured Meats

The three basic methods of restructuring meats include chunking and forming, flaking and forming, and tearing and forming. Excellent reviews of these methods and of the manufacture of UK-style grillsteaks have been previously published (Secrist 1987; Pearson and Gillett 1999; Sheard 2002). In this chapter, the manufacturing of restructured meats from intact muscles or whole-tissue meats is emphasized. In this method of restructuring, grinding, chopping, and emulsification are not used except for the preparation of binders; the raw materials used in the products are section of muscles or muscle strips that are bound by hot- or cold-set binders.

Hot-Set Whole-Tissue Restructuring

The basic steps involved in hot-set restructuring include: (1) raw material selection and preparation; (2) creating a surface protein matrix; (3) molding or shaping; (4) hot-setting of surface protein matrix; (5) portioning; and (6) packaging and storage.

Raw Material Selection and Preparation

All the considerations alluded to in the previous section on raw materials apply here. To reiterate, the choice of raw materials is crucial in the manufacture of restructured meats. Although restructuring is supposed to add value to cheaper cuts, the starting raw materials have to be of high quality in order to be turned into an acceptable end product. An excerpt of the National Meats Groups' raw material specification (Table 23.2) provides a good example of these considerations. National Meats is a New Zealand-based manufacturer of restructured whole-tissue lamb products for retail, H&R, and ready-meal outlets around the world. Their specification clearly shows that very stringent requirements for raw material are imposed on suppliers in order to ensure that the quality and integrity of the finished products are maintained.

If frozen meat is to be used in whole-tissue restructuring, the meat should be tempered to approximately −1.5° to 2°C before use. Sinews, tendons, glands, and excessive amounts of connective tissues and fat should be trimmed from the meat before restructuring (Pearson and Gillett 1999). Depending on the type of whole-tissue restructured meat to be produced, the meat cut may be sectioned, cut into strips of varying sizes, or left intact. If the meat is to be diced into long strips, the cutting should be done along the meat fibers/grains whenever possible. When making steaks, cutting along the fiber is done in order to align the fibers to be perpendicular to the cut surface (Farouk et al. 2005c). High connective tissue cuts could be tenderized using a mechanical tenderizer before restructuring. Mechanical tenderization, also known as blade tenderization or pinning, is the process of physically disrupting the muscle structure by penetrating the meat with closely spaced thin, sharp blades, which disrupt the fibers and sever the connective tissue. This method has been applied to successfully improve the overall tenderness and tenderness variability, especially of lower-value cuts of beef (Jeremiah et al. 1999; Kolle et al. 2004; Pietrasik and Shand 2004; Rosenvold et al.

Table 23.2. Example of raw material specifications. Courtesy National Meats NZ Ltd., Taupo, New Zealand. A manufacturer of restructured whole boneless lamb shoulder roll

1. **SPECIFICATION DETAILS:** Description: Boneless Lamb Shoulder 90VL; Grade: YL-PM grade carcass; State: Chilled
2. **RAW MATERIAL SPECIFICATIONS:** Carcass grade YL-PX (9.0–16.0kg, cold weight). Carcasses must be electrically stimulated within 30 minutes of slaughter, then hung on a cooling floor at 6–10°C for 8 hours before chilling. Marino lamb will not be accepted. Halal certification may be requested.
3. **PREPARATION:** The shoulder is removed from the rack. The boning is done by the shank, foreleg and blade bones being flensed from the rib cage by first marking down either side of the feather bones at the neck end and flensing from the brisket across the ribs to the spine. The shank, foreleg and blade bones are then removed by tunnel boning or slash boning methods. Ensure the shank (including meat) is removed at elbow joint. Remove internal fat and trim excessive outer fat. The prescapular gland and associated fat must be removed. When removing the gland and fat, the knife cut should be continued to remove the adjoining intermuscular fat at the same time. Surface fat must not exceed 5mm thickness. The neck end should be lightly trimmed. All bone pieces, excess fat, paddywack, sinew, blood stains, abscesses, loose pieces, faecal, ingesta, hide, wool fibers and other fine matter must be removed. Product must achieve 90% visual lean content.

   Physical Properties:

Property	Target	Maximum	Minimum	Units	% Within Limits
Chemical Lean	90	95	85	CL	100%

4. **PACKAGING DETAILS:** Bulk packed into lined cartons / bulk bins or vacuum packed (to maximise shelf-life).
5. **DATE REQUIREMENTS:** Slaughter Date; Production Date; Use By Date; Min. Shelf Life at Sale: 3 days (chilled).
6. **LABEL DETAILS:** Minimum requirements—Product description and code; Country of origin; Packers name and address; ME/PH number; Dates of slaughter, production and use by; Traceability bar code; Net weight (Kg); Storage instructions; Visual lean
7. **QUALITY ASSURANCE:**
   Product Defect Tolerance:

Issue	Defect	Tolerance
Foreign Material	Any material other than the natural ingredient e.g. glass, wood, plastic, hair etc.	Nil
Trimming	All bone pieces, excess fat, paddywack, sinew, blood stains, abscesses, loose pieces, faecal, ingesta, hide, wool fibers and other fine matter must be removed.	Nil

   Microbiological standards (log10CFU/g):

Microorganism	Target	n	C	m*	M*
APC (35°C)	<3.63	5	2	4.20	4.92
E.coli	<0.11	5	2	0.70	1.65
Salmonella	Absent	5	0	Absent	Absent

   *Based on 80th and 95th percentiles, respectively, for bulk products, NZFSA National Microbiological Database: National Profile, All data February 1997 to end of April 2006.

   Metal detection

Ferrous	Non-ferrous	Stainless Steel
≤5.0 mm	≤6.0 mm	≤6.35 mm

8. **STORAGE & HANDLING:**
   Storage Temperature: Chilled, ≤4°C (range −1 to 4°C; Delivery Temperature: ≤7°C clean refrigerated vehicle

2006). Enzymes such as collagenase, papain, and ficin have also been used to reduce connective tissue toughness in beef for restructuring (Miller et al. 1988, 1989). Mechanical and enzyme tenderization may have negative effects on the shelf life, color, and drip loss of restructured meats in the raw, and the yield and sensory properties in the cooked states (Miller et al. 1988). Prerigor muscles can be stretched to improve the tenderness of the resultant meat and the restructured whole-tissue products. A muscle-stretching device (Sarcostretch) was used by Farouk et al. (2005a) to stretch prerigor bovine *Mm. semitendinosus*, *semimembranosus*, and *bicep femoris*, and the authors found that the muscles were lengthened by 43% to 97%; overall, stretching improved tenderness, uniformity, presentation, and portion control of the meat, and reduced its drip loss.

Creating a Surface Protein Matrix

In hot-set restructuring using large pieces or intact cuts, the surface protein can be obtained by solubilizing the natural proteins in the meat with salt or by adding nonmeat proteins at the surface. If the natural proteins in the meat are to provide the matrix needed for binding, their extraction can be achieved by mixing, tumbling, or massaging the meat pieces with salt alone or with polyphosphates. The salt can also be sprinkled very lightly on the surface of the muscles without any form of agitation, particularly in restructuring large pieces of cuts with minimum surface-binding area. Some of the equipment used in the extraction process has been discussed by Booren and Mandigo (1987). Extraction of the proteins from meat is affected by, among other factors, the state of rigor development in the meat; the ionic environment and the pH of the system; the temperature history of the meat during rigor onset; the temperature of the mix during extraction; and the age of the meat (King and MacFarlane 1987). Zhang et al. (2005) reported that high pH meat possesses superior functional attributes compared with normal pH meat, regardless of the degree of comminution or storage time. Meat inherently high in pH possesses superior functionality relative to meat whose pH was raised using phosphates (Young et al. 2005). Previous studies in our laboratory indicate that protein extractability generally diminishes over time with some deviations, in that protein extractability in beef and venison stored frozen for 1 month or chilled for 2 to 3 weeks is higher than that of fresh beef just after rigor attainment (Farouk and Wieliczko 2002; Zhang et al. 2005; Farouk et al. 2007; Farouk and Freke 2008). In other words, the optimum time postmortem to maximize the extraction of meat proteins in chilled meat for use in hot-set restructuring is 2 to 3 weeks postmortem. The role of the extracted protein in hot-set restructuring has been discussed in detail by King and MacFarlane (1987). It is widely accepted that myofibrillar proteins, particularly myosin, are responsible for the bind strength of extracted muscle proteins and that sarcoplasmic proteins contribute very little to this process.

The surface protein matrix can also be created by the use of nonmeat protein binders or by the use of pressure, alone or in combination with meat homogenates. The use of pressure is based on the results of a number of studies that indicate that pressure can be used to alter the properties of muscle proteins (Cheftel and Culioli 1997; Colmenero 2002), including increased solubility, aggregation, and gelation of the proteins (Elgasim et al. 1982; Macfarlane et al. 1984). Pressure also affects the physical properties of meat, such as the disruption of myofibrillar structures and increased tenderness and cohesion between meat particles. Farouk and Zhang (2005) described a process of pressure-binding beef steaks and cubes for hot-set restructuring: in this process, semimembranosus muscles were sliced parallel to fiber

length into 2 cm^2 strips; a portion of the mixed strips was minced twice through a 3-mm plate to form a homogenate; the meat strips and homogenate (2.4 kg) were mixed together for one minute and stuffed into a pressure mold with the muscles fibers running along the length of the mold to obtain 12 treatment combinations [3 pressures (1380, 4137, and 6895 kPa) × 4 homogenates (0%, 2.5%, 5%, and 10% wt of meat strips)]. A manual hydraulic press machine was used to apply the required pressures to the mixture of meat homogenate and strips in the mold, and the whole setup was transferred to a −30°C freezer for 3 hours to freeze the molded mixture of meat and homogenate, with the pressure in the mold maintained during the freezing process. The frozen molded meat (logs) were then removed from the mold and sliced into 1-cm thick steaks and 2-cm^3 cubes (sliced to maintain fiber length in one direction) using a band saw. Results obtained using the process indicate that binding of meat pieces for hot-setting could be obtained when pressure ≥1,380 kPa alone was used without binders in restructuring strips of 100 VL beef, which were then sliced into steaks or frozen free-flow cubes; and that the use of meat homogenate using pressure tended to increase bind strength in restructured steaks and cubes.

Regardless of whether the surface protein matrix is inherent to the meat or added from outside sources, sufficient amounts of the binding protein in its optimum functionality should be present at the bind junctions of the meat pieces to provide the binding strength needed to prevent the bound pieces from pulling apart during subsequent processing steps.

Molding or Shaping

Once the surface protein matrix has been created, the meat to be restructured is molded or shaped. The main function of molding is to give the final product its desired shape and to force the pieces of meat into close contact to enable the protein matrix on the surface of the meat pieces to cross-link or bind upon the application of heat. Shaping can be done by forcing the meat pieces into a casing or a mold. The mold could be a simple vacuum bag in which the meat pieces are stuffed and then shaped manually by hand, or a more rigid container made of plastic or metal. Pressure can be applied to the meat pieces during shaping using different press methods, including evacuating the air in the mold using a vacuum machine or by using a hydraulic press in order to improve the contact between meat pieces and the overall binding. Farouk et al. (2005c) used a steel mold and a 240 × 300 mm plastic vacuum bag to shape strips of beef into round and steak-shaped restructured logs, respectively. An important consideration during shaping, particularly when restructuring strips of meat or intact muscles/cuts, is to ensure that the fibers in the meat strips/cuts are aligned in the direction that gives the final product the desired look and texture when portioned. In shaping and deciding on the size of a restructured log, consideration should be given to the method to be used in portioning the final products in order to minimize off-cuts. For instance, the shape and size of a restructured block to be ultimately diced into cubes should reflect the type and the dimensions of the dicer; otherwise, a significant proportion of off-cuts could be generated that may be unable to be reworked.

Hot-Setting/Binding

Heating the protein matrix created on the surface of the meat pieces will coagulate/cross-link the proteins and thereby bind the meat pieces together. The meat pieces should be heated to a final internal temperature of 57° to 68°C to achieve proper binding (Pearson and Gillett 1999). Restructured logs can be hot-set either in their mold or after portioning. To bind the meat pieces together

for portioning and to hold the portioned pieces together for hot-setting, the shaped restructured log should be portioned and cooked while frozen. If the portioned pieces are not held together sufficiently to enable the protein matrix at the bind junctures to be heated to the desired binding temperatures, restructured individual pieces of meat in the portion will fall apart due to shape distortions caused by the shrinkage of the meat proteins. The bind strength at the juncture between meat pieces depends on the nature, amount, and functionality of the protein at the juncture prior to heating. Purslow and his associates (Purslow et al. 1987; Lewis and Purslow 1990; Savage et al. 1990) demonstrated in a series of model studies that the binding of pieces in heated restructured meat was affected by the muscle fiber alignment with respect to the adhesive junction and the size of the meat pieces. The authors observed that the tensile adhesive strength of restructured meat with the fibers at a right angle to the junction in both pieces of meat was three times higher than when one or both pieces of meat contained fibers running parallel to the adhesive junction.

Portioning, Packaging, and Storage of Hot-Set Products

Restructured logs can be portioned into different shapes and sizes using a range of equipment, including slicers, dicers, and saws. The portioning can also be done manually using handheld knives. As mentioned in the previous section, uncooked restructured logs must be deep or surface crust frozen for portioning to maintain the shape of the portioned pieces and to hold the pieces together until hot-set. Restructured logs can be portioned to look like steaks, cubes, or strips like stir-fries. Regardless of the form the portions are to assume, it is important to use appropriate equipment to obtain clean-cut surfaces and to avoid pulling apart the bound pieces in the process. Farouk and Zhang (2005) used a band saw to slice frozen raw restructured hot-set logs of beef into steaks and cubes. When cubes with a more natural rather than mechanically diced appearance are required, frozen logs should be tempered to –2° to –3°C and hand diced using a knife. The portioned pieces can be utilized immediately without the need for further storage, but if merchandized frozen, the raw or cooked portioned pieces can be individually quick frozen and made to free-flow before packaging. The product should be rapidly frozen to ≤–18°C and held at that temperature during storage and distribution (Booren and Mandigo 1987).

Cooked portioned restructured meats can be packed in overwrapped trays or vacuumed and/or gas-flushed in form/fill/sealed pouches, blisters, shrink packs, and skin packs; their frozen counterparts, including steaks, strips, or cubes, can be packaged in bags, pouches, trays, overwraps, and PE-coated paperboards (Harte 1987). In a previous study (Farouk 2001b), hot-set restructured cubes were manufactured from beef clods, boneless lamb, and venison shoulders, and packaged as follows: bulk packaged in a polyethylene bag (to imitate poly-lined cartons); impermeable vacuum bags without vacuum; impermeable vacuum bags with partial vacuum; and impermeable vacuum bags, under a modified atmosphere of nitrogen. They were then stored for one year with no significant deterioration in the physical, chemical, and sensory attributes of the products.

Cold-Set Whole-Tissue Restructuring

The basic steps involved in cold-set restructuring include: (1) raw material selection and preparation; (2) application of cold-set binder; (3) molding or shaping; (4) cold-setting; (5) portioning; and (6) packaging and storage. Steps 1, 3, and 5 are mostly similar to hot-set restructuring. The major difference is that cold-set binding systems do not need

cooking to set. Cold-set products can be sold raw in chilled or frozen forms.

Application of Cold-Set Binders

Ingredients in the cold binding systems are sprinkled and mixed with the meat pieces, rubbed/dusted onto the meat pieces as dry powders, or mixed with water into smooth slurry before mixing with the meat. Regardless of how the binder is applied, it is important to avoid formation of clumps during the application and to make sure surfaces to be bound are coated very well. The ingredients in a binding system may be added sequentially or as a premix. Esguerra (1994) applied three cold-set binding systems as follows: (1) alginate was applied using a fine sieve in order to obtain uniform dispersion of the binder and to avoid pockets in the final product; (2) Fibrimex was mixed with water to form a solution of the binder before mixing with meat; and (3) Pearl F powder was spread evenly on a tray, and meat slices were pressed into the powder. Farouk et al. (2005b) used a premix of alginate binding system (3:3:1, alginate/GDL/$CaCO_3$) and a water solution of Activa to successfully restructure whole-tissue hot-boned beef.

Cold-Setting

Once the cold binder is mixed with the meat pieces and shaped, the molded mix should be held at an appropriate temperature for long enough to enable the binder to set and bind together the meat pieces. An alginate binding system is faster setting than transglutaminase. In order to avoid pre-gelation during processing or before the product is shaped, the calcium source in an alginate binding system is added at the last stage of the process. The setting temperature and time for the commonly used cold-binding systems in meat restructuring is 0° to 4°C and 6 to 24 hours, respectively. Raharjo et al. (1994) held restructured veal steaks using alginate to set at 4°C for 6 hours before freezing. Boles and Shand (1998) restructured beef inside rounds using alginate and Fabrimex™ binding systems, and held the restructured logs from both systems at 4°C for 17 hours to set. Pork rolls restructured with Activa™ and Fibrimex™ binding systems were set at 0° to 4°C for 17 to 18 hours (Flores et al. 2007).

Packaging and Storage of Cold-Set Products

Raw chilled cold-set products can be packaged in overwrapped trays or gas-flushed pouches. With proper packaging and storage temperature, the effective product storage life of whole-tissue restructured products can be similar to their whole-tissue nonrestructured counterparts. Bell et al. (1994) restructured beef steaks using Pearl-F™ and alginate binding systems, determined the chilled product life of the raw restructured steaks under vacuum or carbon dioxide, and found the following: (1) neither the alginate nor the Pearl binding system contributed significantly to the initial microbial contamination or influenced the development of spoilage microflora on the restructured steaks; (2) if products were stored at 3°C, carbon dioxide packaging gave little advantage in respect to restricting spoilage microflora development relative to vacuum packaging; (3) if storage was at –1.5°C, carbon dioxide was better than vacuum packaging in limiting the number and composition of spoilage microflora developing on the stored products; (4) when stored at –1.5°C, Pearl restructured steaks had sufficient microbiological, color, and flavor stability for the retail market, while alginate restructured steaks packaged under CO_2 and stored at –1.5°C had a rather limited color stability and consequently appeared to be more appropriate for hotel, restaurant, and institutional (HRI) trade than for retail distribution; (5) the effective product storage life at –1.5°C under carbon

dioxide was 63 and 105 days for alginate and Pearl bound products respectively.

Frozen portioned, raw, cold-set restructured steaks can be packaged in bags, pouches, trays, overwraps, and paperboards similar to their hot-set counterparts.

Formulations for Restructured Whole-Tissue Meats

Generalized procedures for the manufacture of restructured whole-tissue meats using hot- and cold-set binding systems are shown as flow diagrams in Figures 23.3 and 23.4. The procedures have been simplified to show the fundamental steps involved. The manufacture can be up-scaled using various types of equipment, and ingredients can be added to impart desired attributes to the final product. Binders can be used singly or in combination to achieve a required level of bind; cold- and hot-set binders can even be used together to reinforce the binding effect. For instance, in hot-set restructuring, Activa may be added to increase the bind strength of the protein matrix extracted during mixing with salt. In this chapter, the alginate and transglutaminase (Activa™) cold binding systems are discussed to provide examples of two methods of cold binding involving gelation and enzymatic cross-linking.

An example of a large-scale manufacture of whole-tissue restructured meat is that of Bernard Matthews New Zealand (BMNZ, 2008). Bernard Matthews New Zealand is an added-value further processor and marketer

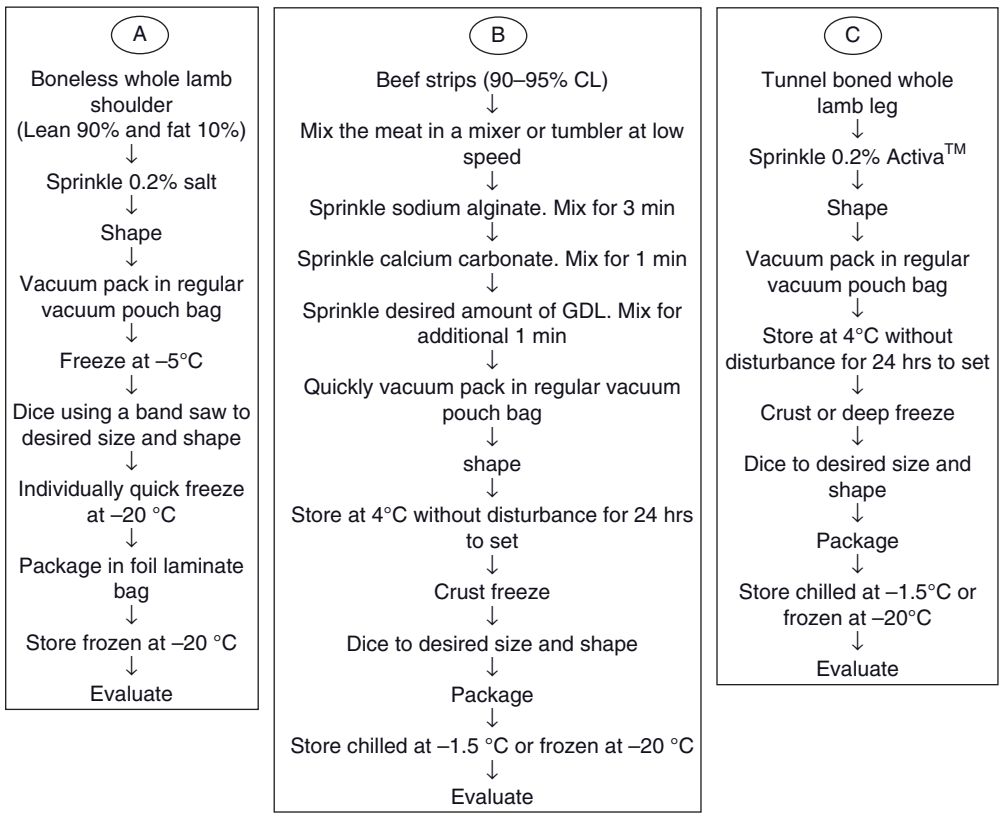

Figure 23.3. Flow diagrams of whole-tissue restructuring using salt as hot-set binder (A), Alginate (B), and Activa™ (C) cold-binding systems.

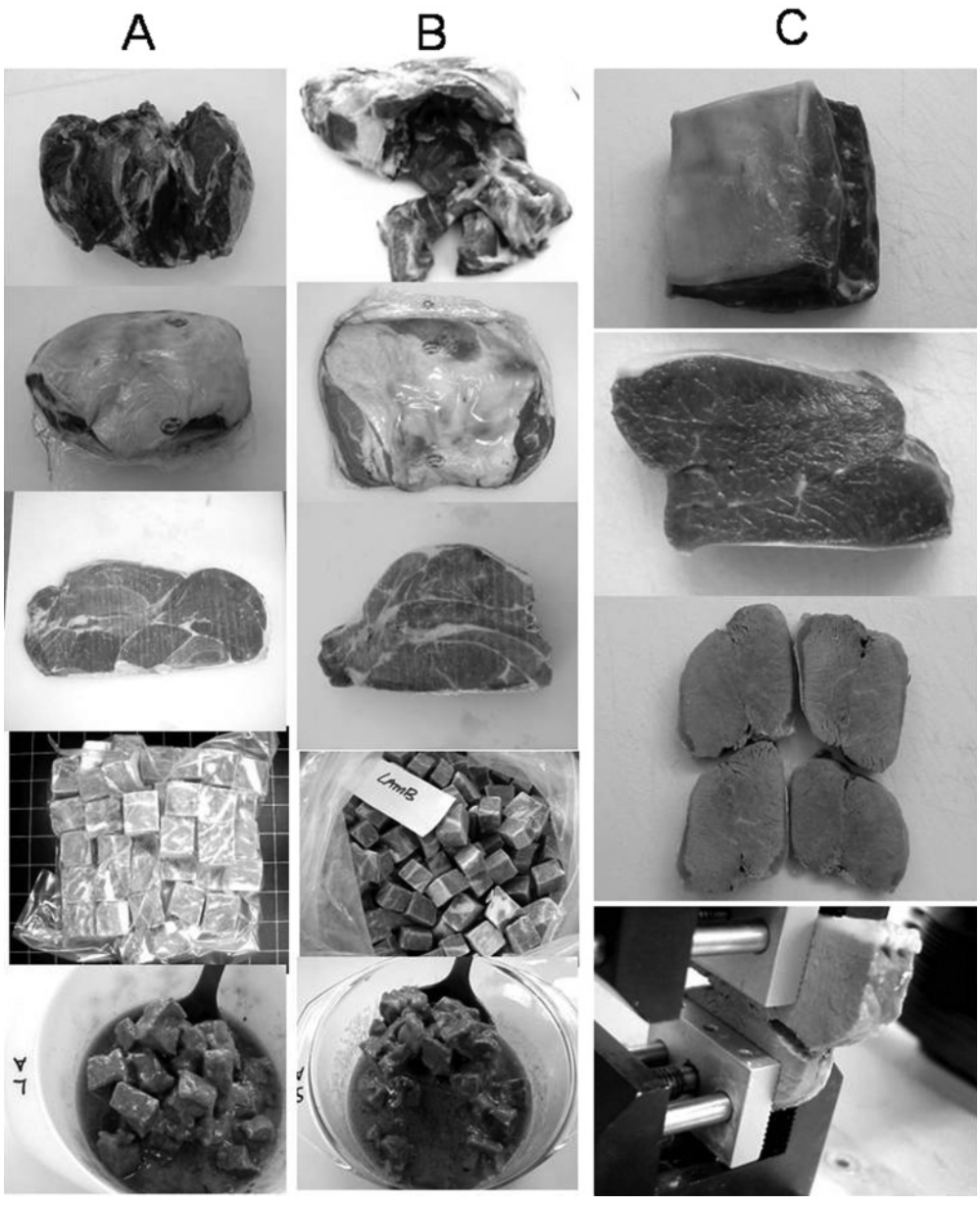

Figure 23.4. Top to bottom = pictorial flow chart of hot-set restructuring of whole boneless lamb leg (A) and shoulder (B) to obtain free-flow cubes; C = Bind strength measurement of restructured lamb loins.

of New Zealand lamb. The company produces a restructured whole-tissue lamb product branded as a Lamb Medallion using a hot-set binding process. Bernard Matthews New Zealand was aligned with the UK-based food company, Bernard Matthews, which holds a patent (Matthews et al. 1989) for the process used to produce the Lamb Medallion. The patented process involves the continuous manufacturing of a restructured lamb product

comprised of a lean meat core and a fatty outer layer through the following steps: (1) slicing whole-muscle meat into small, thin slices (the slices should be held just below 0°C); (2) the thin slices are then agitated and mixed with a hot-set binder (meat-based adhesive liquid) for about two minutes; (3) the binder-coated slices are then loaded into a coextrusion machine and pumped through to the extrusion head; fat-forming fluid (emulsified mixture of fat and meat) is also loaded into the extruder and pumped to another region of the extrusion head; (4) the coated slices and the fat-forming fluid are coextruded in the desired shape onto a moving conveyor; (5) the coextrudate is then conveyed through an elongate freezer to be crust frozen into a partially frozen log that is cut into pieces of desired thickness; (6) the sliced pieces are then finally frozen.

Quality Problems of Restructured Whole-Tissue Meats

The common objective of restructuring is to achieve a product that not only imitates but also possesses the attributes of a whole-tissue product, including: aesthetic-related attributes (color, appearance, and overall visual appeal); oral-related attributes (texture and tenderness); and other sensory related attributes (flavor and aroma/odor).

Aesthetic-Related Quality Problems

Color and appearance are important factors in consumers' point-of-purchase decisions. An undesirable color reduces the acceptability of restructured meats and is a major problem for manufacturers (Hunt and Kropf 1987). The factors that affect the color of restructured meats include: (1) raw material condition, such as the oxidative-reductive state of the meat used in restructuring, meat pH, and microbiological condition; (2) ingredients, such as the level of salt and its negative effect on color, and phosphates and their beneficial effect on color; and (3) processing factors, such as boning time (hot versus cold), rigor state (pre- versus postrigor), particle size reduction, blade tenderization, temperature, pressure, and packaging (Hunt and Kropf 1987). Color problems in restructured meats are related to the method of restructuring and the type of binder used. The color and overall appearance of raw slices of beef rolls restructured using Activa™ binding system were preferred over those restructured using the alginate binding system (Farouk et al. 2005b). Color and appearance problems may not be as important in hot-set restructured products, since they are likely to be sold in cooked or frozen form compared with cold-set restructured products that could be sold uncooked and/or chilled. For instance, consumers in a previous study found the color of cooked hot-set restructured roast beef prepared from nonuniform colored raw material (including prerigor, postrigor, grass-, and grain-finished beef) equally acceptable (Farouk and Swan 1997). The use of nonuniform-colored meats, as in the study by Farouk and Swan (1997), or mixing high- and normal-pH meats or muscles with predominantly white fibers and those with red fibers, or restructuring meats with fibers aligned at different directions to the cut surface of steaks, can all result in the reduced acceptability of cold-set raw restructured meat products. The appearance of the steaks shown in Figure 23.5 is a good example of an aesthetic-related problem. The cooked beefsteak does not look natural because of the cross striations on the steak caused by the alignment of muscle fibers parallel instead of perpendicular to the surface of the steak during restructuring. The offal steak had a mottled appearance and looked like a jigsaw puzzle, due to the use of raw materials of nonuniform texture and color during restructuring.

The effect of fiber alignment on the appearance of restructured steaks was demonstrated by Farouk et al. (2005c) in a study in which raw restructured beef steaks with

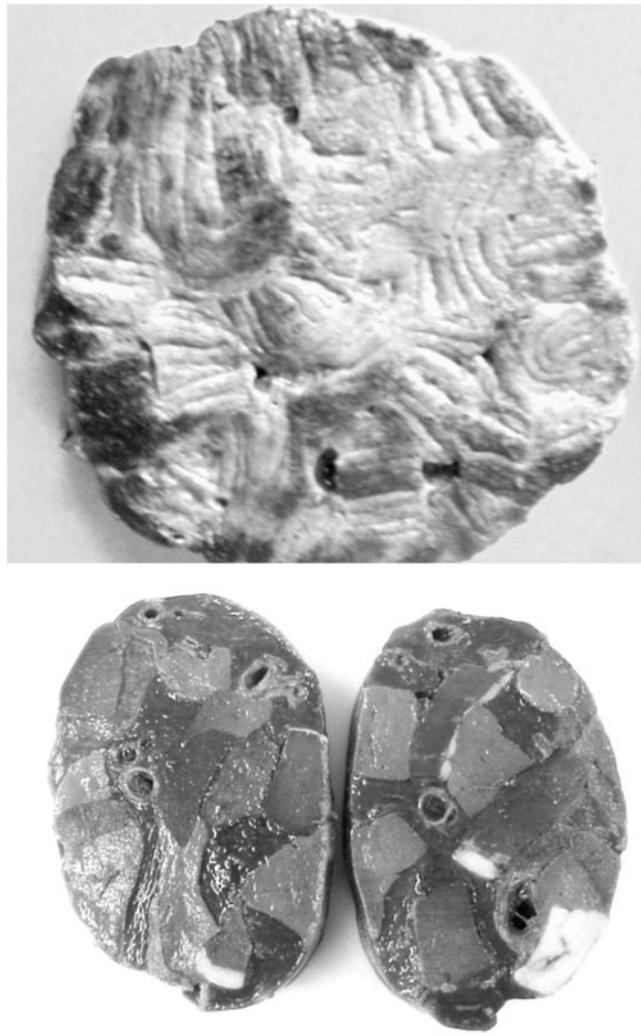

Figure 23.5. Aesthetic-related appearance problems in whole tissue restructured cooked beef steak (top) and raw offal steak (bottom), depicting the improper alignment of muscle fibers and the nonuniformity of meat pieces during restructuring, respectively.

fibers aligned parallel to the cut steak surface were ranked highest in acceptability by a consumer panel compared with steaks with fibers aligned perpendicularly or mixed; the panelists ranked the raw steaks in the following order of acceptability: parallel > perpendicular > equal mixture of parallel and perpendicular. The panelists' comments indicated the reasons for their choice of the most preferred raw steak were influenced by the color and the appearance of the cut steak surface. Five of the representative comments used by the panelists for the most preferred raw steak with fibers parallel to the cut surface include "most uniform appearance," "the most consistent color," "least obviously restructured," "looks more natural," and "closest to real meat." The five comments for

the least preferred raw steak with mixed fiber alignment include "too patchy," "looks like a mosaic," "mottled appearance and chunky looking," "looks like a jigsaw," and "more obviously processed/processed looking." The panelist ranking of the visual acceptability of the restructured steaks changed on cooking. In cooked steaks, those with fibers running parallel to the cut surface of the steak were ranked lowest compared with the perpendicular and mixed steaks, which did not differ in visual appeal. The reason for the change in the visual appeal of the parallel steaks from being the highest ranked in the raw state to the lowest in the cooked state is because, in the raw state, color had more influence on the decision of the panelists, as it was more difficult to see the direction of the fibers in relation to the cut steak surface. However, due to the shrinkage and the thickening of the fibers in the cooked steaks, the effect of the fiber alignment became more obvious and the parallel steaks lost their natural-look appeal. The comments of the panelists regarding the cooked parallel steaks include "the least natural," "looked patchwork," "looks very restructured," "slightly strange," or "lots of crossed grain bits" (see Fig. 23.5).

Other visual appearance issues may arise from the poor dispersion of binders during restructuring. Esguerra (1994) and Mikkelsen and Esguerra (1996) restructured beef steaks and cubes using alginate and found that undissolved encapsulated acid appeared as small white spots in the steaks, and poorly dispersed alginate appeared as red gel spots in raw chilled or thawed restructured cubes.

Texture/Tenderness

The texture of restructured whole-tissue steaks should simulate that of a real steak; otherwise, they will be rejected by the consumer. Some of the common terminologies used by consumers to describe texture problems in restructured meats include rubbery, spongy, soft, mushy, crumbly, chewy, loose, and aerated (Mikkelsen and Esguerra 1996; Flores et al. 2007).

According to Berry (1987), texture problems of restructured meats may be related to excessive or insufficient bind, lack of uniformity of texture, excessive connective tissue, distortion of cooked product, excessive crust formation, layering, and/or formation of pockets inside the product during cooking. Sheard (2002) suggested three factors might affect the eating quality of restructured meats: (1) the nature of the meat pieces' orientation and composition (such as their size, shape, surface morphology, and fiber direction); (2) the amount and composition of the surface protein matrix; and (3) the relative proportion of the meat pieces to the surface matrix. Boles and Shand (1998) determined the effect of particle size on the acceptance of restructured beefsteaks produced using alginate-binding systems and found that particle size had no effect on the consumer acceptability of the texture of the restructured steaks. Flores et al. (2007) found no effect of binders on the consumer acceptability of the texture of pork restructured with Activa™, Fibrimex™, or phosphates. However, Esguerra (1994) reported that alginate-bound steaks were more tender than Pearl F-bound steaks. Farouk et al. (2005b) also reported that consumers preferred the tenderness of beef rolls restructured using alginate binding system relative to Activa™-bound ones. Previous studies indicated that muscle fiber alignment in whole-tissue steaks from different species of animals affected the texture of the steaks measured objectively or subjectively (Guenther 1989; Poste et al. 1993; Otremba et al. 1999). Results of these studies show that cooked intact whole-tissue meat samples sheared longitudinal/parallel to the direction of meat fiber or masticated with the grain were more tender (lower shearforce values) than those sheared tranverse/perpendicular to fiber direction or masticated across the grain. Purslow and his associates (Purslow

et al. 1987; Lewis and Purslow 1990; Savage et al. 1990) demonstrated in a series of model studies that the texture of cooked restructured meat was affected by: the muscle fiber alignment with respect to the adhesive junction; the degree of adhesion between meat pieces; and the size of the meat pieces in the restructured meat. Farouk et al. (2005c) cold-set restructured beefsteaks with the meat fibers aligned parallel, perpendicular, or an equal mixture of parallel and perpendicular (mixed) in relation to the cut steak surface. The authors subjected the steaks to sensory evaluation and found that consumers preferred the texture and tenderness of the steaks with fibers running perpendicular or an equal mixture of parallel and perpendicular to the face of the steaks compared with those with fibers running parallel only. The steaks with fibers running parallel also ranked significantly lower than the others (perpendicular or mixed) in overall eating quality.

Flavor and Odor

One of the major causes of deterioration in the flavor of restructured meats is lipid oxidation. There are a number of terms, such as "stale," "rancid," "musty," and "barnyard," used to characterize oxidized flavor and odor. The oxidized or rancid flavor that develops rapidly during refrigerated or frozen storage of precooked or partially cooked meat products or meats in which the membranes are broken down such as in restructuring is termed warmed-over flavor (Pearson and Gray 1983). Love (1988) reported that warmed-over flavor can develop in fresh meats. According to Gray and Pearson (1987), lipid oxidation and warmed-over flavor development in restructured meats is influenced by the raw materials used in restructuring, reduction in particle size, and cooking and/or heating of the product. Undesirable flavors and odors can also arise due the ingredients and additives used in restructuring. The use of whey protein, wheat gluten, soy protein isolates, carrageenan, and tenderizing enzymes in meat restructuring has been reported to affect the finished product flavor (Miller et al. 1988; Chen and Trout 1991; Demos et al. 1994). Fraser et al. (1993) used a variety of hot-set binders in restructuring lamb roast and found that undesirable flavors increased with increased storage time. Esguerra (1994) reported the presence of slight liver-like foreign flavors in beefsteaks restructured using Fibrimex™. Similarly, Flores et al. (2007) reported that the flavor of pork restructured with Activa™ and Fibrimex™ were described by some consumers as having a "bad after-taste" or "liver-taste off-flavor," and one consumer in particular described the flavor as having an "iron flavor" or "strong pig flavor." The presence of high numbers of spoilage microorganisms will lead to the development of off-odors in chilled restructured meat products before any flavor changes are detected by the consumer (Kotula et al. 1987).

Improving Product Quality

There are a number of ways to improve the quality of restructured meat products. The use of any process or additive must be balanced with the need to maintain the overall quality of the final product and not just a few attributes at the expense of the others. Factors such as cost, potential risk to health, environmental effect, and even carbon and energy footprint should be considered while deciding on ways to improve product quality.

Visual Appeal

For whole-tissue restructured steaks to have the appearance that closely resembles that of a real steak, the muscle fibers/fiber bundles in the restructured steaks should be aligned (Guenther 1989), and the color and other visual attributes of the meat should be as uniform as possible. To achieve this, the meat fibers/fiber bundles should be aligned

so that the fibers are perpendicular to the cut steak surface. This is very important, as the aim of the restructuring is to produce restructured steaks that imitate steaks from the more expensive cuts, such as the cuberoll, striploin, or tenderloin, especially when the consumer will have the opportunity to view the cooked steak before consumption. A number of the muscles in the fore- and hind-quarters of carcasses, with fibers running parallel to the length of the muscle or with mixed fibers, could be used to produce acceptable looking steaks (Farouk et al. 2002). The use of larger pieces of meat or intact muscles will improve the appearance of restructured meats relative to the use of smaller-sized meat pieces. To minimize distortion of cooked whole-tissue restructured products, muscles skinned of surface connective tissue should be used or high connective tissue cuts should be tenderized using a mechanical tenderizer before restructuring.

Oral Appeal

The texture and tenderness of restructured whole-tissue meats can be improved by reducing the amount of connective tissue in the meat to be restructured. Berry et al. (1988) restructured beefsteaks to have extra-high, high, or low levels of connective tissue and subjected the steaks to consumer sensory analysis, and found that the texture and toughness of extra-high connective tissue steaks was undesirable. Texture and tenderness can be improved by aligning the muscle fibers or fiber bundles to be perpendicular or a mixture of 50:50 perpendicular and parallel fibers relative to the face of the steak (Farouk et al. 2005c). The use of enhanced meat in hot-set restructuring will improve tenderness and juiciness of the final product. The injection of beef with up to 20% marinade (mostly a solution of salt and phosphates) to enhance tenderness and palatability is becoming a common practice in many countries (Maca et al. 1997; Vote et al. 2000; Hoogenkamp 2003; Robbins et al. 2003). Vote et al. (2000) injected intact U.S. choice and select strip loins with up to 15% solution containing phosphate, lactate, and chlorides, and then subjected the steaks to sensory evaluation using a trained panel, and found that injecting the loins with the solution improved the tenderness, juiciness, and cooked beef flavor. In a more recent study, Robbins et al. (2003) injected strip loins and rounds up to 10% with a solution containing sodium tripolyphosphates and sodium chloride, then evaluated the steaks using a consumer panel, and found that the injected steaks were more acceptable than the controls. The addition of 3% or 8% water in restructured beef cubes formulation improved the tenderness and texture of the cubes significantly over that of control with no added water (Mikkelsen and Esguerra 1996).

Flavor/Aroma Appeal

The control of flavor deterioration in restructured meats due to lipid oxidation can be accomplished to varying degrees of success by using chemical compounds such as antioxidants and chelating agents, as well as by the exclusion of oxygen. The following were shown to inhibit or retard oxidation: EDTA and ascorbic acid (Liu and Watts 1970); 156 ppm nitrite, 0.5% tripolyphosphate, and 2% EDTA (Igene and Pearson 1979); extract of eggplant tissue and yellow onion peels (Younathan et al. 1980); catechol, EDTA, DTPA, sodium polyphosphate, and sodium tripolyphosphate (Shahidi et al. 1986); rice bran oil (Kim et al. 2000); the aqueous extract of rosemary, sage, and thyme (Mielnik et al. 2008); grape seed extract and pine bark extract (Ahn et al. 2002); and Chinese five-spice ingredients composed of cinnamon, cloves, fennel, pepper, and star anize (Dwivedi et al. 2006). Reverte et al. (2003) and more recently Stika et al. (2008) added

propyl gallate and a beef flavoring in the formulation of restructured beef steaks from forage- and grain-fed cattle and matured cows respectively, and demonstrated that the strong grassy flavor of forage-finished beef steaks detected by a sensory panel was masked by the beef flavoring agent, thereby improving consumer acceptance of the restructured steaks. The use of propyl gallate retarded lipid oxidation and the development of rancid flavors in restructured steaks from matured cows, but was unable to overcome the low acceptability of steaks with inherent off-flavors. The use of propyl gallate in combination with the beef flavoring agent helped mask mature forage-fed off-flavors.

Future Trends

To achieve a more natural look in restructured whole-tissue products, meat fibers must be aligned so that they are perpendicular to the face of the steak. Currently, the alignment is achieved manually, and we are not aware of any equipment that automates the process. If the process of fiber alignment during restructuring can be mechanized, it will reduce the cost of producing restructured meat products and help the industry to capture the current trend in the increased consumption of steaks and the demand for convenience by the consumer. Meat and Livestock Australia has taken out a provisional patent on a "meat strip alignment technology" and indicated in their 2006–07 annual report (MLA 2008) that they were trialing the technology in conjunction with an Australian added-value meat processor. The outcome of this trial has not yet been reported in the public domain for the technology to be assessed.

Cold-set restructured whole-tissue meats currently have to be crust or deep frozen before fabrication in order to get a clean-looking steak surface or shaped product, and also to avoid breaking the bind between meat pieces. Research into technologies that can slice chilled, raw, restructured meats should be undertaken. Success in this regard will ensure better appearance for restructured products and a saving in cost.

Current trends indicate consumers are demanding more natural products with no additives of any kind if possible. This challenge should be taken up by the industry and researchers to produce restructured whole-tissue meats that meet this requirement. There are indications (Farouk and Zhang 2005) that pressure alone without any binder can be used to create enough surface protein matrices on whole-tissue meat to achieve reasonable bind strength on hot-setting. More research should be done to explore this and other possibilities of achieving binding of whole-tissue meats without the use of external binders.

Enhancement of meat and the stretching of prerigor muscles are recent techniques employed to improve the eating quality and consistency of meat from whole-tissue muscles. Research should be done to determine if these techniques can be used to improve the texture and tenderness of the raw materials used for whole-tissue restructuring.

The problem of warmed-over flavor and aroma in restructured meats has been recognized for a long time. The use of natural antioxidants to control warmed-over flavor and lipid oxidation is promising. However, research that seeks to combine natural antioxidants and packaging to solve flavor and odor issues in whole-tissue restructured meats is not being given the attention it deserves and should be encouraged.

More whole-tissue restructured meats are produced in shapes and sizes that are different from the traditional steak products. For example, restructured free-flow individually quick-frozen cubes from whole muscles or boneless cuts are manufactured for use in ready meals and stroganoff-type products.

While objective methods of measuring the binding strength of steak-type restructured products have been developed, it appears that little research effort is going into developing methods to evaluate the new products that are being produced. This work is needed for the proper quality control of these products.

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Chapter 24

Functional Meat Products

Keizo Arihara and Motoko Ohata

Introduction

Since meat contains an abundance of proteins with high biological value, meat is categorized with fish and eggs as a protein food group in dietary food guides (Lachance and Fisher 2005). In other words, in terms of nutrition, meat is an excellent diet source of essential amino acids. Meat also plays an important role in supplying our diet with minerals and vitamins, such as iron, zinc, selenium, and B vitamins (Mulvihill 2004; Biesalski 2005). As well as these basic nutritional components, studies have revealed that meat contains several bioactive compounds, such as conjugated linoleic acid, carnosine, and L-carnitine (Williams 2007; Arihara and Ohata 2008).

However, consumers often associate meat and meat products with a negative health image. This regrettable image of meat is mainly due to its content of fat, saturated fatty acids, and cholesterol, and their association with chronic diseases, such as cardiovascular diseases, some types of cancer, and obesity (Chan 2004; Ovesen 2004a, 2004b; Fernández-Ginés et al. 2005; Valsta et al. 2005). Also, intake of sodium chloride from meat products has been linked to hypertension (Ruusunen and Puolanne 2005). Such a one-sided view concerns us, since it ignores the important role of meat in the maintenance of human health. In addition to accumulation of scientific evidence, there is a need to inform consumers of the exact nutritional value of meat and meat products.

The most important factor for evaluation of foods is their "primary" function (i.e., their role in providing standard nutrient components). The "secondary" function of foods, which is defined in terms of sensory properties such as taste, flavor, appearance, and texture, is also important for consumers and the food industry. In addition to these basic functions of foods, the "tertiary" function of foods has attracted considerable attention due to increasing concerns about health in developed countries (Heasman and Mellentin 2001; Dentali 2002; Sloan 2008). Tertiary functions are the roles of food components in preventing diseases by modulating physiological systems. Examples of tertiary functional properties are antioxidative, antihypertensive, anticarcinogenic, immunomodulating, and antiaging activities. Foods utilizing or emphasizing such tertiary functions are regarded as "functional foods."

Although there has been extensive research and development of functional foods in the dairy industry (Chandan 2007; Chandan and Shah 2007), little attention has been paid to functional meat products until recently. However, efforts have been directed in recent years to research of functional meat products (Jiménez-Colmenero et al. 2001, 2006; Arihara 2004, 2006a, b; Fernández-Ginés et al. 2005; Jiménez-Colmenero 2007a, b; Arihara and Ohata 2008). Since meat products are important in the diet, the development of novel healthier meat products will contribute to human health. This chapter provides overviews of functional

foods, functional meat products, and bioactive compounds in meat. The development of novel functional meat products is also discussed.

Functional Foods and Functional Meat Products

Functional Foods

Although there is no universal definition of "functional food," a typical and simple definition is "processed foods having disease-preventing and/or health-promoting benefits in addition to their nutritive value" (Arihara 2004). The term functional food was coined in Japan in the early 1980s (Arihara 2006b). Japan is also the first country to have formulated a specific regulatory approval process for functional foods. In 1991, the concept of foods for specified health use (FOSHU) was established by the Japanese Ministry of Health and Welfare. FOSHU are foods that, based on the knowledge of the relationship between foods or food components and health, are expected to have certain health benefits and have been licensed to bear a label claiming that a person using them may expect to obtain that health use through the consumption of these foods. The majority of FOSHU products utilize functional ingredients to maintain the health of the human body. Such functional food ingredients are listed in Table 24.1. As of August 2009, 894 FOSHU products have been approved in Japan. The market scale of FOSHU in Japan in 2007 was about seven billion US$.

Regulations for functional foods have not yet been established in many countries. International considerations of functional foods were reviewed by Fitzpatrick (2007). Also, a recent book (Bagchi 2008) provides the regulations of functional foods in many countries, including the United States, European Union, Australia, and Japan.

Functional Meat Products

Numerous low-fat or fat-free meat products have been developed in many countries, with the United States at the head of the list (Jiménez-Colmenero et al. 2006). Recently, sugar-free meat products, such as roast ham and sausages, have been developed in Japan (Fig. 24.1). In addition to these "free" and

Table 24.1. Representative food ingredients used for FOSHU products

Health claims	Functional food ingredients
Intestinal disorder	Oligosaccharides Xylo-oligosaccharide, Fructo-oligosaccharide, Galacto-oligosaccharide, Lactulose, Raffinose Dietary fiber Indigestible dextrin, Indigestible starch, Sodium alginate, Corn fiber Bacterial strains *Lactobacillus* sp., *Bifidobacterium* sp., *Propionibacterium* sp., *Bacillus subtilis*
Cholesterol level	Vegetable sterol, Vegetable steroid ether, Soy protein, Sodium alginate, Chitosan, Catechin
Blood pressure level	Peptides Casein dodeca peptide, Sardine peptide, Isoleucyl tyrosine, 4-Aminobutyric acid
Mineral (Ca/Fe) level	Caseinphospho peptide, Calcium citrate malate, Heme iron
Bone density	Soy isoflavone, Milk basic protein, Vitamin K2, Polyglutamic acid
Dental caries	Xylitol, Maltitose, Palatinose, Tea polyphenol
Blood sugar level	Indigestible dextrin, Arabinose, Wheat albumin
Blood neutral fat level	Diacylglycerl, Globin hydrolyzate, Catechin

Figure 24.1. Sugar-free meat products "Zero" (Nippon Meat Packers, Inc., Japan). Left, sliced roast ham; right, sliced half bacon.

"low" type of products, meat products with additional physiologically functional properties have been introduced in some countries. Such functional ingredients, including vegetable proteins, fibers (e.g., oats, sugar beet, soy beans, apples, peas), antioxidants, and probiotics (intestinal *Lactobacillus* and *Bifidobacterium*), have been utilized for meat products (Jiménez-Colmenero 2007a; Fernández-Ginés et al. 2005; Jiménez-Colmenero et al. 2006). Research articles about meat and meat products with functional ingredients have been summarized by Fernández-Ginés et al. (2005). Other functional food ingredients have also been reviewed (Playne et al. 2003; Chandan and Shah 2007; Jackson and Paliyath 2007).

Dietary fibers and soy proteins have been utilized as functional ingredients in FOSHU meat products in Japan (Arihara 2004). For example, pork sausage products containing indigestible dextrin, a water-soluble dietary fiber prepared from potato, are claimed to have beneficial effects on intestinal disorders. Another product is a sausage containing soy proteins. It is claimed that acceptable blood cholesterol levels can be maintained by consuming this product. In addition to the approved FOSHU products, meat products with additional functional food ingredients, such as fibers, vegetable proteins, and minerals (e.g., calcium), have been developed in Japan. Soy proteins are popular vegetable proteins for their various health-enhancing activities (e.g., prevention of cardiovascular diseases, cancer, and osteoporosis). A sausage with additional potato starch was developed in the United States (Pszczola et al. 2002). Such dietary fibers improve intestinal microflora as prebiotics, as described in a later section, and they contribute to the reduction of fat intake. Healthier lipid formulation is also a critical approach for developing meat-based functional foods. Technological options for replacement of meat fats with various nonmeat fats (i.e.,

plant and fish fats) were reviewed extensively by Jiménez-Colmenero (2007b).

Although meat is less allergenic than common allergy-inducing foods, such as milk, eggs, and soy (Tanabe and Nishimura 2006), meat products (e.g., sausages) often contain vegetable, egg, and/or milk proteins. People with allergies are often affected by allergens in such ingredients. A series of meat products named Apilight (including sausages, hamburger steak, and meat balls, Fig. 24.2) are beneficial for such people. These products are made with a formulation that eliminates ingredients causing allergic symptoms and have been approved as allergen-free products by the Japanese Ministry of Health and Welfare. Also, gluten-free and/or lactose-free meat products have been produced in some countries (Jiménez-Colmenero et al. 2006). On the other hand, there is increasing evidence that even meat can cause allergic symptoms in sensitized patients. In Japanese children with food allergies, the prevalence of chicken allergy was the highest (4.5%) among various meats (Iikura et al. 1999). It has also been reported that beef allergy occurs with an incidence of 3.3% to 6.5% in children with atopic dermatitis (Fiocchi et al. 2000). Heat and enzymatic treatments have been shown to be effective methods for reducing the allergenicity of meat allergens (Tanabe and Nishimura 2006). It has also been shown that antigenicity of beef proteins can be changed by high-pressure treatment (Han et al. 2002).

Approaches for Designing Functional Meat Products

Various possible strategies for developing healthier meat and meat products, including functional foods, are listed in Table 24.2. All aspects of animal production and product processing have to be considered for devel-

Figure 24.2. Allergen-free meat products "Apilight" (Nippon Meat Packers, Inc., Japan). Left, hamburger steak; right, meatballs.

Table 24.2. Diverse possible strategies for developing healthier meat and meat products

1. Modification of Carcass Composition
2. Manipulation of Meat Raw Materials
3. Reformulation of Meat Products
Reduction of contents (e.g., fat)
Modification of components (e.g., fatty acid)
Addition of functional ingredients

oping functional meat products. As described later, through modification of animal feed, the composition (e.g., conjugated linoleic acid) of animal products can be improved. Also, the functional characteristics of meat products can be changed by introducing food ingredients considered to be beneficial for health or by eliminating components that are considered harmful. Possible approaches for functional modification in meat products suggested by Fernández-Ginés et al. (2005), Jiménez-Colmenero et al. (2006), and Jiménez-Colmenero (2007a) are summarized in Table 24.3.

Table 24.3. Possible approaches for functional modification in meat products

Control and Reduction
Sodium chloride
Fat
Cholesterol
Allergens (vegetable & egg proteins)
Biogenic amines
Modification
Fatty acid (selection of breeds)
n-6 : n-3 PUFA (linseed feed)
Addition
Vegetable oils
Fish oils
Conjugated linoleic acid
Plant-based proteins (soy protein)
Natural extracts
Vitamins C & E
Minerals (Ca,Se,Fe,Mg,Mn)
Plant sterols
Phytate
Probiotic lactic acid bacteria
Dietary fiber
Oligosaccharides
L-carnitine

Bioactive Compounds in Meat

Utilizing or emphasizing physiological activities originating from meat is a promising approach for developing functional meat products. Attractive meat-based bioactive substances have been studied for their physiological properties. Such substances include conjugated linoleic acid (CLA), histidyl dipeptides (carnosine and anserine), L-carnitine, glutathione, taurine, coenzyme Q10, and creatine. These bioactive compounds in meat have recently been reviewed by Arihara and Ohata (2008).

Since studies have shown that feeding conditions of animals affect the contents of CLA and L-carnitine in meat (Krajcovicova-Kudlackova et al. 2000; Mir et al. 2004), healthier meat and meat products could be created through modification of animal feed. As representative meat-based bioactive compounds, CLA and histidyl dipeptides are described here.

Conjugated Linoleic Acid

Conjugated linoleic acids (CLAs) have attracted considerable attention as nutraceutical compounds found in foods. CLAs were initially found in cooked ground beef by Pariza et al. (1983). Later, they were found in the meat and milk of ruminants (Gnadig et al. 2000; Watkins and Yong 2001; Nagao and Yanagita 2005). CLA is most abundant in the fat of ruminant animals, such as cattle and sheep, since CLA is converted from linoleic acid by the isomerase of rumen bacteria. CLA produced in the rumen is transported to muscles and mammary tissue of animals. For example, beef fat contains 3.1 to 8.5 mg CLA per g of fat (Hasler 1988).

CLA is composed of a group of positional and geometric isomers of octadecadienoic acid. The most common CLA isomer found in beef is octadeca-*c*9,*t*11-dienoic acid (Fig. 24.3, top). Much attention has been paid to

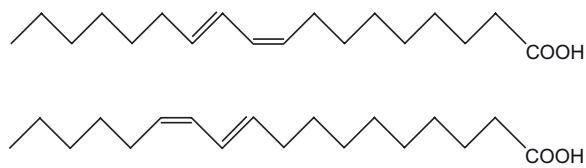

Figure 24.3. Structures of conjugated linoleic acid isomers. Top: *c*9, *t*11-isomer; bottom: *t*10, *c*12-isomer.

this CLA isomer due to its anticarcinogenic activity. Commercially available supplements utilized for many studies are usually mixtures of several CLA isomers (e.g., *c*9,*t*11: 41%; *t*10,*c*12: 44%; *t*9,*t*11/*t*10,*t*12: 7%). Some studies have shown that the *t*10,*c*12 isomer (Fig. 24.3, bottom) exhibits stronger physiological activities than those of the *c*9,*t*11-isomer. However, most animal products such as beef and cow's milk contain only trace amounts of the *t*10,*c*12 CLA isomer.

The CLA content of animal products is changed by several factors, such as breed, age, and feed composition (Dhiman et al. 2005). CLA content in grass-fed animal products is more than three times greater than that in products from animals fed a diet of 50% hay and silage with 50% grain. CLA content was also reported to be higher in beef from cattle fed a diet containing soy oil (Lorenzen et al. 2007). CLA content of foods is increased by heat treatments, such as cooking and processing (Herzallah et al. 2005). Also, lactic acid bacteria promote the formation of CLA. The effect of lactic acid bacteria on the formation of CLA in media and fermented dairy products has been studied (Alonso et al. 2003; Coakley et al. 2003; Sieber et al. 2004; Xu et al. 2005). Such bacterial conversion would be expected in fermented meat products.

Epidemiological studies have suggested that high intakes of high-fat dairy foods and CLA may reduce the risk of colorectal cancer (Larsson et al. 2005). Besides anticarcinogenic activities, CLA has anti-artherioscleroticic, antioxidative, and immunomodulative activities (Azain 2003). CLA may also play a role in the control of obesity, reduction of the risk of diabetes, and modulation of bone metabolism.

Histidyl Dipeptides

Various endogenous antioxidants, including tocopherols, ubiquinone, cartenoids, ascorbic acid, glutathione, lipoic acid, uric acid, spermine, carnosine, and anserine, have been found in skeletal muscle (Decker et al. 2000). Both carnosine (β-alanyl-L-histidine) and anserine (N-β-alanyl-1-methyl-L-histidine) are antioxidative histidyl dipeptides (Fig. 24.4) and are the most abundant antioxidatives in meats. Consumption of antioxidant-rich foods prevents oxidative damage in our body (Lindsay 2000). This action is attributed to neutralization and reduced release of free radicals by antioxidants (Langseth 2000). Antioxidant activities of carnosine and anserine may result from their ability to chelate transition metals such as copper (Brown 1981).

The concentrations of carnosine and anserine vary depending on animal species and the parts of the meat. The concentration of carnosine in meat ranges from 500 mg/kg in chicken thigh to 2,700 mg/kg in pork shoulder. Anserine is especially abundant in

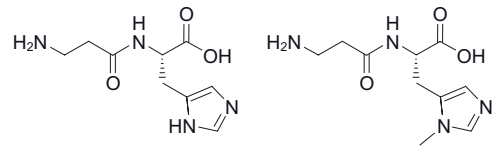

Figure 24.4. Structures of carnosine (left) and anserine (right).

chicken muscle (e.g., 980 mg/kg in skeletal muscle). These peptides have been reported to play roles in wound healing, recovery from fatigue, and prevention of diseases related to oxidative stress. Since anserine is more resistant to digestion than is carnosine, the physiological function of anserine would be more effective than carnosine in the human body. For this reason, functional food ingredients with high concentrations of anserine (approx. 98%) purified from fish extracts have been developed in Japan.

Park et al. (2005) demonstrated the bioavailability of carnosine by determining its concentration in human plasma after ingestion of beef. Also, increasing attention to these meat-based bioactive compounds has resulted in the development of a new sensitive procedure for determining these compounds (Mora et al. 2007).

Table 24.4. Representative functions of bioactive peptides derived from food proteins

Body systems	Bioactive peptides
Cardiovascular	ACE-inhibitory Antihypertensive Antioxidative Antithrombotic Hypercholesterolemic
Digestion	Antimicrobial Mineral binding Prebiotic
Immune	Immunomodulatory Cytomodulatory
Nerve	Opioid agonist Opioid antagonist

Utilization of Meat Protein-Derived Peptides

Bioactive Peptides Generated from Food Proteins

Carnosine and anserine are attractive bioactive peptides in meat as described above. In addition to these peptides, meat protein-derived peptides are another group of bioactive components of meat (Arihara 2006a; Arihara and Ohata 2008). It has been reported that numerous bioactive peptides are generated from food proteins, such as milk, soy, fish, and meat proteins (Korhonen and Pihlanto 2003, 2007; Pihlanto and Korhonen 2003; Mine and Shahidi 2005; Gobbetti et al. 2007). Representative bioactivities of such peptides are summarized in Table 24.4.

Of such bioactive peptides, angiotensin I-converting enzyme (ACE) inhibitory peptides have been studied most extensively (Vermeirssen et al. 2004; Meisel et al. 2005). Since some of these peptides have antihypertensive effects by oral administration, they have been utilized for pharmaceuticals and functional foods (Arihara 2004, 2006a). However, to date, functional meat products with bioactive peptides, including ACE inhibitory peptides, have not been developed.

Generation of Peptides from Meat Proteins

Most food proteins, including meat proteins, contain bioactive sequences. However, those bioactive sequences are inactive within the parent proteins. Peptides with respective bioactivities are generated from native proteins by proteolytic digestion. Processes of protein digestion for generation of peptides from meat proteins include gastrointestinal digestion, aging, fermentation, and protease treatment (Fig. 24.5).

Meat proteins are attacked by proteolytic enzymes (e.g., pepsin, trypsin, chymotripsin, elastase, and carboxypeptidase) during gastrointestinal digestion (Pihlanto and Korhonen 2003). Although there has been no clear evidence that bioactive peptides are generated from meat proteins in human intestinal tracts, their generation has been shown in several *in vitro* studies. For example, some gastrointestinal digestive enzymes generated ACE

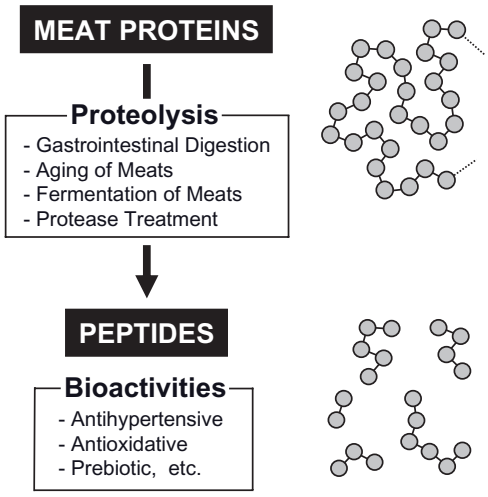

Figure 24.5. Generation of bioactive peptides from meat proteins.

inhibitory activity from pork proteins (Arihara et al. 2001). Also, ACE inhibitory activity was generated from meat proteins (myosin, actin, tropomyosin, and troponin) by pancreatic protease treatment (Katayama et al. 2003a).

Since meat contains various muscle endogenous proteases, meat proteins are hydrolyzed by these enzymes during aging (Etherington 1984; Koohmaraie 1994; Toldrá 2007). Thus, the content of amino acids and peptides increases in aged meat (Nishimura et al. 1988; Mikami et al 1995). Although enzymatic hydrolysis of meat proteins during aging results in improvement in sensory properties, there has been no report about the generation of bioactive peptides in meat during aging or storage. Studies would be expected to reveal the novel meaning of meat aging for improving its bioactivities.

Meat proteins are hydrolyzed during the fermentation and ripening of dry sausages. Both endogenous muscle and microbial proteolytic enzymes are involved in fermentation of meat products and contribute to the development of sensory properties of fermented meat products (Hierro et al. 1999; Hammes et al. 2003; Toldrá 2004). ACE-inhibitory and antihypertensive activities were generated from porcine skeletal muscle proteins by lactic acid bacteria (Arihara et al. 2004). Since small peptides have been identified in dry-cured ham (Sentandreu et al. 2003), some such peptides generated from meat proteins could have bioactivities. Also, Sentandreu and Toldrá (2007a, b) suggested that the proteolytic action of porcine muscle dipeptidyl peptidases during the ripening period of dry-cured ham could contribute to the generation of ACE inhibitory peptides.

The most common procedure for producing bioactive peptides on an industrial scale is enzymatic treatment. Various commercially available proteases have been utilized for the production of peptides from food proteins (Pihlanto and Korhonen 2003). Also, many bioactive peptides have been experimentally prepared by using commercial proteases (Korhonen and Pihlanto 2003, 2007). Proteolytic enzymes have been used for meat tenderization in the meat industry (Dransfield and Etherington 1981). Although peptides having bioactivities might be generated in meat treated with enzymatic tenderization, efforts have not been directed to such studies. Since effects of commercial proteases on meat protein breakdown and sensory properties of fermented sausages have been demonstrated (Bruna et al. 2000), such treatment could be used for developing functional meat products containing bioactive peptides. Meat protein hydrolyzates (peptides) could also be food ingredients, as they have bioactivities for functional foods.

Meat Protein-Derived Bioactive Peptides

Although many bioactive peptides have been isolated from various food proteins as described above, information on meat-derived peptides is still limited. As for other food proteins, ACE-inhibitory peptides have been studied most extensively among the

bioactive peptides derived from meat proteins (Vercruysse et al. 2005; Arihara and Ohata 2006a, 2008). Table 24.5 shows a summary of bioactive peptides, including ACE-inhibitory peptides, generated from meat proteins.

Some ACE inhibitory peptides generated from meat proteins showed antihypertensive activity when administrated orally to spontaneously hypertensive rats (Arihara et al. 2005a; Fujita et al. 2000; Nakashima et al. 2002). For example, two ACE inhibitory peptides (Met-Asn-Pro-Pro and Ile-Thr-Thr-Asn-Pro) found in the sequence of myosin heavy chain showed antihypertensive activity (Nakashima et al. 2002).

Several antioxidative peptides have been identified in enzymatic hydrolyzates of meat proteins (Saiga et al. 2003b; Arihara et al. 2005b). Hydrolyzates from porcine myofibrillar proteins generated by papain or actinase E exhibited high levels of antioxidative activity (Saiga et al. 2003b). Asp-Ala-Gln-Glu-Lys-Leu-Glu, which is found in the sequence of actin, showed the highest level of activity among five identified peptides. In another study, three antioxidative peptides (Asp-Leu-Tyr-Ala, Ser-Leu-Tyr-Ala, and Val-Trp) were isolated from enzymatic hydrolyzates of porcine skeletal muscle (Arihara et al. 2005b). These peptides had an antifatigue effect when orally administered to mice in an experiment using a treadmill.

In addition to the bioactive peptides described above, prebiotic (Arihara et al. 2006) and hypocholesterolemic (Morimatsu et al. 1996) peptides have been studied. Apart from bioactivities, meat protein-derived peptides also contribute to organoleptic properties of meat (Nishimura and Kato 1988; Nishimura et al. 1988). Peptides generated from meat proteins have a potential to produce novel functional ingredients with good organoleptic properties.

Table 24.5. Bioactive peptides derived from meat and meat-related proteins

Bioactivity	Protein source	Sequence[a]	References
Antihypertensive (ACE inhibitory)	Chicken muscle	IKW	Fujita et al., 2000
	Chicken muscle creatine kinase	LKA	Fujita et al., 2000
	Chicken muscle aldolase	LKP	Fujita et al., 2000
	Chicken muscle	LAP	Fujita et al., 2000
	Porcine muscle actin	VWI	Arihara et al., 2005a
	Porcine myosin	ITTNP	Nakashima et al., 2002
	Porcine myosin	MNPPK	Nakashima et al., 2002
	Chicken muscle myosin	FQKPKR	Fujita et al., 2000
	Bovine muscle	VLAQYK	Jang & Lee, 2005
	Chicken muscle creatine kinase	FKGRYYP	Fujita et al., 2000
	Fermented pork myosin	VFPMNPPK	Arihara et al., 2004
	Chicken muscle actin	IVGRPRHQG	Fujita et al., 2000
	Porcine muscle troponin C	RMLGQTPTK	Katayama et al., 2003b; 2004
	Chicken muscle collagen	GFXGTXGLXGF	Saiga et al., 2003a
Antioxidative	Porcine muscle	VW	Arihara et al., 2005b
	Porcine muscle	DLYA	Arihara et al., 2005b
	Porcine muscle	SLYA	Arihara et al., 2005b
	Porcine muscle actin	DLQEKLE	Saiga et al., 2003b
Opioid	Bovine blood hemoglobin	VVYPWTQRF	Zhao, et al., 1997
	Bovine blood hemoglobin	LVVYPWTQRF	Zhao, et al., 1997
Savory taste-enhancing	Beef treated with papain	KGDEESLA	Hau, et al., 1997
Sourness-suppressing	Cooked pork loins	APPPPAEVHEV	Okumura et al., 2004

[a] The one-letter amino acid codes were used

Bioactive peptides generated from food proteins, such as milk and soy proteins, have been utilized for functional ingredients. For example, several food products containing ACE inhibitory peptides have been marketed for hypertensives (Arihara 2006b). Although bioactive peptides have not yet been utilized in the meat industry, such peptides are promising candidates for ingredients of functional meat products. Also, bioactive peptides generated from meat proteins could be developed as novel functional food ingredients.

Functional Fermented Meat Products

Rediscovery of traditional fermented meat products as functional foods is an interesting direction. In the dairy industry, traditional fermented dairy products have been rediscovered and reborn as functional foods (Farnworth 2003). Recently, Ansorena and Astiasarán (2007) described the possibilities of development of novel healthier dry-fermented sausages that could minimize the negative features of meat. For producing such dry-fermented sausages, they mentioned the following items: (1) modification of mineral content, (2) fat modifications, (3) incorporation of fiber into formulation, and (4) utilization of probiotic bacteria. This section focuses on the utilization of probiotic bacteria for the development of functional meat products. Along with probiotics, prebiotics and synbiotics will be discussed here.

Probiotics

Probiotics is defined as "live microorganisms which, when administered in adequate amounts (as part of food), confer a health benefit on the host" (Stanton et al. 2003). Thus, probiotic foods are regarded as functional, if they have been satisfactorily demonstrated to beneficially affect target physiological functions in our body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being or a reduction of the risk of disease.

Probiotic bacteria, mainly intestinal *Lactobacillus* and *Bifidobacterium*, show various physiological functions, such as modulation of intestinal flora, prevention of diarrhea, improvement of constipation, lowering faucal enzyme activities, lowering blood cholesterol level, modulation of immune responses, prevention of food allergies, prevention of cancer, and adjuvant in *Helicobacter pylori* treatment (Stanton et al. 2003; Agrawal 2005). Desirable properties of probiotic strains (Brassart and Schiffrin 2000) are:

- human origin
- resistance to acid and bile toxicity
- adherence to human intestinal cells
- colonization of the human gut
- antagonism against pathogenic bacteria
- production of antimicrobial substances
- immune modulation properties
- history of safe use in humans

Probiotics and Meat Fermentation

Although the concept of probiotics has not been well recognized in the meat industry, the possibility of probiotic meat products has been discussed in recent years (Hammes et al. 2003; Työppönen et al. 2003; Arihara 2004, 2006b; Kröckel 2006; Ammor and Mayo 2007; Ansorena and Astiasarán 2007; Cocconcelli and Fontana 2008; De Vuyst et al. 2008; Leroy et al. 2008). Target meat products with probiotic bacteria would be mainly dry sausages, since they are processed without heat treatment. Although the market for probiotic meat products is still very limited, some probiotic meat products have been marketed in Germany and Japan (Arihara 2006b). A German producer devel-

oped a salami product containing intestinal bacterial strains (*Lactobacillus casei*, *Lactobacillus casei*, *Bifidobacterium* spp.) in 1998. In the same year, a Japanese producer also launched a meatspread product (Fig. 24.6) fermented with intestinal lactobacilli (*L. rhamnosus* FERM P-15120). *L. rhamnosus* FERM P-15120 has been screened from the collection of human intestinal lactobacilli (Sameshima et al. 1998).

Arihara et al. (1998) have shown that *L. gasseri* JCM1131 is applicable for meat fermentation as a potentially probiotic strain. Erkkilä et al. (2000, 2001a, b) tested the applicability of probiotic strains *L. rhamnosus* GG, LC-705 and VTT-97800 to dry sausage fermentation. They found that strains GG and E-97800 are suitable for use as probiotic starter cultures in fermenting dry sausage. Furthermore, several studies demonstrated the possibility of utilizing probiotic strains of lactic acid bacteria and bifidobacteria for meat products (Leroy et al. 2006; Pennacchia et al. 2004, 2006; Klingberg et al. 2005; Klingberg and Budde 2006; Rebucci et al. 2007; Ruiz-Moyano et al. 2008). Muthukumarasamy and Holly (2006, 2007) studied the effectiveness of a microencapsulation technique for protecting probiotic bacteria during sausage processing.

Most studies on utilization of probiotic strains for meat fermentation have focused on the growth of bacteria in meat and their influence on the sensory properties and inactivation of pathogenic bacteria. Bunte et al. (2000) and Jahreis et al. (2002) carried out studies on the utilization of probiotic lactobacilli for moist types of sausages. Their studies using healthy volunteers demonstrated that the ingestion of such products fermented with probiotic strains of *L. paracasei* LTH2579 had some beneficial physiological effects. The levels of CD4 T helper cells were elevated and the phagocytosis index increased after ingestion of the product. Further assessment of the relationship between ingestion of meat products with probiotic bacteria and human health are needed from various viewpoints.

Prebiotics and Synbiotics

In addition to probiotics, much attention has been paid to prebiotics in the food industry. Prebiotics is initially defined as "non-digestible food ingredients that beneficially

Figure 24.6. Fermented meat spread product "Breadton," utilizing the intestinal lactobacilli (Prima Meat Packers, Ltd., Japan).

affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve the health of the host" (Gibson and Roberfroid 1995). Later, this definition was updated as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits" (Gibson et al. 2004). As representative prebiotic substances, oligosaccharides and dietary fibers have been utilized to enhance the growth of probiotic bacteria (Holzapfel and Schillinger 2002; Tanaka and Sako 2003; Roberfroid 2008). In addition to oligosaccharides and dietary fibers, the presence of prebiotic peptides has been reported (Liepke et al. 2002; Arihara 2006a). Arihara et al. (2006) found that the hydrolyzate of porcine skeletal muscle proteins enhanced the growth of *Bifidobacterium strains*. One of the corresponding prebiotic peptides was identified as Glu-Leu-Met.

Alteration of the gut microflora by the ingestion of prebiotics has the following beneficial effects on health status (Tanaka and Sako 2003):

- suppression of harmful bacteria
- reduction of putrefactive substances
- reduction of carcinogenetic substances
- stimulation of bowel movement
- optimization of immune responses
- improvement of mineral absorption
- activation of colonocytes
- acidification of caecal and faecal contents
- improvement of lipid metabolism

Also, desirable attributes of functionally enhanced prebiotics listed by Rastall (2000) are: (1) targeting specific probiotics (*Lactobacillus* and/or *Bifidobacterium*), (2) active at low dosage with lack of side effects, (3) persistence through the colon, (4) protection against colon cancer, (5) enhancement of the barrier effect against pathogens, and (6) inhibition of adhesion of pathogens.

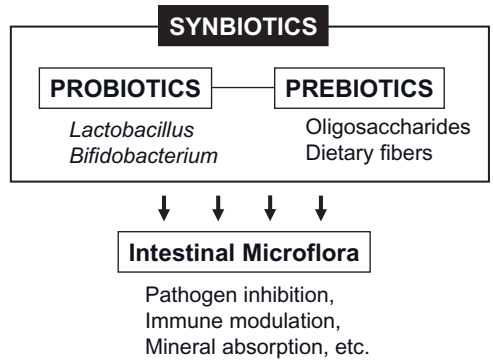

Figure 24.7. Concept of probiotics, prebiotics, and synbiotics.

Gibson and Roberfroid (1995) also proposed the concept of synbiotics, which is a mixture of probiotics and prebiotics (Fig. 24.7). Synbiotics are foods containing both probiotic bacteria and prebiotic substances to provide a diet in which the growth of the probiotic bacteria is enhanced by the prebiotics, thus promoting the chance of the probiotic bacteria becoming established in the gut and conferring a health benefit (Ziemer and Gibson 1998). Along with probiotics, the concepts of prebiotics and synbiotics are expected to be utilized for the development of novel meat products.

Concluding Remarks

Increasing attention has been paid to the physiological functions of meat and functional meat products in recent years (Jiménez-Colmenero et al. 2001, 2006; Arihara 2004, 2006a, b; Fernández-Ginés et al. 2005; Jiménez-Colmenero 2007a, b; Arihara and Ohata 2008). Since meat and meat products are important in the diet in most developed countries, healthier meat and meat products would contribute to human health. Although development of novel functional meat products is still limited, scientific information for designing functional meat products has been accumulating, and it has become technically possible to produce various meat products.

Utilization of meat-based bioactive compounds, including bioactive peptides, is a possible approach for the development of such products. Also, probiotics and prebiotics are promising food ingredients for fermented functional meat products. Such novel meat products would open up a new market in the meat industry.

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Part III

Controls

Chapter 25

Physical Sensors for Quality Control during Processing

Marta Castro-Giráldez, Pedro José Fito, Fidel Toldrá, and Pedro Fito

Introduction

The meat industry constitutes one of the main sectors in the developed countries due to its important protein contribution to the human diet. The meat market has markedly changed during the past several years, and the trend is toward elaborate high-quality meat products. For this reason, the industry must obtain meat quality information during the production process, in order to maintain quality standards. Over the past few years, certain physical sensors have been developed in order to provide reliable meat quality information. These sensors can be used in the process line to obtain objective information in a fast and nondestructive way. The most important are mainly based on electromagnetic energy, ultrasounds, and resonance techniques. These sensors can directly provide important information about meat composition and structure; sometimes, the information provided by the sensor does not allow discrimination of low-quality pieces, and it has to be implemented with additional information on the product.

This chapter presents an overview of the main electromagnetic physical methods for controlling meat products throughout the production process. Moreover, a brief summary of the most important marketed sensors is presented.

Methods Based on Electromagnetic Energy

There exist different kinds of sensors based on the interaction of food with electromagnetic waves, particularly: sensors using visible radiation, ultraviolet or infrared, microwaves, radiowaves, x-rays, or very high frequency waves (Nuclear Magnetic Resonance). Some applications have been available in the market for several years, particularly for laboratory use.

There exist also in-line applications for foods: detection of foreign components through the application of x-rays or control of food composition by using Near Infrared (Holm 2003). Concretely, microwave spectroscopy is currently used for the determination of moisture content in some cereals and fruits (Kraszewski 1991, 1996; Kraszewski and Nelson 1992, 1993a, b, 1994; Kraszewski et al. 1998; Nelson and Bartley 2000), density determination (Kress-Rogers and Kent 1987; Kraszewski and Nelson 1992), or, for example, fruit ripening (Nelson et al. 1993, 1994, 1995, 2006, 2007). There also exist in-line applications for the meat sector—for example, the evaluation of meat tenderness by using x-ray absorptiometry (Kröger et al. 2006). Some of these applications will be described throughout this chapter.

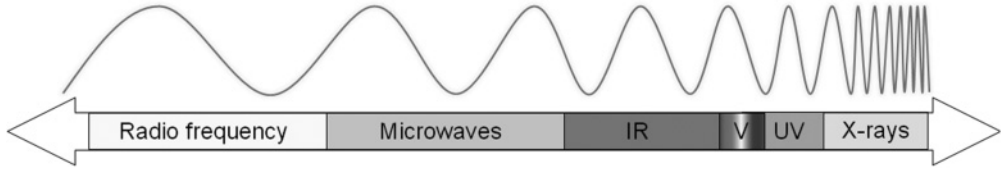

Figure 25.1. The electromagnetic spectrum.

Figure 25.1 presents the electromagnetic spectrum, which is characterized by different types of radiation on the basis of wavelength and frequency.

Electromagnetic waves are composed of an electric and a magnetic field. Due to the fact that foods do not present components that can interact with the magnetic field, it is possible to assume that food permeability is similar to that of free space ($\mu_0 = \mu = 4\pi\ 10^{-7}$ H/m) (Regier and Schubert 2005) and to consider only the complex permittivity (ε_r) as the dielectric property that describes the behavior of the food when it is subjected to an electromagnetic field (Metaxas and Meredith 1993; Nelson and Datta 2001). Complex permittivity is defined by the next equation:

$$\varepsilon_r = \varepsilon' - j \cdot \varepsilon'' \qquad (25.1)$$

In this equation,

$$j = \sqrt{-1}$$

the real part of complex permittivity is called the dielectric constant (ε') and the imaginary part is called the loss factor (ε''). The dielectric constant is related with the capacitance of the material and its ability to store energy (polarization). Foods are nonideal dielectrics and polarization has associated dissipation phenomena, producing energy absorptions and the decay of the dielectric constant. The parameter that reflects the absorption and dissipation of electromagnetic energy is the loss factor. The subscript "r" indicates that the values are relatives to air, and for this reason, the variable is dimensionless (Eq. 25.2).

$$\varepsilon_r = \varepsilon^* / \varepsilon_0 \qquad (25.2)$$

In this equation ε_0 represents the air permittivity ($8{,}8542 \times 10^{-12}$ F/m).

When complex permittivity is drawn as a vector (Fig. 25.2), real and imaginary parts are diphase 90°. The sum vector forms a δ angle with real axis (ε'). The ratio between real and imaginary parts of permittivity represents another important parameter, the loss tangent (Eq. 25.3, which represents a measure of the food ability to the energy dissipation (Ponne and Bartels 1995; Içier and Baysal 2004).

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} = D = \frac{1}{Q} \qquad (25.3)$$

In Equation 25.3, D is called the dissipation factor, and Q the quality factor. Tan δ can be defined as the energy lost per cycle divided by energy stored per cycle (Grimnes and Grøttem-Martinsen 2008).

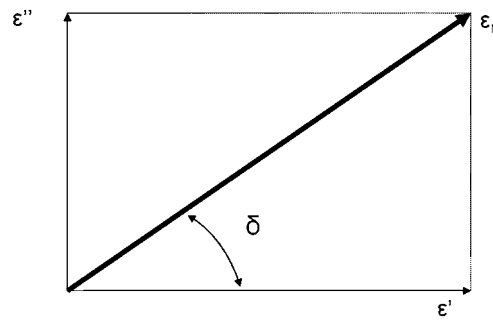

Figure 25.2. Diagram of loss tangent vector.

Dielectric Mechanisms in Cellular Systems

There are different mechanisms affecting the dielectric behavior of cellular food systems. These mechanisms are divided into two classes: resonance and relaxation processes. Resonance phenomena happen when the applied electric field has a frequency that matches the natural oscillation frequency of the material. It includes electronic polarization and atomic polarization, which are produced at the highest microwave frequencies and above them (Fig. 25.3). Electronic polarization results from the displacement of the electronic cloud with respect to the nucleus, whether in single atoms or in molecules (Grimnes and Grøttem-Martinsen 2008). The effects of this particular polarization can be observed in the visible part of the spectrum, caused by light refraction (von Hippel 1954; Kent 2003). Atomic or ionic polarization appears when atoms or ions are displaced in a molecule. These displacements are typically associated with changes in chemical bounds into the molecule, producing resultant dipolar moments. A distortion of the molecule's natural vibration is produced, and, therefore, this polarization is also called vibrational (Kao 2004). This phenomenon is mainly produced at the infrared region.

On the other hand, relaxation phenomena are produced at microwave and radio frequencies, and characterize, with the conductivity, the dielectric behavior of practically all tissues at these frequencies. In meat products, these relaxation phenomena are the result of the interaction between electromagnetic radiation and tissue constituents (at the cellular and molecular levels).

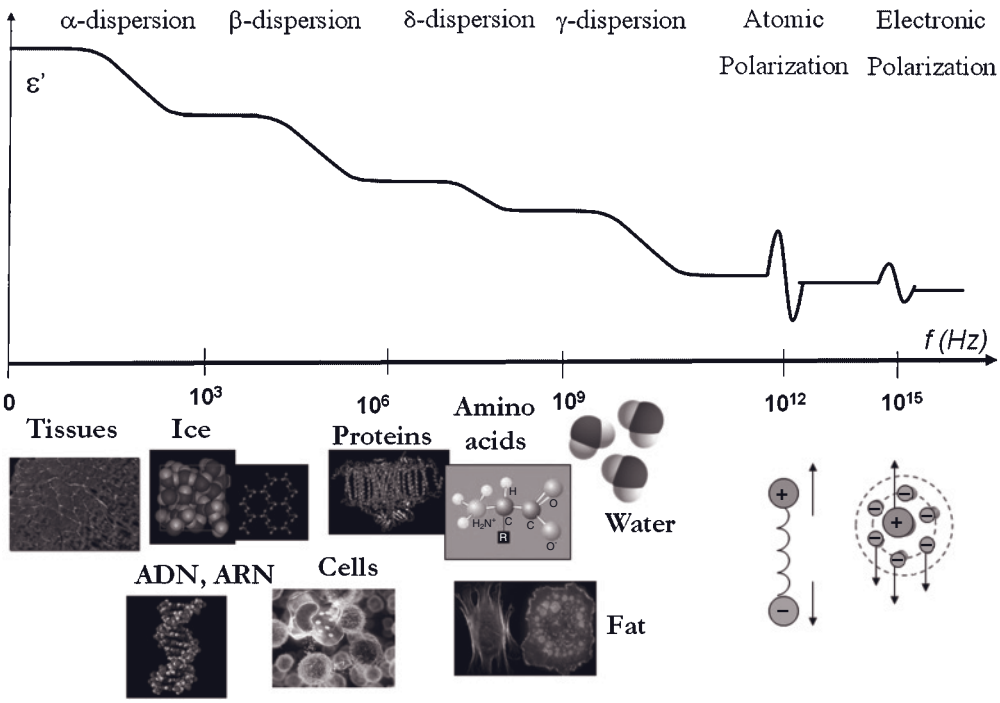

Figure 25.3. Ideal representation of dielectric constant spectrum in biological systems. The four relaxation regions that can be presented in these systems are also represented: α, β, δ and γ. (Adapted from Castro-Giráldez et al. 2008.)

The conductivity of most tissues rises from a low value at low frequencies that depend strongly on the volume fraction of extracellular fluid up to a plateau in the 10–100MHz frequency range, which mainly corresponds to the conductivity of intra- and extracellular ions. Conductivity then rises dramatically, due to the dielectric relaxation of water (Rigaud et al. 1996) (Fig. 25.4).

This increase in conductivity is associated with a decrease in permittivity, from very high values at low frequencies in different steps called dispersions. It is important to highlight that these dispersions are not produced instantaneously and are characterized by the correspondent relaxation phenomena (Schwan 1988). In biological systems, there are four main relaxation regions: α, β, δ and γ (Fig. 25.3). Each of these steps characterizes a type of relaxation that occurs in a specific frequency range and which allows the identification of different phenomena. The dispersions of biological systems have been widely studied by many authors (Grant et al. 1978; Schanne and P-Ceretti 1978; Pethig 1979; Stuchly 1979; Schwan and Foster 1980; Stuchly and Stuchly 1980; Schwan 1981; Foster and Schwan 1986; Pethig and Kell 1987; Duck 1990; Foster and Schwan 1996; Gabriel 1996, 2006; Gabriel and Gabriel 1996; Gabriel et al. 1996a, b, c).

The γ-dispersion, also called orientation polarization, is located at the GHz region, and it is due to the polarization dipoles, fundamentally free water molecules. The β-dispersion or interfacial polarization is mainly due to the Maxwell-Wagner effect. This effect is produced due to interfacial phenomena on heterogeneous materials (Feldman et al. 2003). Other dispersions can be produced by proteins or other macromolecules at frequencies between the β and γ dipersions, depending on the size and charge of the molecules (Gabriel 2006). Another additional relaxation (δ) is located between the β and γ dispersions. This relaxation is caused by the rotation of amino acids, the rotation of charged side groups of proteins, and the relaxation of protein-bound water (Schwan 1981). The α-dispersion dominates between millihertz and a few kilohertz, and is not yet completely understood. Some hypotheses remark the counter-ion effects near the mem-

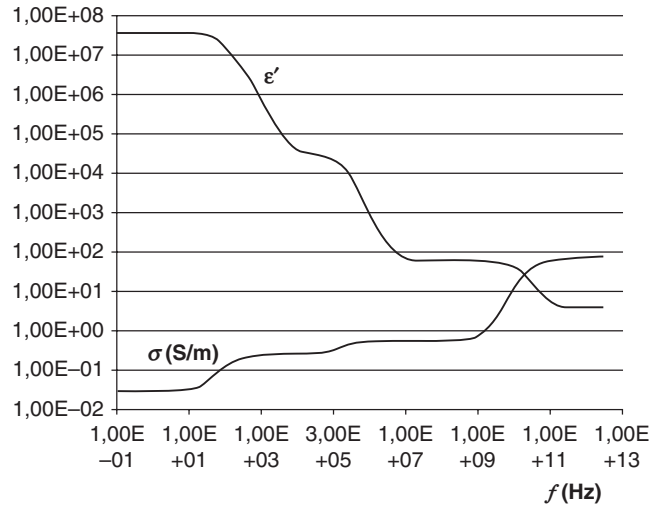

Figure 25.4. Ideal representation of electric conductivity and dielectric constant spectra.

brane surfaces, the active cell membrane effects, and gated channels, caused mainly by the intracellular structures or the ionic diffusion (Grimnes and Grøttem-Martinsen 2008).

In some cases, it could be useful to analyze the energy dissipation of these relaxation phenomena in terms of loss factor spectra instead of conductivity spectra (Fig. 25.5). Loss factor can be expressed by Equation 25.4, which reflects the different phenomena contributing to the loss factor spectrum, depending on the frequency range. It is important to highlight that ionic conductivity only introduces losses into the material when exposed to electromagnetic energy.

$$\varepsilon'' = \varepsilon''_d + \varepsilon''_{MW} + \varepsilon''_e + \varepsilon''_a + \frac{\sigma}{\varepsilon_0 \omega} \quad (25.4)$$

where:

ε_d'' represents the loss factor caused by the dipolar orientation or dipolar relaxation.

ε_{MW}'' represents the loss factor due to the Maxwell-Wagner effect.

ε_e'' represents the loss factor relative to electronic polarization.

ε_a'' represents the loss factor caused by atomic polarization.

$\sigma/\varepsilon_0\omega$ represents the loss factor due to the effect of ionic conductivity, where σ, ε_0 and ω are the conductivity of the material, the dielectric constant in vacuum, and the angular frequency, respectively.

Electric Impedance Spectroscopy (Bioimpedance)

The application of bioelectrical impedance (electrical impedance in the medical field)

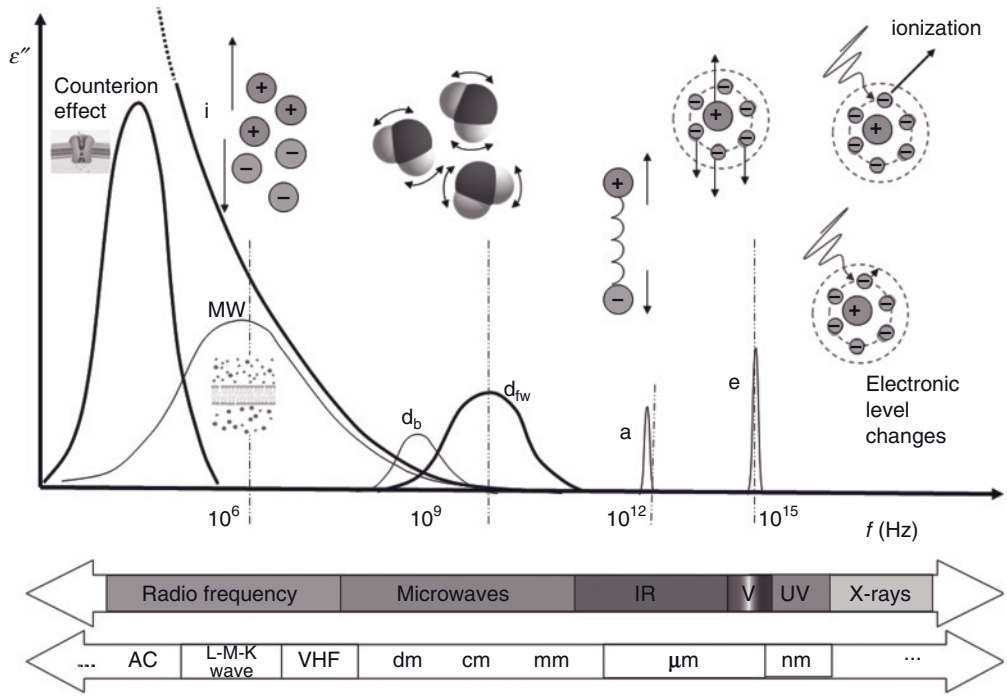

Figure 25.5. Schematic representation of the electromagnetic spectrum (in logarithm scale) of the different effects that contribute to effective loss factor (adapted from Castro-Giráldez et al. 2008), where i represent the ionic losses; MW means Maxwell-Wagner effect; d_{fw} is related to the dipolar losses of free water; d_b is related to the dipolar losses of bound water; a is related to the atomic losses; and e is related to the electronic losses.

was developed in the sixties and mainly concerned the follow-up of variations in the composition of the human body (Charnet et al. 1999). Bioimpedance was used for the first time in the control of meat products and processes in the eighties (Damez and Clerjon 2008).

The principle of the impedancemetry is based on the ability of a medium to pass an alternating electrical current. When the impedance is not depending on the frequency, the system works as a resistive media; otherwise, as in the biological tissue and colloidal systems, the impedance has resistive, capacitive, and inductive components (Damez and Clerjon 2008). Meat tissue is composed of cells with an internal liquid phase (cytoplasm), surrounded by the external liquid phase with a different composition than the internal liquid phase, because the cell membrane is between both phases (Chenoll et al. 2007). Cell membrane is a dielectric material, working at a low frequency as an isolator, behaving like a capacitor (Damez et al. 2007). Chemical, physical, and structural transformations produce changes in the electrical properties of the muscle tissue, and the impedance variation permits the control of some quality aspects, such as types of fresh pork meat (freshness, tenderness, fat content) or the detection of frozen meat, and safety aspects, such as a microbial detection or chemical contaminations. As was explained in the beginning of this chapter, the dielectric dispersions measured in the impedance of meat with capacitor equipment (Fig. 25.6) are produced at different ranges of the frequency of the electric field imposed on a meat sample. At high frequency, the dispersion produced is in the dipolar mechanisms (such as water molecules), which consist of the orientation of the molecules, spin rotation, and transport of charges in the way of the field, increasing the conductivity of the media. Next affected is the ionic motion of the charged molecules (anions and cations). At medium frequency, the dispersion produced is the Maxwell-Wagner effect, which depends on the varia-

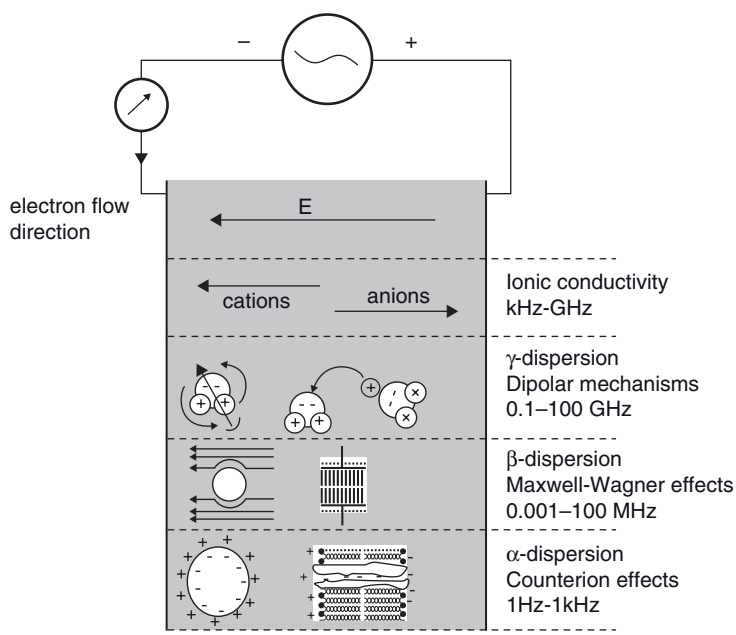

Figure 25.6. Basic capacitor equipment and different dispersion in the dielectric properties of meat.

tion of the intensity of the electric field by the passive cell membrane capacitance, intracellular organelle membranes, and protein molecules. At low frequency, the dispersion produced is the counter-ion effect, where the electric field produces changes in the transport channels of the cell membrane to activate the anion inflow through the membrane, changing the polarity of the extra- and intracellular liquid phase of cells (Grimnes and Grøttem-Martinsen 2008).

In assessing meat quality, some authors used bioimpedance spectroscopy to separate the PSE (pale, soft, exudative) meat, DFD (dark, firm, dry) meat, and RFN (red, firm, nonexudative) meat (Swatland 1999; Castro-Giráldez et al. 2007a). Castro-Giráldez et al. (2007a) published that an amount of ATP variation exists in storage for each type of pork meat and related the ion activity of ATP with the ionic dispersion in the dielectric spectra. Figure 25.7 shows the three dispersions of normal pork meat and the electric conductivity variation. Castro-Giráldez et al. (2007a) showed the variation of the dielectric spectra before and after rigor mortis, developing a method to determine the freshness of pork meat using the β-dispersion measure, because Maxwell-Wagner dispersion is affected by the structural protein's degradation throughout meat maturation. The interaction of the protein degradation in the β-dispersion has been used to determine the tenderness of pork meat (Byrne et al. 2000) and bovine meat (Lepetit et al. 2002).

Some chemical compounds or group of compounds have been related to the dielectric properties as fat content, analyzed with electrodes inserted in the muscle before rigor mortis, in reference to the a-dispersion variation (Madsen et al. 1999). In salted and cured pork meat, some authors have developed methods to control the salting level and moisture. Castro-Giráldez et al. (2007b, c) explain a thermodynamic model coupled with dielectric measures in the range of the g-dispersion to control the shear out of salt and water, and establish the water adsorbed in meat matrix and the salt involved in the protein meat degradation. Some commercial sensors have been developed recently to control physical and chemical properties, such as the

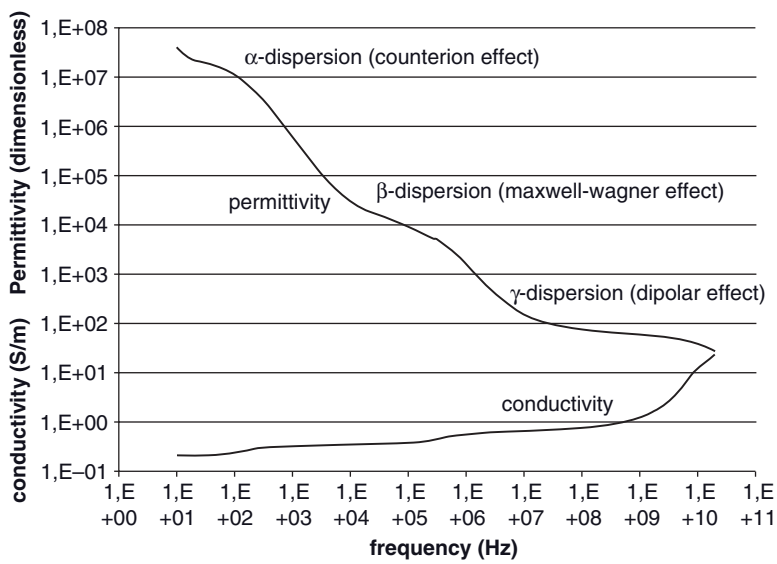

Figure 25.7. Dispersions of meat tissue with fibers in transversal direction (Castro-Giráldez et al. 2007a).

Meat Qualitymeter® from Tecpro (U.S.), which analyzes some chemical and physical properties as an ATP content using low-frequencies or RFM1000 from Process-sensor Corporation, which uses high frequencies to analyze chemical properties as moisture.

On the safety side, bioimpedance spectroscopy has been used to determine microbial levels in meat. Kim et al. (2008) developed an interdigitated microelectrode sensor that works at low frequencies to detect *salmonella enteritidis* in pork meat. Ong et al. (2002) developed an electrical conductivity sensor that works at low frequencies to control *E. Coli*, *Pseudomonas putida,* and *Bacilus subtilis*. Radke and Alocilja (2005) developed a conductivity sensor to control *E. Coli* counts in pork meat pieces, with the new methodology previously published (Radke and Alocilja 2003).

Microwave Spectrometry

Microwaves include waves with wavelengths from 3m to 3mm. The corresponding frequency range extends from 100MHz to 1mmGHz (Kent 2001). In the electromagnetic spectrum, microwaves are located between radio waves at low frequencies and infrared at higher frequencies (Fig. 25.1).

The interaction of microwaves and meat products produces the so-called γ-dispersion (Fig. 25.3), which is closely related to the water content and its state (Kent and Jason 1975). But the dielectric spectrum at these frequencies also depends on food composition, which obviously influences the water state. Moreover, the presence of ions produces ionic conductivity, which affects the loss factor spectrum (Fig. 25.4), and also the electrical charges of proteins; the presence of free amino acids or the pH variations are some of the factors that can affect the dielectric spectrum at these frequencies (Gabriel 2006).

The application of this technique to food quality control has been widely studied. One application is to detect the fraudulent addition of water in meat products (Kent et al. 2000, 2001, 2002). It was also employed to measure water activity in proteic gels (Clerjon et al. 2003). A microwave sensor for controlling meat and fish freshness was developed, based on the change in dielectric properties due to the reduction of muscle anisotropy during meat and fish aging (Clerjon and Damez 2007). It is also possible to predict the fat content in fish or minced meat (Kent 1990; Kent et al. 1993; Boggaard et al. 2003). A nondestructive meter for measuring fat in fish (Kent 1990), fat in meat (Kent et al. 1993), and fish freshness (Boggaard et al. 2003; Tejada et al. 2007) is already on the market (Distell Company®, West Lothian, Scotland). A Guided Microwave Spectometer® (Thermo Electron Corporation, U.S.) has been developed for on-line measurements of moisture and fat content in ground meat. There exists also an on-line sensor for measuring the fat-to-lean ratio in pork middles (Keam Holden Ltd., New Zealand).

Near Infrared Spectroscopy

Near infrared (NIR) spectroscopy is a technique that uses a portion of the electromagnetic spectrum, from 780 to 2500nm (Datta and Almeida 2005). NIR spectra are composed of overlapping absorptions, corresponding to overtones, and combinations of vibrations involving C-H, O-H, and N-H chemical bonds (Osborne 2000). This technique provides information about the molecular bonds and chemical constituents of the sample (Belton 1997), and allows a complete picture of the organic composition of the analyzed material (Van Kempen 2001). It can be considered one of the most powerful analytical techniques to analyze final meat quality (Van Kempen 2001). On the other hand, although this technique gives information on the molecular level, some research also showed the utility of determining macro-

scopic structural changes of meat (Damez and Clerjon 2008).

More efforts have been made in order to investigate the ability of NIR to predict meat quality and composition: in pork (Brøndum et al. 2000; Forrest et al. 2000; Chan et al. 2002; Geesink et al. 2003; Meulemans et al. 2003; González-Martin et al. 2005; Barlocco et al. 2006; Savenije et al. 2006; Ortiz-Somovilla et al. 2007), in beef (Hildrum et al. 1994, 1995; Thyholt and Isaksson, 1997; Byrne et al. 1998; Park et al. 1998; Rødbotten et al. 2000, 2001; Liu et al. 2001, 2003; Leroy et al. 2004; Shackelford et al. 2004, 2005; Andrés et al. 2008; Naganathan et al. 2008a, b; Ripoll et al. 2008; Sierra et al. 2008;), in poultry meat (Valdes and Summers 1986; Cozzolino et al. 1996; Rannou and Downey 1997; Ding et al. 1999; Fumière et al. 2000; Lyon et al. 2001), in lamb (Cozzolino et al. 2000; Andrés et al. 2007), in oxen (Prieto et al. 2006, 2008a, b), and in kangaroo (Ding and Xu 1999). The main applications of this technique are for determining moisture, fat, protein, and in some cases, minerals in meat and meat products (Gonzalez-Martin et al. 2002a, b, 2005; Alomar et al. 2003; Realini et al. 2004; Barlocco et al. 2006; Sierra et al. 2008). It is also used to determine meat quality, pH, appearance and color, and muscle characteristics, such as water-holding capacity, intramuscular fat, tenderness, and microbial spoilage (Byrne et al. 1998; Brøndum et al. 2000; Geesink et al. 2003; Liu et al. 2003; Garcia-Rey et al. 2005; Hoving-Bolink et al. 2005; Shackelford et al. 2005; Barlocco et al. 2006; Savenije et al. 2006; Andrés et al. 2007; Rust et al. 2008). Moreover, NIR is used to detect the adulteration of meat and meat products and to identify frozen/thawed meats (Downey and Beauchêne 1997a, b; McElhinney et al. 1999a, b; Ding and Xu 2000; Lyon et al. 2001). An extensive overview of the applications of infrared spectroscopy in foods (Ozaky et al. 2007) and in meat and meat products (Prevolnik et al. 2004; Hildrum et al. 2006; Reddy-Gangidi and Proctor 2008) was reported.

Some commercialized sensors based on infrared spectroscopy techniques already exist. For example, the QualitySpec® BT system (ASD Inc, U.S.) utilizes NIR technology in order to analyze beef carcasses for predicting tenderness on-line. Other examples are the APIS Meat Optimizer® (Prediktor AS, Norway) or the DA7200 multi-purpose NIR Analyser (Perten Instruments AB, Sweden), which allow on-line analysis of meat and meat product composition by using NIR technology. Other sensors use the FT-NIR to analyze the composition of meat and poultry (Quadra Chem Lavoratories, Ltd., U. K.). The FOP analyzer® (Tecnilab, Spain) utilizes the NIR with a fiber-optic probe for measuring proteins, moisture, and fat in processed meats. Other sensors available for real-time measurements of fat, moisture, and protein in meat products are the in-line Foods Gauge (NDC Infrared Engineering, U.S.) and the MCT 360 (Process Sensors Corporation, U.S.).

In conclusion, NIR spectroscopy offers a number of important advantages with regard to traditional methods of determining meat and meat product quality. This technique allows for a fast, simple, nondestructive, and noninvasive analysis (Büning-Pfaue 2003). It requires minimal or no sample preparation and offers accurate results (Niemöller and Behmer 2008). Its greatest disadvantage is a weak sensitivity to minor constituents and the complicated spectra data interpretation (Prevolnik et al. 2004).

Conclusions

The improvement of meat products' quality is one of the most important challenges for the industry in order to satisfy human desires, to minimize economic losses, and to optimize meat plant processes. Meat quality can be based on technological, sensory, and

safety aspects. These quality factors are directly or indirectly related to the composition or structural aspects of the meat and, for this reason, can be well predicted by physical sensors. These sensors are mainly based on novel technologies, such as spectroscopic methods, nuclear magnetic resonance, ultrasounds, or electrical measurements, and they present several advantages when compared with traditional laboratory assessment. The main advantages are that these measurements are nondestructive and very fast, and some of these techniques can even be noncontact. Moreover, these measurements can be implemented in the production line, allowing product information to be obtained in real time.

In summary, the use of physical sensors has high potential and real possibilities for controlling meat quality in the industry. Some of these sensors have already been adopted in process lines, but others need further research to be implemented in-line and commercialized.

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Chapter 26

Sensory Evaluation of Meat Products

Geoffrey R. Nute

Introduction

The definition of what constitutes a meat product has been documented in different government guidelines and statutes in various countries around the world. In the United Kingdom, definitions of meat products are given in Statutory Instrument number 2075; however, from a sensory point of view, a more appropriate definition could be that given in EC Council Directive 92/5/EEC (1992), which basically states that meat products are derived from or contain processed meats so that the characteristics of fresh meat are no longer evident in the product.

Meat products can be produced by many processes, such as heating and cooking, smoking, salting, brining, curing, drying, and aging. A fuller description of food processing technology is contained within a book by Fellows (2000), who gives an extensive review of the many processes available to food manufacturers.

The essential aim of producing meat products is to increase or vary the many aspects of texture, flavor, and odor/aroma available to consumers. As far as sensory properties are concerned, the approaches used are based on the quest for information about some aspect of the products produced. Sensory methodology and its applications for meat are adequately described by Deliza and Gloria (2009). Further approaches in sensory analysis by way of typical sensory descriptions used in meat studies and sensory analysis of meat are given by Nute (2002, 2009).

The following sections give examples of work that includes sensory methods as a major part of each study across a wide range of meat products.

Cooked Ham

Ham usually refers to muscles removed from the hind leg of pork that has undergone a curing process, although in modern times, ham is often used in a generic way to describe a process rather than a specific part of a pig (e.g., turkey ham, shoulder ham, etc.).

The American Meat Science Association Committee produced "Guidelines for Sensory, Physical and Chemical Measurements of Ham" circa 1982. They listed the important sensory descriptors covering flavor (6), aroma (5), appearance (9), and texture/mouthfeel (7). They also recommended using eight-point category scales for the color of cured ham (pale to dark), cured flavor intensity, juiciness, cured pork aroma, and firmness.

Rhodes and Nute (1980) used category scales, a sensory panel, and a consumer panel to investigate the acceptability of canned ham differing in water content. Polyphosphate concentration was varied from 0 to 0.80% on a wet weight basis. The intent was to produce hams without the foreign flavors associated with increasing polyphosphate levels while water content was increased. There was no significant difference attributable to increas-

ing polyphosphate concentration. The amount of added water ranged from 0% to 24%. The panel were able to distinguish between 0% added water and 24% added water but not between 10% and 20% added water in texture. Juiciness did not differ between 0% and 10% added water, but was significantly different at 20% and 24% added water when compared to 0% and 10%.

This approach was aimed specifically at researchers working on aspects of production.

Nute et al. (1987) used a trained panel to produce a map of UK hams purchased at retail that included sensory characteristics, composition, and instrumental texture analysis. In all, 52 hams were examined and their descriptions at retail, which ranged from different packaging types, canned, loose wrapped, vacuum packed, and different product descriptors (mild cure, gammon cure, honey roast, sugar roast, smoked, and country of origin, British, Danish).

The sensory profile generated covered three areas: appearance, texture, and flavor. The resultant map based on a combination of Generalized Procrustes Analysis and principal component analysis showed that the first axis accounted for 33% of the total variation and presented a contrast between hams that were gelatinous, of a plastic texture, and plastic appearance against hams that were flaky, cohesive, and firm, with a stronger ham flavor. This same axis, when instrumental and chemical measurements were projected into the sensory space, showed that canned hams tended to have more expelled fluid and higher water content. The second axis, which accounted for 17% of the total variation, was related to the texture descriptors, which include: rubbery, firm, plastic, and cohesive. The third principal axis, accounting for a further 10% of total variation, was related to the dominant color of the ham slices, fatness, and saltiness.

When hedonic ratings were included, the first principal axis accounted for 91% of the total variation and showed that preferred hams were firmer and meaty.

Dry-Cured Ham

In Spain, dry cured hams are a popular commodity because of their flavor and long shelf life. Work by Flores et al. (1997) developed a lexicon of descriptors that could be used to study flavor characteristics. The approach centred on using an intensity reference scale and training as outlined by Meilgaard et al. (1991). This method gives examples of different products with differing characteristics, that is, sweet with an intensity rating of 1.0 using a 1% sucrose solution as against sweet with an intensity rating of 16.5 given by frosted raspberry pop-tarts from the Kellogg Company. Higher intensity ratings of 18.0 are also given by frosted blueberry pop-tarts from the Kellogg Company. A major disadvantage with this approach is that the same products are often not available in Europe, or even if given the same name, may not relate to the same intensity.

After this initial screening, assessors were given examples of Italian dry-cured hams, country-style ham, and Spanish "Serrano" ham. The final lexicon contained three categories: aromatics (10 descriptors), comprising fat complex, boar taint, barnyard, haylike/musty, brown spice, pickling spice, smoky, pork, serum, pungent; taste (3 descriptors), comprising sour, salty, bitter; feeling factors (3 descriptors), comprising astringent, metallic, and mouthfilling. For each of these terms, a description was given (e.g., boar taint: the aromatic associated with boar meat; hormone-like (skatole); mouthfilling: mouthfeel associated with monosodium glutamate).

This lexicon was then used to compare the long and short processing times of "Serrano" dry-cured ham. The results of a univariate analysis indicated that there were differences between drying times of 7 months and 12 months, with significant increases in boar

taint, barnyard, smoky serum, and pungent aromatics; increases in sour, salty tastes; and an increase in astringency in the mouth. It was postulated that the longer processing time of 12 months would allow more time for underlying biochemical changes to take place that could produce increases in volatile fatty acids.

Multivariate analysis (factor analysis) was also used to investigate the development of dry-cured flavor. After 12 months, three main factors were identified, "cured flavor," "off-flavor," and "pork flavor," whereas at 7 months, the dry-cured flavor was not fully developed. In this trial it was interesting to note that "boar taint" increased over the dry-curing time. Boar taint, which is used as a general term, has at least two compounds responsible, which differ in their origin. Androstenone, a hormone-like compound, is produced in the testes and then migrates to the salivary glands in the pig. It is also a good indicator of boar taint in back fat as a result of the lipophilicity and hydrophobicity of androstenones. Skatole, the other major contributor to boar taint, is produced by the breakdown of tryptophan in the hind gut of the pig. The compound is both fat and water soluble. An extensive study of how boar taint is perceived by assessors is given by a series of papers by Annor-Frempong et al. (1997a, b, c, 1998) and Nute et al. (1988). Although a full description of these works is outside the scope of this present chapter, these papers may be of interest to those working on boar taint.

Work described by Banon et al. (2003) investigated the differences in boar taint in cooked pork loin and dry-cured ham from the same pigs as assessed by a trained panel. Meat in this study came from entire males and castrates from two lines of pigs, Large White x Hungarian Duroc females crossed with Landrace x Danish Duroc males and Landrace x Large White females crossed with Large White males. All pigs were slaughtered at around a liveweight of 105 kg. Samples of fat were analyzed for androstenone and skatole and fat samples, then grouped according to levels of androstenone and skatole. In terms of androstenone, there were low, medium, and high levels, with concentrations of androstenone of $<0.5\,\mu g\,g^{-1}$, 0.5 to $0.99\,\mu g\,g^{-1}$, and $0.99\,\mu g\,g^{-1}$ respectively. For skatole, the definitions of low and medium were $<0.8\,\mu g\,g^{-1}$ and $\geq 0.08\,\mu g\,g^{-1}$. Assessors rated samples on a five-point scale where 1 was minimum and 5 was maximum for each sensory attribute of boar odor and boar flavor. Samples were compared across six combinations of androstenone and skatole, using the convention of LL (i.e., low androstenone and low skatole), LM, ML, MM, HL, and HM. The analysis showed that cooked pork boar odor and flavor increased significantly in LM and MM groups when compared to control castrate samples, while in the dry cured ham only HM samples were differentiated from the control group. A further analysis compared cooked pork loin and dry-cured ham from entires and castrates, and as expected, boar odor and flavor was lower in castrates and higher in entires in cooked pork. In the equivalent dry-cured ham samples, boar odor was higher in entires than in castrates. However, generally low ratings were given for boar flavor. Dry-cured ham from castrated pigs had improved aroma and taste. It was concluded that in cooked pork loin, boar taint was much more intense than in dry-cured ham from the same pigs. Therefore, the process of curing (which in this case used a curing solution of 98% sodium chloride plus 2% potassium nitrate and sodium nitrate in equal proportions, and then dry salting with coarse sea salt) demonstrated the changes in meat sensory characteristics that occurred during processing.

Earlier reference was made to the work of Nute (1987) in producing a sensory map of ham products. A similar approach has been adopted by García-González et al. (2008) when assessing dry-cured ham, where the emphasis is on the relationship between vola-

tiles and sensory attributes. In this study, 41 samples were obtained from several different areas of France and Spain. The project was designed to simulate the dry-cured ham variability that the consumer would find in the market place. The hams comprised 30 white hams from different crossbreeds, eight Iberian hams, and three Gasconne and Basque hams. The curing time varied between ham types, where the French hams were cured for less than 12 months apart from the Bayonne hams. The Spanish white hams were cured for periods varying from 10 to 18 months, and the Iberian hams cured for more than 18 months. A descriptive sensory profile contained 27 descriptors that were grouped into appearance, texture, and flavor. Nine-point structured scales were used throughout this study. An initial analysis comparing white hams versus Iberian hams showed that for 17 sensory attributes, there were significant differences between the hams. Of these descriptors, nine were related to odor or flavor: cured ham flavor, rancid odor, acorn odor, rancid taste, acorn flavor, raw meat flavor, pungent flavor, fat rancid flavor, and fat pungent flavor. Relationships between sensory attributes and volatile compounds were then explored using a combination of PCA analysis based on sample configuration, into which were mapped the concentration values of the volatiles. This approach identified those volatiles associated with sensory descriptors. Stepwise regression analysis was then used to relate volatiles to specific attributes. It was found that 70% of acorn odor could be explained by benzaldehyde, 2-heptanone, and 3-methylbutanol; 77% of acorn flavor by 3-methylbutanal, hexanol, 3-methylbutanol, and 2-nonanone; 60% of rancid odor by hexanal, pentanol, and hexanol; 82% of rancid taste by 3-methylbutanal, hexanol, and octanol; 86% of fat rancid flavor by octanol, 3-methybutanal, and limonene; and 78% fat pungent flavor by octanol and limonene. The integration of volatile data, sensory data, and mathematical procedures showed that many of the sensory components that give dry-cured ham its unique flavor could be explained by a small number of volatiles. These approaches have increasingly led to the relatively new area of science called "chemometrics."

The developments described above give some indication of quality-control procedures that could be applied to dry-cured hams that could lead the way to standardization of the process. However, this may not be possible with the interest in PGI status (Protected Geographical Indication status). So far, there may well be a number of variations for standardized procedures in the preparations of dry-cured ham. There already exists a procedure for maturation after the drying stage. Indeed, this is part of the traditional process for Iberian ham.

Work by Cilla et al. (2005) investigated the change in sensory attributes and consumer acceptability of twenty-eight 12-month dry-cured hams obtained from four different manufacturers (each supplied seven hams) that complied with the specifications required for "Designation of Origin Teruel" (Boletin Oficial del Estado 1993). The samples were greased with pork lard and hung at 18°C and 75% relative humidity. Samples, one from each manufacturer, were assessed at 2-month intervals for 12 months of drying, except for 22 months, when the next sample point was at 26 months.

Acceptability as assessed by the consumer panel did not find any significant differences between the 12- and 26-month hams. The sensory panel found differences in acceptability after 22 months.

The sensory profile indicated that aroma was at a maximum around 18 months of maturation, and flavor and saltiness showed results similar to aroma. Throughout the maturation period there was no significant change in rancidity. The main changes that occurred in the texture involved attributes of hardness, crumbliness, pastiness, fibrousness, and adhesiveness. Most of the changes

occurred after about 18 months, and these were attributed to proteolysis, which agreed with biochemical maturation indices.

The continuing theme of PG1 status and quality labeling of traditional products led to work in France, where Rason et al. (2007) used a sensory profiling technique and a cluster analysis approach to identify groups of traditional sausages produced in the Massif Central and establish the underlying dimensions of production and processing. A total of 108 producers took part in the trial, and each producer supplied details of their production method based on a questionnaire. The results from the questionnaire were analyzed using multiple correspondence analysis combined with a hierarchical cluster analysis technique. Six groups or clusters were identified, separating the differences in manufacturing, which were mainly due to the gender and slaughter age of the pigs, the amount and type of fat used in the batter, and the type of drying.

The sensory panel used 26 attributes that were subdivided into five categories as follows: appearance, four descriptors; texture by fingers, three descriptors; texture by mouth, four descriptors; aroma, nine descriptors; and flavor, six descriptors. Initial validation of the data showed that two descriptors were removed from the analysis: "hole" was removed as it was a quality defect atypical of the products, and "the aroma of mushroom," which had a nonsignificant *F*-value for the product.

The remaining 24 attributes and their ratings for each product were submitted to correspondence analysis, which showed that the first dimension was mainly related to fat attributes and aroma ratings linked to the raw material. The second dimension mainly consisted of texture descriptors, contrasting with aroma and flavor. The overall conclusion was that the main effect on the second dimension was related to texture attributes, which in turn were linked to the drying time of the sausages.

Further analysis of this data set using canonical correlation analysis was an attempt to gain further insights into the relationship between the manufacturing data of traditional dry-cured sausages and sensory attributes. This analysis showed that the relationship between the first two axes of the sensory data and the manufacturing data was highly correlated. Discriminant analysis showed that the traditional dry sausages were correctly classified in their manufacturing groups. The methodology could lead to the production of quality labels that could correctly reflect both production and geographical territory. It was postulated that a further experiment would be justified to link consumer choice with both sensory and manufacturing processes. This would lead to the underlying dimensions of consumer choice.

Sausages

UK-Style Sausages

In the UK, expenditure on UK-style sausages increased by over 8% between 2003 and 2005, with a market value of £503.1 million (MLC 2005). However, there has been very little published work on UK sausages, which are unique to the UK and differ from sausages produced in Europe. The sensory characteristics of UK-style sausages were described by Jones et al. (1989). A range of 12 different brands of sausages were purchased from retail outlets. Two cooking procedures were used, oven-baked at 180°C until sausages reached an internal core temperature of 100°C, or grilled, turning every 3 minutes, to the same core temperature. Assessors agreed on a list of 24 descriptors, which also included five hedonic scales. There were four groups of descriptors, based on the appearance of the outside of the sausage (four descriptors), the inside (six descriptors), texture (nine descriptors) and flavor (six descriptors). Data from nineteen intensity scales were submitted to PCA and

GPA analysis, which showed that 86% of the total variation was related to five independent components. The first axis was related to skin toughness, firmness, and meatiness. The second principal axis was related to juiciness and fattiness.

A later UK study by Hope (2007) revisited the work of Jones et al. (1989) and looked at 36 brands of pork sausages, purchased from 10 different retailers. Of the brands studied, five were among the ten most popular sausages in the UK. The objective was to link sensory and declared compositional data. Instrumental texture analysis was not included in this study. The sensory descriptors used were very similar to those quoted earlier and included skin toughness, firmness, meatiness, particle size, and bitterness.

Sausages in this work were grilled, turned every 3 minutes, and cooked to an internal core temperature of 100°C.

Declared meat content was demonstrated to be significantly related to price, skin toughness, firmness, pork flavor, meatiness, particle size, and perceived saltiness.

Fat content was related to fattiness, sweetness, and acidity.

Salt content was related to skin toughness, firmness, meatiness, and particle size, but interestingly not saltiness. It was suggested that this could be that perceived salt content could be influenced by other components in the sausage, such as herbs, spices, MSG (monosodium glutamate), fat content, and lean meat content. The hedonic term "overall liking" was influenced by price, firmness, juiciness, pork flavor, meatiness, and particle size.

PCA analysis of the data set, including compositional, labeling, price information, and sensory attributes showed that the first two principal components accounted for 51% of the total variation. The first principal axis revealed high negative weights related to percentage of meat content, price per kilogram, and the sensory attributes of firmness, meatiness, and particle size. Positive weightings were related to percentage of salt, percentage of fat, and the sensory attributes of cohesiveness and sweetness. The second principal axis gave high negative weightings to fat, and the sensory attributes of juiciness and sweetness, with high positive weightings to the sensory attributes acidic and bitter.

Fermented Sausages

In Northwest Spain a traditional product is the Galician chorizo. It is usually eaten shortly after manufacture, or, for a more extended shelf life, it is often vacuum packed. A more traditional way of extending its storage time is to immerse it in vegetable oil or cover it in pork fat.

Fernández-Fernández et al. (2001) looked at four different ways of preserving chorizo: refrigeration, freezing, oil immersion, and vacuum packing. All chorizos were obtained from the same manufacturer. Sensory panels assessed these products before storage and weekly for a period of six weeks, using a 28-attribute descriptive profile. After four weeks, there was a significantly higher rancid aroma in refrigerated samples. It was concluded that the use of oil immersion and vacuum packing may have masked some of the sensory properties. The most effective method of conservation was freezing, which did not reduce the sensory properties; rancidity was not evident, even after 6 weeks of storage.

There has been considerable interest in improving the desirable fatty acid profile of meat by enrichment with *n-3* PUFA. Valencia et al. (2006) investigated enrichment of dry fermented sausages by substituting pork fat with deodorized fish oil. Total fatty acids in g/100 g of products showed significant differences in the sum of mono-unsaturated fatty acids at 12.3 and 11.98 for control and modified sausages, respectively, and polyunsaturated fatty acids at 4.65 and 5.62, respectively. The n-6/n-3 ratio was 13.86 and 2.97 for control and modified sausage, respectively.

Assessors rated sausage odor, color intensity, juiciness, fish taste, and overall acceptability on 0 to 5 line scales, where 0 is equivalent to the lowest intensity of the attribute and 5 was the highest intensity. Juiciness was slightly higher in the modified products. Some assessors detected a low intensity of fishy taste and also a less intense sausage odor; however, the overall conclusion was that the general acceptability was similar in both products. The conclusion was that it is possible to produce sausages with enriched PUFA n-3 and with a favorable n-6/n-3 without reducing sensory quality.

The use of alternative meat from lesser-used species has also been of recent interest. Soriano et al. (2007) investigated the use of ostrich meat in the production of salchichon, a Spanish sausage. Ten batches of ostrich sausages were obtained from two different Spanish production facilities in Northern Spain. Four batches contained lean ostrich meat, pork belly, salt, black pepper, natural spices, sucrose, and nitrifying salt. Three batches contained ostrich meat, pork ham, salt, black pepper, natural spices, sucrose, and nitrifying salt. A further three batches were obtained from the same facility, but pork ham was omitted from the formulation.

Sixteen assessors took part in these trials, using free choice profiling and rated their descriptors on a 10 cm unstructured line scale from weak to strong. Individual vocabularies varied from 21 to 36 terms. A consensus map was produced after GPA analysis, and the first two dimensions were represented by three different samples groups. Group 1 comprised the first four batches; the next group comprised batches 5, 6, and 7; and the third group comprised batches 8 to 10. Batches 1 to 4 were characterized by a brighter surface and were juicier. Intensity of black pepper was also higher in these samples. Batches 5 to 7 had a less intense taste and odor than other batches, while batches 5 to 7, which included pork ham in the formulation, showed higher intensities for odor and taste. Overall assessors were able to discriminate between the different batches of sausages. Sausages containing ostrich meat were well accepted, and inclusion of pork ham and pork fat in the formulation had positive benefits on the sensory characteristics.

Bacon

Differences in endpoint cooking temperature have been shown to affect texture, flavor, and juiciness. Since bacon is often thinly sliced, there are problems with controlling its final temperature.

In a study (Taylor et al. 1982) on hot curing Wiltshire bacon, where the aim was to produce an acceptable product in 5 days, a combination of expert judges and a sensory panel was used to assess the eating quality of both bacon and gammon joints.

Vacuum-packed samples of collar bacon, both hot and cold cured, stored for either 20 days at 5°C or up to 15 days at 15°C and back bacon that had been stored at 5°C for 35 days were assessed for odor upon opening the packs by a small panel of expert assessors. They concluded that all packs of back bacon were acceptable. However, two packs of hot cured bacon and two packs of cold-cured bacon were judged unacceptable after 20 days at 15°C.

A trained panel was used to assess the cooked bacon for raw appearance of the fat and lean, cooked color, saltiness, flavor, and overall liking.

The method used for cooking the bacon consisted of threading the bacon slices on a wire frame contained within a casserole, such that the bacon was suspended. The casserole dish was then placed in an oven set at 175°C for 35 minutes.

Assessors did not find any significant difference in eating quality attributes. This same study also investigated consumer responses to cooked gammons from hot- and cold-cured treatments. The consumer test used

491 consumers who were asked to assess appearance, flavor, texture, and juiciness. The appearance of hot-cured gammon was judged acceptable by 474 consumers and unacceptable by 17; the corresponding figures for cold-cured gammon were 469 and 22 respectively. On eating the gammon, there were no significant differences for any of the eating-quality attributes.

The general conclusion was that hot curing could produce bacon and gammons of equivalent eating quality to those produced under the more traditional cold-cured process.

White Exudate

A particular problem that can occur in bacon concerns the amount of "white exudate" that is present on cooking. Initially, it appears as a milky white liquid that eventually turns brown and leaves an unsightly residue that adheres to the pan.

From a sensory analysis viewpoint, it is difficult to accurately provide good sensory information when the amount of exudate varies betweens cooking sessions; assessors also need to be present at the time of cooking.

A possible solution was to photograph successive cooking trials of bacon and build up what was essentially a photographic standard. This procedure was adopted by Sheard et al. (2001). Assessors were given 20 photographs of bacon that had been fried. Assessors were asked to rank the photographs in increasing order of exudates. The results followed the procedures given in the British Standard BS5929. This ranking revealed differences in bacon types in relation to the amount of exudates produced. The amount of exudate produced by the different bacon types from least to most was: dry-cured, Wiltshire cured untempered, Wiltshire cured tempered, rapid cured untempered, and rapid cured tempered. A further experiment confirmed the effect that the Wiltshire cured bacon had less exudate loss than the rapid-cured bacon.

Irradiation

This process has been the cause of much dispute among both consumers and regulators, and consequently an impasse has been reached where at present, bacon that has been irradiated is not for sale. However, there has been some research by Risvik (1986) into the effects of irradiation on bacon quality. Bacon was produced, vacuum packed, and then irradiated using a Co^{60} source with doses varying from 0, 1, 2.5, 5.0, and 10.0 KGy.

A ten-descriptor sensory profiling procedure was adopted covering appearance of the fat and lean, fatness, firmness, juiciness, chewing resistance, off-taste, rancidity, and sweetness. Assessors rated the samples on a nine-point linear intensity scale where 1 is at a low intensity and 9 is a high intensity for the attribute. Tests were conducted within one week of irradiation and again after 3 months. Nonirradiated bacon was classified as juicier, sweeter, and with less off or metallic taste. It was noted that off-flavors such as rancidity increased with increasing doses of irradiation. Doses above 1.0 KGy were identified as significantly different from nonirradiated samples.

Pâté

Siret and Issanchou (2000) investigated the influence of traditional processing and nontraditional processes on the eating quality of pâté. The essential differences in production between traditional and nontraditional processes are that in the former, the raw materials used are more diverse; nitrate is used instead of nitrite. The precuring process is longer, comminution is coarser, eggs are used as binding agents, thickeners are not used, and cooking is done in a dry atmosphere. The expectation of the product was that the traditional process would produce pâtés with larger pieces of fat, less smooth texture, and a more complex flavor profile.

A 30-attribute profile was used in this study, which included seven appearance attributes, comprising brown, pink, cohesive, size of fat pieces, size of lean pieces, proportion of fat pieces among all pieces, and proportion of pieces in the stuffing. Thirteen flavor attributes comprised bitter, salty, sweet, alcohol, fat, liver, meat, onion, pepper, seasoning, spicy, thickener, and warmed over. Ten texture attributes comprised sliceability, cohesive, doughy, dry, elastic, fat, firm, granular, heterogeneous, and smooth.

A PCA plot of appearance variables of the pâtés showed that the first two dimensions accounted for 80.53% of the total variation. The traditional pâtés were browner and had a higher proportion of fat pieces. The nontraditional pâtés were pinker, which was probably a result of nitrite being used in this product.

A PCA plot of texture and flavor attributes showed that the first two dimensions accounted for 81% of the total variation. Traditional pâtés were drier, less smooth, and less sweet than those pâtés produced in a nontraditional way. Traditional pâtés also were more elastic, more granular, stronger in fat flavor, and lower in onion flavor.

These same pâtés were also used to study consumer expectations based on information and preconceived knowledge about pâtés. It was found that consumers did not show a preference for traditional pâtés, although when given information, it was shown that the traditional process evoked a favorable response to these products, which led to a higher expectation of quality compared with the nontraditional product, which generally evoked a low expectation. Interestingly, the overall liking for these products was not changed significantly. On eating, it was shown that flavor and texture had a major impact on perceived quality. Consumers did not completely assign their expectation against actual product quality, and therefore it is likely that in a normal retail situation, they could reappraise their selection based on previous product quality experience.

Fat is a major component of pâtés, but in the current climate there are concerns about obesity, heart disease, cholesterol, and the need to reduce fat in the diet. It would therefore be advantageous if some of the fat in pâté could be replaced without impinging on eating quality. A study by Viana et al. (2005) investigated the influence of using bovine globin and plasma as fat replacers. Four ham pâtés were produced, a control containing 26.2% fat, and three other types, 10% globin, 10% plasma, and a combination of 5% globin and 5% plasma that replaced 38.2% of the total fat content.

Twenty-five assessors rated their preference on five-point hedonic scales, where 1 = dislike and 5 = like very much, for color, taste, aroma, and consistency. Results showed that color was affected by treatment. The control pâtés were preferred over those treatments with fat replacer. In terms of taste, aroma, and consistency, there were no significant differences attributable to the fat replacer treatments. The conclusion was that fat replacement by globin, plasma, or a combination could be beneficial in producing quality ham pâtés.

Restructured Meats

It is widely known that texture is an important attribute of any meat product. This is the case with restructured steaks that can range in texture from almost the original muscle through what is almost a beef burger-type product. In all cases, there are many different systems that can produce a wide variety of textural properties; these may be centered on particle size and binding systems.

An essential requirement is the development of a sensory analysis procedure that can provide the tools to evaluate the properties of restructured steaks. Berry and Civille (1986) produced a very detailed procedure to evaluate the texture of steaks that were made from

meat pieces of varying particle sizes. They initially recruited over 135 people; after telephone interviews, this was reduced to 35. These were then given texture profile training following the method of Civille and Szczesniak (1973). This reduced the number of assessors to 15, and further training reduced this number to 10. The final texture profile had five sections: visual; partial compression (placing sample pieces between the molars); first bite, including hardness, cohesiveness, moisture release, and uniformity; mastication, including sample breakdown, juiciness, size of chewed pieces, gristle, cohesiveness, uniformity of mass, webbed connective tissue, number of chews, overall gristle impression, and overall connective tissue; after swallow, including toothpack and mouthcoating.

Using this profile, assessors were able to provide data that classified meat particle size into large, intermediate, and small sizes. Size (rather than shape of chewed particles) after a set number of chews showed a close relationship between processing and raw material variations. It was found that steaks made from large meat particles were rated as having more gristle than either the intermediate or small particles.

One of the consequences of "restructured steaks" is the requirement to bind the meat pieces together so that they do not break up under cooking and can therefore be presented to the consumer as a product similar to an intact steak. Savage et al. (1990) investigated the use of myosin gels to produce adhesion between the meat pieces. Previous mechanical tests had shown that this procedure could produce differences in total adhesive strength. Part of this study used a sensory panel to establish a relationship between added myosin and texture. Ten assessors took part in this study and used a consensus profile method and shortened texture profile that included tactile information: firmness on cutting, crumbliness on cutting, and fibrous particles on cutting. During eating, the assessment included rubberiness, ease of fragmentation, degree of comminution, tenderness, and moistness.

Five formulations were used in this study, with 0%, 1.75%, 3.5%, 5.25%, and 7.0% added myosin. The assumption was that increasing myosin percentages would lead to increasing bind, and indeed the profile accurately tracked this change in texture. It was shown that on cutting, the highest concentration of added myosin did produce an increased impression of firmness. There were fewer crumbs at the cut surface and fewer fibrous particles. The 7.0% myosin gel produced steaks that were more rubbery, less likely to fragment, less tender, and had increased moisture in all the myosin treatments compared with the 0% treatment.

A small in-house consumer test on these same steaks did not produce clear differences in acceptability, indicating that the market for these products was probably segmented.

This question of market segmentation had previously been investigated by Nute et al. (1988) in a study of eight formulations of restructured steaks varying in salt, fat, temper, and blend time using a half replicate of a 2^4 fractional design.

Steaks were made according to the process outlined by Jolley and Rangeley (1986). A hall test approach was used in the north of the UK (72 consumers) and in the south of the UK (70 consumers). At each venue, a mobile sensory testing laboratory was used in conjunction with market researchers who recruited consumers according to their usage of restructured steaks. Using an internal preference mapping approach, the underlying dimension of acceptability was influenced by salt content in the north, but in the south, two dimensions were identified that were related to both salt and fat content. When individual consumers were mapped into the configuration, it was apparent that segmentation was important and no single formulation of restructured steak would satisfy every consumer.

Conclusion

This chapter attempts to give examples of sensory analysis and the results obtained over a range of different meat products. It is by no means an exhaustive review of all meat products, but it does serve to indicate that no single approach will answer questions on composition and sensory characteristics. Sensory properties are wide and varied across these very different products. It is therefore necessary at the onset to understand the questions that are being asked.

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Chapter 27

Detection of Chemical Hazards

Milagro Reig and Fidel Toldrá

Introduction

The development of modern analytical technologies linked to epidemiologic studies and investigations on the safety aspects of food components has revealed in recent decades that some toxic compounds may be present or generated in certain types of meat and processed meat. Three groups of compounds have received great attention because they are potential carcinogens, and, even when not present in the raw meat, they can be generated during further processing or cooking. These groups are nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. N-nitrosamines can be generated under certain processing conditions when using nitrite as a preservative. The polycyclic aromatic hydrocarbons (PAH), which are generated in certain smoking processes, can also be produced when cooking at high temperatures. This is also the case with the heterocyclic amines, which are generated when cooking at high temperatures. In fact, diets associated with meats cooked at high temperatures may increase the risk of certain types of cancer (Jakszyn et al. 2004). Other compounds that can also exert adverse effects on consumers' health are the biogenic amines that are generated by the microbial decarboxylation of certain amino acids, which have potential vasoactive or psychoactive toxicological effects. Amines may also constitute a source of secondary amines that can react with nitrite and produce nitrosamines with potential carcinogenic activity. Finally, other toxic compounds may result from the oxidation of lipids and proteins, as well as other products that may be present in the raw materials used for processing (veterinary drug residues, environmental contaminants, etc.). The main routes for the presence of these compounds in meat are summarized in Table 27.1.

Controls and effective corrective measures (i.e., control of raw materials, reduction in the addition of nitrite, control of microbial decarboxylase activity, etc.) can been taken to minimize this problem in cooked, cured, and dry-cured meat products. All these hazardous compounds and the main analytical techniques used for their control are briefly described in this chapter.

N-nitrosamines

Nitrosamines are N-nitroso compounds that have received much attention in the last 50 years due to their recognition as potential carcinogenic compounds. Nitrosamines are formed in cured meats through the reaction of nitrous acid in its dissociated form, generated from nitrite, with secondary amines. Some of the most important nitrosamines detected in cured meats are N-nitrosodimethylamine, N-nitrosopirrolidine, N-nitrosopiperidine, N-nitrosodiethylamine, N-nitrosodi-n-propylamine, N-nitrosomorpholine, and N-nitrosoethylmethylamine. Some are carcinogenic in a wide range of animal species. In addition, a large number of nonvolatile nitroso compounds, higher in

Table 27.1. Potential sources of exposure to contaminants and conditions affecting their presence in meat and processed meat

Group of compounds	Exposure source	Conditions affecting
Nitrosamines	Cured meats	Available residual nitrite in cured meats with presence of secondary amines and catalyzed by temperature
Biogenic amines	Fermented meats or non-hygienic meats	Hygiene and/or type of microbial flora: decarboxylation of certain amino acids
Heterocyclic amines	Cooked meats at high temperature	Type of cooking of meat, heating temperature and time
Polycyclic aromatic hydrocarbons	Smoked meats	Smoking, especially traditional smoking at high temperature for long time. Smoke flavorings if not adequately treated
Lipid oxidation	Cooked or processed meats	Chemical reactions during storage or processing catalyzed by oxygen, salt, hydrogen peroxide, radiations, lypoxigenase, temperature.
Protein oxidation	Cooked or processed meats	Chemical reactions during processing catalyzed by oxygen reactive species and temperature
Veterinary drugs	All meats	Intentional addition to feed or water given to animals
Growth promoters	All meats	Intentional addition to feed or water given to animals
Environmental contaminants	All meats	Contaminants already present in primary ingredients formulated in feeds given to animals

molecular weight and more polar, have also been reported. Some of the most important are N-nitrosoaminoacids, such as N-nitrososarcosine and N-nitrosothiazolidine-4-carboxylic acid; hydroxylated N-nitrosamines; N-nitroso sugar amino acids; and N-nitrosamides, such as N-nitrosoureas, N-nitrosoguanidines, and N-nitrosopeptides (Pegg and Shahidi 2000).

Nitrite is the main additive used as a preservative in cured meats because of its powerful inhibition of the outgrowth of spores of putrefactive and pathogenic bacteria such as *Clostridium botulinum*. Nitrite has other additional advantages, including the generation of nitrosylmyoglobin that gives the typical pink cured color formation and its contribution to the oxidative stability of lipids and indirectly to cured meat flavor (Ramarathnam 1998).

However, the main concern is related to the residual nitrite remaining in the meat product, because it can be a source of nitrous acid and thus of nitrosamines if secondary amines are also present (Toldrá et al. 2009). The amount of nitrous acid increases when the pH of the product approaches the pKa of nitrous acid (pKa = 3.36). The amount of N-nitrosamines in meat products depends on many variables, such as the amount of added and residual nitrite, processing conditions, the amount of lean meat in the product, heating if any, and the presence of catalysts or inhibitors (Hotchkiss and Vecchio 1985; Walker 1990). A database with the nitrosamine content in 297 food items from 23 countries was recently created with the aim of facilitating the quantification of dietary exposure to potential carcinogens and their relation to certain types of cancer (Jakszyn et al. 2004).

There was intense discussion in the 1970s about residual nitrite in cured meats and the generation of N-nitrosamines in certain cured meat products. The generation rate of nitrosamines depends on many variables, such as the amount of remaining nitrite, presence of nitrosation catalysts or inhibitors, the processing temperature, pH of the product, time, storage conditions, and the addition of reducing substances like ascorbate or isoascorbate. The presence of microorganisms able to generate nitrite from nitrate via nitrate reductase activity or able to produce amines

can also contribute. In general, nitrite rapidly decreases during processing and thus remains at low residual content in the final product (Hill et al. 1973). It was recommended to reduce the levels of nitrites and add ascorbate or erythorbate to favor the reduction of nitrite to nitric oxide and thus, the inhibition of nitrosamines formation (Cassens 1997). Ascorbate is better than ascorbic acid because it reacts with nitrite 240 times faster (Pegg and Shahidi 2000). As an example, the residual nitrite content in fermented sausages was found to be below 20 mg/kg in most of the products surveyed in the late 1990s and early 2000s in Europe (EFSA 2003). Nitrosodimethylamine and nitrosopiperidine were reported as the main nitrosamines found at levels above 1 µg/kg. The levels of nitrosamines were found to be rather poor or even negligible in European fermented sausages assayed in the framework of a European project (Demeyer et al. 2000). Some N-nitrosamines appear to be generated in packaged dry-cured ham because of the reaction of nitrite with amine additives present in the rubber nettings (Sen et al. 1987). Regulations on nitrate and nitrite have been recently modified in the European Union according to the Directive 2006/52/EC of 5 July 2006 that modifies previous Directive 95/2/EC on additives other than colors and sweeteners.

Different types of extraction can be used for the separation of nitrosamines from the meat matrix. These techniques include steam distillation, liquid-liquid extraction, solvent extraction, solid phase extraction, and supercritical fluid extraction (Fiddler and Pensabene 1996; Raoul et al. 1997; Rath and Reyes 2009). Once extracted, volatile N-nitrosamines, or nonvolatile nitrosamines previously derivatized by acylation or trimethylsilylation, are usually analyzed by gas chromatography coupled to a thermal energy analyzer or mass spectrometry detectors in case a specific identification and confirmation is necessary. Nonvolatile nitrosamines are analyzed with liquid chromatography atmospheric pressure chemical ionization mass spectrometry and tandem mass spectrometry (Eerola et al. 1998; Rath and Reyes 2009).

Biogenic Amines

Biogenic amines are produced through microbial decarboxylase activity against precursor amino acids. Consequently, tyramine is produced from tyrosine, tryptamine from tyrptophan, histamine from histidine, phenylethylamine from phenylalanine, cadaverine from lysine, agmatine from arginine, and putrescine from ornithine. Polyamines spermine and sperdimine follow a different generation route, usually originated from putrescine. Low amounts of amines consumed in meats are generally degraded in humans by the enzyme monoamine oxidase (MAO) through oxidative deamination reactions. However, when significant amounts are consumed, some risk situations like hypertensive crisis may appear due to their vasoactive and psychoactive properties.

The presence of these amines in meat may be an indication of its hygienic quality. Thus, the presence of cadaverine and/or putrescine may indicate the presence of contaminating meat flora. In other cases, the processing conditions are very important for the generation of biogenic amines, especially in fermented meats where the decarboxylase activity in any of the microorganisms of natural flora or microbial starters must be carefully controlled. For instance, certain lactic acid bacteria with decarboxylase activity can generate tyramine from tyrosine (Eerola et al. 1996). The estimated tolerance level for this amine is 100–800 mg/kg (Nout 1994), but larger ingested amounts may result in higher blood pressure and the risk of hypertensive crisis (Shalaby 1996). Other amines like phenylethylamine may cause migraine and increases in blood pressure.

Ways to control amine generation are based on the use of starter cultures unable to produce amines but competitive against amine-producing microorganisms; the use of microorganisms having amine oxidase activity; the selection of raw materials of high quality; and good manufacturing practices (Talon et al. 2002; Vidal-Carou et al. 2007).

The analysis of biogenic amines includes a liquid extraction with acid solutions or organic solvents followed by cleanup of the extract. Amines are then analyzed by high performance liquid chromatography with either ion-exchange or reverse-phase, with ion pairs followed by ultraviolet-visible or fluorescence detection. Amines are usually derivatized either pre- or post-column (Vidal-Carou et al. 2009).

Heterocyclic Amines

These amines are formed by the reaction of amino acids, alone or with creatine or creatinine, under high heating such as that achieved in certain types of meat cooking. The main two classes of heterocyclic amines (HAs) are aminoimidazol-quinolines and aminoimidazol-pyridines. The exposure to HAs is mostly due to 2-amino-1-methyl-6-phenylimidazol(4,5,b)pyridine (PhIP) and 2-amino-3,8-dimethylimidazo(4,5,f)quinoxiline (MeIQx). Other minor compounds are 2-amino-9-H-pyrido(2,3,b)indole (AC); 2-amino-3,4-dimethylimidazo(4,5,f)quinoline (IQ); and 2-amino-3,4,8-trimethylimidazo(4,5,f)quinoxiline (DiMeIQx) (Jakszyn et al. 2004). All these amines are closely related to the development of certain types of cancer (Bogen 1994; Augustsson et al. 1999). The assessment of the intake of AHs is difficult because its content in meat depends on the type of cooking, temperature, and time (Bjeldanes et al. 1983). Frying, broiling, and grilling/barbecuing are the cooking methods that produce the largest amounts of HAs because of the very high temperatures used (Sinha et al. 1998). Lower levels of HAs are formed in oven roasting and baking at low temperatures. In general, the direct contact of meat with the heating source facilitates the formation of higher levels of HAs than when the meat is in less intimate contact with the heating device.

Polycyclic Aromatic Hydrocarbons (PAH)

Smoking has been used for centuries as a means of meat preservation and more recently it has been mainly used for its sensory effects on the meat. It has traditionally consisted in the exposure of meat products to the smoke generated by controlled combustion of certain natural hard woods, sometimes accompanied by aromatic herbs and spices. The pyrolysis of wood generates smoke through different oxidation routes. Moist wood chips can also be used for direct generation of smoke. The smoke is condensed and adsorbed on the surface of the meat product. The penetration rate into the product depends on the conditions of the process (temperature, humidity, volatility, and velocity of the smoke). Further information on smoking, its production and application as well as its sensory and antimicrobial effects on meat, may be read in chapter 12.

There are some flavoring substances that exert typical smoke flavor, but the smoke may also contain some health-hazardous compounds like polycyclic aromatic hydrocarbons (PAH), phenols, and formaldehyde (Bem 1995). Polycyclic aromatic hydrocarbons are generated during the thermal decomposition of the wood (over 500°C) when oxygen is limited (Simko 2009a). The most important polycyclic aromatic hydrocarbons are listed in Table 27.2. Some polycyclic aromatic hydrocarbons, especially benzo-a-pyrene, are known to possess cancer-inducing and carcinogenic properties. Most of the PAH have been classified as 2A by the International Agency of Research on Cancer. Information about PAH content in 313 food

items in 23 countries was recently created (Jakszyn et al. 2004). Formaldehyde has been identified as promoting cancerous tumors. Some smoke phenols could react to form highly toxic nitrosophenols that could further react to form toxic reaction products, such as nitrophenols, polymeric nitrosic compounds, and other toxic compounds, or even catalyze the formation of nitrosamines (Bem 1995). The worst situations are found in heavily smoked meat products with old or inadequate smokehouses where the PAH levels could reach amounts near 100 µg/kg (Simko 2009a). In any case, the content in PAH is highly variable because it depends on many variables, including the use of direct or indirect smoking, the type of generator used, the type and composition of wood and herbs, the accessibility to oxygen, and the temperature and time of the process. When technology is correctly applied, the PAH content is below 1 µg/kg. Benzo-a-pyrene (BaP) is being used as an indicator of the presence of PAH in the meat. In fact, the EC Regulation limited its amount to 5 µg/kg in meat and meat products.

Some alternative processes have been designed to reduce the contamination of the smoked meat products with hazardous compounds. Some of these strategies that can reduce significantly the PAH content in smoked meat products consist of the filtration of particles, the use of cooling traps, lower temperatures, and/or reducing the duration of the process. Alternatively, liquid smoke can be obtained through distillation and subsequent condensation of volatile compounds and then applied to the surface of the meat product. This last strategy with extended use consists of the use of the primary products (primary smoke condensates and primary tar fractions) that may be further processed to produce smoke flavorings applied in or on foods. These smoke flavorings can be incorporated at concentrations within the range 0.1%–1.0%. These flavorings are produced from primary products obtained from different woods after specific pyrolisis conditions and extraction protocols. In addition to forming part of smoke flavorings, these primary products can be used as such in foods. Smoke flavorings have a wide variability of compounds, including polycyclic aromatic hydrocarbons (Jennings 1990; Maga 1987). The toxicological effects of smoke flavorings can vary significantly among preparations because these effects depend on many factors, such as the production process of the primary products, the qualitative and quantitative composition, the concentration used in the flavoring, and the final use levels (SCF 1995). Recently, the application of smoke-flavored primary products has been controlled in the European Union through the Council Regulation 2065/2003 on smoke flavorings used or intended for use in or on foods. Under this regulation, the use of a primary product in and on foods will be authorized only if it has been sufficiently demonstrated that it does not present risks to human health. The European Food Safety Authority (EFSA) is in charge of issuing a list of primary products allowed for use as such in/or on food and/or for the production of derived smoke flavorings. Studies on subchronic toxicity and genotoxicity must be performed to evaluate the potential toxicological effects of the primary products used for smoke flavoring.

Regulation 627/2006 implements Regulation 2065/2003 regarding quality criteria for validated analytical methods for sampling, identification, and characterization of primary smoke products (E. C. 2006). The detection of PAH compounds can be performed with gas chromatography, coupled with a flame ionization detector or high-performance liquid chromatography coupled with ultraviolet or fluorescence detectors. The identification and confirmation of PAH may be performed by using mass spectrometry detectors coupled with either gas chromatography or HPLC chromatography. The description of methods of analysis for the

detection and identification of PAH in meat products has been recently reviewed (Simko 2009b).

Oxidation

Lipid-Derived Compounds

Lipid oxidation is a cause of major deterioration in meat and meat products. Triacylglycerols, phospholipids, lipoproteins, and cholesterol constitute the main lipid compounds in meat that are susceptible to oxidation. Phospholipids are very susceptible to oxidation due to their high content in polyunsaturated fatty acids. Oxidation may be induced by light, metal ions (i.e., iron, copper, cobalt, manganese, etc.), or enzymes like lipoxygenase. In the case of induction by lipoxygenase, this enzyme needs activation by a preformed hydroperoxide (Honikel 2009). Lipid oxidation may also be induced by hydrogen peroxide generated by peroxide-forming bacteria during meat fermentation. Lipid oxidation follows a free radical mechanism consisting of 3 steps: initiation, propagation, and termination. Hydroperoxides are the primary products of oxidation, but they are relatively unstable and odorless, while the secondary products of oxidation can contribute to off-flavors, color deterioration, and potential generation of toxic compounds (Kanner 1994). These compounds are aldehydes, ketones, alkanes, alkenes, alcohols, esters, acids, and hydrocarbons. The development of rancid taste is associated with lipid oxidation, mainly aldehydes that have low threshold values. Some products of lipid oxidation may be chronic toxicants, and high levels have been reported to contribute to aging, cancer, and cardiovascular diseases (Hotchkiss and Parker 1990).

There are several methods for the measurement of lipid oxidation in meat products. One of the most common methods is the TBARS that consists of the spectrophotometric determination of malondialdehyde (MDA) formation as an index of the oxidative status. This method is not specific and is subject to some errors. An interesting alternative that is quite usual is based on the analysis of aldehydes, especially hexanal, by static headspace-gas chromatography, dynamic headspace-gas chromatography, or solid phase microextraction-gas chromatography (Ross and Smith 2006).

Cholesterol oxidation may occur through an autoxidative process or in conjunction with fatty acid oxidation (Hotchkiss and Parker 1990). Cholesterol oxides are considered to be prejudicial for health due to their role in arteriosclerotic plaque but can also be mutagenic, carcinogenic, and cytotoxic (Guardiola et al. 1996). Cholesterol oxides may be formed when reheating chilled meat or during the chilling storage of meat. No cholesterol oxides were reported to be detected after the heating of pork sausages (Baggio and Bragagnolo 2006), but studies made on European sausages revealed the generation of up to 1.5 μg/g of cholesterol oxides, even though the percentage of cholesterol oxidation was below 0.17. The major cholesterol oxide found in an Italian sausage was reported to be 7-ketocholesterol, while 5,6α-5,6-epoxycholesterol was the major end product in other analyzed sausages (Demeyer et al. 2000). The reported values were below the toxic levels, as concluded with assays performed with laboratory animals (*in vivo* tests) (Bösinger et al. 1993).

Protein-Derived Compounds

Muscle proteins may be oxidized by reactive oxygen species—for instance, the hydrogen peroxide generated by certain bacteria during meat fermentation. Oxidative damage of proteins may result in degradation or polymerization of myofibrillar proteins and alter their functionality in properties such as gelation, emulsification, solubility, and water-holding capacity (Ooizumi and Xiong 2004). The main modifications of amino acids by oxida-

tion, especially proline, arginine, lysine, methionine, and cysteine residues, consist of the formation of carbonyl derivatives (Giulivi et al. 2003). The formation of carbonyl compounds can be used as a kind of measurement of protein damage by oxygen radicals under processing conditions. Other oxidative mechanisms consist of thiol oxidation and aromatic hydroxylation (Morzel et al. 2006). Sulfur amino acids of proteins are those more susceptible to oxidation by peroxide reagents, like hydrogen peroxide. Consequently, cystine is oxidized only partly to cysteic acid, while methionine is oxidized to methionine sulfoxide and methionine sulfone in small amounts (Slump and Schreuder 1973). Sulfinic and cysteic acids can also be produced by direct oxidation of cysteine (Finley et al. 1981). The oxidation of homocystine can generate homolanthionine sulfoxide as a main product (Lipton et al. 1977). Peptides such as reduced glutathione can also be oxidized by hydrogen peroxide. The oxidation rates increase with the pH, and most of the cysteine in the glutathione is oxidized to the monoxide or dioxide forms.

A method used for the quantification of carbonyl compounds in meat and meat products is based on the derivatization of carbonyl protein groups with the 2,4-dinitrophenylhydrazine (DNPH) to form hydrazones, and then the absorbance is measured at 370 nm (Oliver et al. 1987). Another method to evaluate protein oxidation is based on the conjugated fluorophores resulting from reactions between lipid oxidation products (aldehydes) and amino groups. This fluorescence can be detected at excitation and emission wavelengths of 350 and 450 nm, respectively (Viljanen et al. 2004). But these methods are unspecific and may give gross margins of error. Recently, a method based on the measurement of α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively) was considered to be a good alternative to measure specific biomarkers of oxidative damage (Estévez et al. 2008). Both semialdehydes are formed as the main carbonyl products from metal-catalyzed oxidized proteins. This method uses liquid chromatography-electrospray ionization mass spectrometry and was recently applied to a survey of protein oxidation in different meat products. The results showed that dry-cured ham and dry-cured sausages had the highest amount of GGS, followed by liver pâté and cooked sausages. Ground meat had the lowest GGS levels (Armenteros et al. 2009).

Veterinary Drugs and Growth Promoters Residues

Veterinary pharmaceutical drugs have been used for a long time in animal production as therapeutic agents to control infectious diseases or as prophylactic agents to prevent outbreaks of diseases and control parasitic infections (Dixon 2001). Meanwhile, growth-promoting agents like the anabolic agents are added to improve the feed conversion efficiency by increasing the lean-to-fat ratio, while antimicrobial agents are added to make more nutrients available to the animal and not to the gut bacteria. In recent years, there has been an increasing concern regarding the development of increased bacterial resistance to certain antibiotics due to the abuse of antibiotics consumption (Butaye et al. 2001).

Most veterinary drugs have been banned in the European Union for use in farm animals because of fears about health effects (genotoxic, immunotoxic, carcinogenic, or endocrine) from their residues in animal tissues. These substances can only be administered to animals for therapeutic purposes under strict control of a responsible veterinarian (Van Peteghem and Daeselaire 2004). Antibiotics were banned due to concerns about the development of antimicrobial resistance (Reig and Toldrá 2009a).

The main veterinary drugs and substances with anabolic effect are listed in Table 27.2.

Table 27.2. List of veterinary drugs and substances with anabolic effect according to classification in Council Directive 96/23/EC (Toldrá and Reig, 2007)

Group A: Substances having anabolic effect	Representative substances
1 Stilbenes	Diethylstilbestrol
2 Anthithyroid agents	Thiouracils, mercaptobenzimidazoles
3 Steroids	
Androgens	Trenbolone acetate
Gestagens	Melengestrol acetate
Estrogens	17-β-estradiol
4 Resorcycilic acid lactones	Zeranol
5 β-agonists	Clenbuterol, mabuterol, salbutamol
6 Other substances	Nitrofurans
Group B: Veterinary drugs	
1 Antibacterial substances	Sulfonamides, tetracyclines, β-lactam, macrolides (tylosin), quinolones, aminoglycosides, carbadox and olaquindox
2 Other veterinary drugs	
Antihelmintics	Benzimidazoles, probenzimidazoles, piperazines, imidazothiazoles, avermectins, tetrahydropyrimidines, anilides
Anticoccidials	Nitroimidazoles, carbanilides, 4-hydroxyquinolones, pyridinols, ionophores
Carbamates and pyrethroids	Esters of carbamic acid, type 1 and 2 pyrethroids
Sedatives	Butyrophenones, promazines, β-blocker carazolol
Non-steroidal anti-inflammatory drugs	Salicylates, pyrazolones, nicotinic acids, phenamates, arylpropionic acids, pyrrolizines
Other pharmacologically active substances	Dexamethasone
Group B: Contaminants	
3 Environmental contaminants	
Organochlorine compounds	PCBs, compounds derived from aromatic, cyclodiene or terpenic hydrocarbons
Organophosphorous compounds	Malathion, phorate
Chemical elements	Heavy metals
Mycotoxins	Aflatoxins, deoxynivalenol, zearalenone
Dyes	
Others	

The illegal addition of any of these substances to farm animals may imply that their residues could remain in the animal-treated derived foods, constituting a risk. As a result, the presence of these substances in farm animals and foods of animal origin must be monitored (Croubels et al. 2004). The presence of these substances in foods and the number of samples to be tested each year is regulated in the European Union by EC Directive 96/23/EC on measures to monitor certain substances and residues in live animals and animal products. The analytical methodology for the monitoring of compliance was given in Decisions 93/256/EEC and 93/257/EEC. The Council Directive 96/23/EC was recently implemented by the Commission Decision 2002/657/EC, which provides rules for the analytical methods to be used in testing official samples and specific common criteria for the interpretation of the analytical results of official control laboratories for such samples. In the United States, the Food Safety and Inspection Services (FSIS) establishes the surveillance programs, including the National Residue Program, the exploratory residue testing programs, and inspector-generated in-plant

residue test samples (Croubels et al. 2004). The FDA Center for Veterinary Medicine issues the analytical criteria. The control of residues of these substances in meats exported to the European Union was further assured by an Additional Testing Program designed by the USDA (Croubels et al. 2004).

The detection of these substances is quite complex due to the large number of samples and the low levels of the substances to be detected. The control is usually based on screening tests like ELISA test kits, antibody-based automatic techniques, or chromatographic techniques (Reig and Toldrá 2008). In the case of antibiotics, microbiological tests such as the European Four Plate Test can be used. Screening tests are useful because they are rapid, but they are unable to confirm the results because they can only give qualitative or semiquantitative data. The next step for suspicious samples (suspected of being noncompliant) is a confirmatory analysis through gas or high performance liquid chromatography coupled with mass spectrometry or other sophisticated methodologies for accurate identification and confirmation of the substance (Toldrá and Reig 2006). The description of methods of analysis for the detection and identification of growth promoters and veterinary drug residues in meat and meat products has been recently reviewed (Reig and Toldrá 2009b, c).

Environmental Contaminants

There are a wide variety of environmental contaminants. The main concern is that they may be present in the feeds consumed by farm animals and thus contaminate the resulting meats. Some well-known contaminants are dioxins, organophosphorous, and organochlorine pesticides. These contaminants are quite extensive worldwide, making their control very difficult. The term "persistent organic pollutants" (POPs) was defined by the United Nations Environment Programme as those persistent chemical substances that can accumulate in foods and cause adverse effects to consumers. Most of the organochlorine pesticides were banned during the 1970s and 1980s, but they are persistent and stable and may remain in the environment for many years, constituting a risk of long-term exposure (Moats 1994). These substances tend to be accumulated in the fatty tissue of living organisms. Current maximum residue limits in the EU for the organochloride pesticides that can be present in animal products are within 0.02 and 1 mg/kg of fat, while in the United States they are established between 0.1 and 7 mg/kg fat (Iamiceli et al. 2009).

The contaminants described above, as well as polychlorinated biphenyls (PCBs) (a family of 209 compounds that were used in lubricating oils and heat exchange fluids), mycotoxins produced by molds and marine toxins, and heavy metals, among others, can be present in feeds used for farm animals. The reasons for such contamination are varied: use of contaminated ingredients, lack of control of the ingredients, inadequate processing, growth of molds in feed grains and meals, etc. (Croubels et al. 2004). Environmental contaminants are rather difficult to control, even though they can exert potential toxicity in the product (Heggum 2004). There are recent reviews on the methods of analysis for the detection and identification of persistent organic pollutants in meat (Iamiceli et al. 2009) and polychlorinated byphenils in meat products (García-Regueiro and Castellari 2009).

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Chapter 28

Microbial Hazards in Foods: Food-Borne Infections and Intoxications

Daniel Y. C. Fung

Introduction

Food is essential for human survival. Food is also essential for microorganisms to grow and thrive. Therefore, for the protection of human health and well-being, it is important to understand the microbial hazards in foods. All through history, human beings were no doubt affected by a great variety of food-borne diseases through their consumption of water and food.

No one knows for certain the number of cases of food-borne intoxications and infections occurring annually in the world. There are an estimated 76 million cases of food-borne illnesses in the United States every year, resulting in $5–17 billion in economic and productivity losses annually. Food-borne diseases caused more than 5,000 deaths a year over the last 10 years. In countries with poor sanitation, one can only surmise that the number of food-borne disease cases is much higher.

Efforts have been made to ensure better food safety and to control all kinds of pathogens. In the United States, consumers have a heightened awareness of the role of food-borne diseases due to some sensational outbreaks of food-borne diseases affecting large numbers of people, including children who died after consuming undercooked hamburger, tainted cheeses, or some other contaminated food. As a result, the U.S. government has implemented tighter monitoring programs and Hazard Analysis Critical Control Point (HACCP) procedures in the food industry to attempt to curtail the outbreaks and protect the safety of consumers. Consumers are much more aware of the great potential for large-scale food-borne outbreaks and demand a safer food supply. At the same time, consumers are also demanding more fresh foods, minimally processed food, and organic foods, where control of food-borne pathogens is more difficult. There is also a drastic demographic change in society where more and more people live longer, and yet aging also tends to weaken the immune system, making these individuals more vulnerable to food-borne pathogens. People with immunocompromising diseases are also surviving longer, but they too are more susceptible to food-borne disease. Meanwhile, food distribution systems have also been greatly improved, and thus production of food in one location can be transported hundreds and thousands of miles in a short time. When a problem occurs, the amount of food involved can be astronomical, such as the case where 25 million pounds of ground beef were recalled due to one contamination source of *Escherichia coli* O157 : H7; the company involved is no longer in existence as a major player in food supply. Another important development is international trade. Vast amounts of food are regularly shipped from one country to another with minimal monitoring of the microbial safety of the food involved. To complicate matters further, new microorganisms are

emerging or reemerging in the food supply, which makes tracking and controlling these organisms more difficult. In addition, the outbreaks can transcend national boundaries. In 2008 in the United States, an outbreak of food-borne illness involving spinach consumption resulted in an international effort to trace the origin of the disease, which was eventually tracked back to Mexico. Peanut products also became a national sensational case and many products were recalled in the United States in 2008. *Salmonella* was the culprit in that particular case. The list is long; suffice it to say that outbreaks are constantly evolving, while microbiologists are kept busy tracking down these food-borne diseases.

Fortunately, new developments in microbial detection offer new methods and systems to detect these organisms. Also, there are better and more efficient intervention strategies and food-processing methods to control unwanted microorganisms. Thus, food microbiologists, food scientists, epidemiologists, medical personnel, public health workers, and consumer educators are charged with the responsibility of studying the occurrence, enumeration, isolation, detection, characterization, prevention, reporting, and control of food-borne microorganisms in food, water, and the environment—and then educating the public in order to reduce microbial hazards in food nationally and internationally.

Synopsis of *Introduction to Food Microbiology* by Fung (2009b)

Microorganisms are ubiquitous in our environment, and they affect our daily lives through their prolific biochemical activities under ideal growth conditions. All living things less than 0.1 mm in diameter fall into the microscopic world of microbes. The microbial world includes viruses, bacteria, yeasts, molds, protozoa, algae, and other organisms that at different growth stages are too small to be seen without a microscope. On the one hand, microorganisms can be beneficial to humans because of their role in various geochemical cycles, such as the phosphorous cycle, carbon and oxygen cycles, nitrogen cycle, and sulfur cycle; without them, the earth would not be livable for humans. They are also important in various fermented foods, such as wine, cheese, beer, vinegar, bread, and soybean products, and in the production of industrially important acids, solvents, antibiotics, steroids, enzymes, etc. They can even be eaten as foods such as yeasts and single-cell proteins. On the other hand, microorganisms can spoil our food supplies and cause devastating disease in animals and humans; if unchecked, they could even destroy the human race.

From the standpoint of the microorganisms, however, they are simply trying to fulfill their biological need to grow and perpetuate themselves via sexual and asexual reproduction. Like humans, they need water, carbohydrates, protein, fat, mineral, vitamins, and the right combinations of gases, temperature, pH, and other conditions in order to grow and multiply and survive. Therefore, there are no "good" microorganisms or "bad" microorganisms in nature; we can consider them harmful or beneficial according to how they affect us.

Definitions

There are three major microbial hazards in food: spoilage microorganisms, food-borne intoxications, and food-borne infections.

Spoilage Microorganisms

When large numbers of undesirable microorganisms are present in raw, contaminated cooked, or fermented food supplies, they compete with the space and utilization of the food's nutrients; these are considered to be spoilage microorganisms. Occasionally, the

effects of consuming the food are minimal. But if the contamination of bacteria, yeasts, and fungi levels are too high, then there will be undesirable physical, chemical, and biochemical changes, such that the food involved becomes unappealing to human senses and not suitable for consumption.

Food-Borne Intoxication

Toxic compounds in foods may come from chemical contamination or be preformed by toxigenic microorganisms. When these toxins are consumed, susceptible persons will become ill.

Food-Borne Infection

Food-borne infection takes place when microorganisms are ingested from food, and then continue to grow in the gastrointestinal tract, causing susceptible persons to become ill. For this to happen, usually the number of microorganisms present in the food needs to be around one million live organisms per gram of food, but there are cases when as low as 100 cells and even 10 or fewer cells per gram of food can cause infection.

Food Poisoning

Food poisoning is the ingestion of contaminated food containing either live microbes that produce toxins in the gastrointestinal tracts of susceptible persons or chemical preformed toxins, causing the individual to then become ill.

Food-Borne Outbreak

A food-borne outbreak is the consumption of contaminated food from one source by two or many people who later became ill. A food-borne outbreak can have two cases or 100,000 cases, with the exception of botulism, in which case, one individual with botulism is considered one outbreak.

Food-Borne Disease Cases

A food-borne disease case is the consumption of contaminated food by one susceptible person who later became ill.

Endemic

An endemic occurs when a particular illness affects an entire community.

Epidemic

An epidemic is an unusually large number of cases of a particular illness from a single source in a community.

Pandemic

A disease is said to be pandemic when it affects the entire world.

Epidemiology

Epidemiology is the study of diseases in a population using statistical methods. An epidemiologist studies patterns of diseases and their causative agents in terms of a population, whereas a physician treats individual patients.

Etiologic Agent

An etiologic agent is the agent that caused a specific disease.

Food-Borne Intoxications

Chemical intoxications are usually the result of accidents. People have been poisoned by inorganic compounds such as antimony, arsenic, cyanide, cadmium, lead, selenium, and mercury. The symptoms usually occur rapidly (in a few minutes or hours), and in cases of ingestion of large doses of the toxic compounds, reactions are usually violent. Immediate medical assistance is essential for the victims in such cases.

Long-term chemical intoxication is also possible from the ingestion of small amounts of toxins in food or water over many years, which eventually causes the person to become ill.

There are many naturally occurring chemical toxins in foods as well as unintentionally added chemical toxins, such as pesticides and chemical residuals from packaging materials and the environment.

Bacterial and Microbial Intoxication

This form of intoxication is the result of susceptible persons consuming microorganisms in food, which then form toxic substances in the gut. The effects can be rapid (within hours), but usually the process is longer than chemical intoxication (such as from cyanide or arsenic).

Clostridium botulinum

The first recorded outbreak of botulism was in 1793, involving sausages (botulus) in Germany. Since that time, many outbreaks all over the world have been reported. From 1899 to 1977, there were 766 outbreaks, involving 1,961 cases and 999 deaths. In the United States between 1971 and 1985, three outbreaks were recorded with 485 cases and 55 deaths. In 1990, there were 12 outbreaks, 22 cases, and 5 deaths, and in 1994 there were 42 cases of food-borne botulism, 86 cases of infant botulism, and 11 cases of wound botulism with no fatality. In 2002, the CDC (U.S. Centers for Disease and Control, Atlanta, Georgia) reported that there were 13 outbreaks, 56 cases, and 1 death. (This information was gathered from 1993 to 1997.) Up-dated information, organized according to etiology, about the number of reported food-borne diseases, outbreaks, cases, and deaths from all reportable food-borne disease in the United States can be obtained at the CDC's website. Due to food professionals' and consumers' growing awareness of this disease, the outbreaks of botulism have been decreasing in recent years. Because of botulism's high fatality rate, the general public usually panics at reports of botulism.

The organism is a Gram-positive, anaerobic, spore-forming rod that can grow at temperatures from 3.3°C to as high as 50°C. Most strains will grow well at 30°C with optimum temperature at 37°C. To control the growth of the organism, the pH must be below 4.6, salt content needs to be 10% or more, and a water activity less than 0.94. The vegetative cells of this organism are easily killed by heat, but the spores formed by this cell are far more resistant to heat, cold, acid and basic chemicals, radiation, and other forms of preservation methods. Thus, control of botulism is geared to the destruction of the spores. Time and temperature combinations for canning all foods are designed to kill the most heat-resistant spores of *Clostridium botulinum*.

The spores formed by this organism can reside in soil, water, and the environment, and can be transmitted to foods. Foods involved in botulism cases usually are improperly home-canned, medium- or low-acid foods. Information since 1899 indicates that about 70% of the outbreaks can be traced to improperly processed, home-canned foods and 9% to commercially processed food, with the other outbreaks from unknown sources.

Symptoms develop from 18 to 96 hours after the ingestion of toxic foods. They include vomiting, nausea, fatigue, dizziness, vertigo, headache, dryness of mouth, muscle paralysis, and death by asphyxiation. Since the toxin affects peripheral nerves, the patient is alert until the moment of death. There are several types of botulin toxins (types A, B, C_1, C_2, D, E, F, and G). These are large molecular weight proteins (about 1 million dalton). The important toxins affecting human beings are toxins A, B, E, and rarely F. These are among the most toxic materials

produced by a biologic system. It has been estimated that one pure ounce of toxin can kill 200 million people. Treatment is by the administration of monovalent E, bivalent AB, trivalent ABE, or polyvalent ABCDEF antisera.

The toxins can be detected by animal tests using mice, as well as immunologic tests using specific antibodies (gel diffusion tests, ELISA, RIA tests, etc). Recently, a rapid Polymerase Chain Reaction (PCR) method has been employed to detect the botulin gene harbored by cultures isolated from foods. The information, however, does not directly imply that the food is toxigenic and harbors the toxin.

Fortunately, the toxins are heat sensitive. Boiling of the toxin for 10 minutes will destroy it. The key to preventing botulism is to know the composition (pH, A_w oxidation-reduction potential, presence of inhibitory compounds, etc.) of the food and to utilize proper time and temperature for processing as well as correct packaging and storage of the processed food. All high-moisture, low-acid foods processed and then stored under anaerobic conditions, whether in cans, glass bottles, or pouches, should be subject to close scrutiny to avoid the possibility of *C. botulinum* surviving and later germinating and producing the toxins. Since there are proteolytic and nonproteolytic strains of *C. botulinum,* the absence of off-odor from a suspected canned food cannot guarantee the safety of the food. Never taste a suspected food or use food from swollen, dented, or deformed cans. When in doubt, always boil the suspected food for 10 minutes before discarding it. The use of nitrites in fermented meat such as sausages is to prevent the germination of the *Clostridium botulinum* spores.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive, facultative anaerobic coccus occurring in clusters. The organism is ubiquitous and can be found on human skin, nose, hair, and many food items. The organisms, when allowed to grow in food, may produce a class of low molecular weight (ca 30,000 daltons) protein toxins called staphylococcal enterotoxins (A, B, C_1, C_2, C_3, D, E, and maybe others). These toxins, when ingested by a susceptible person, will cause severe nausea, vomiting, abdominal cramps, diarrhea, and prostration about 4 to 6 hours after consumption. Recovery is about 24 to 72 hours. Victims usually will not die, but they may wish they had, as the reactions are very violent. Also, there is no immunity against the toxins; thus a person can have staphylococcal intoxication repeatedly. Along with *Salmonella* and *Clostridium perfringens*, staphylococcal intoxication has ranked among the top-three agents of food-borne disease in the past 40 years. In 2002, there were 42 outbreaks, involving 1,413 cases and 1 death. Since this is a nonreportable disease, many more outbreaks and cases occur regularly without being known to public health officials.

These toxins are heat stable. Once formed in food, the toxin is very hard to destroy. Heating the toxins at 80°C for 5 hours will not destroy the toxin. Boiling for 3 hours will destroy the toxin, and cooking under pressure (121°C) will inactivate the toxin in 30 minutes. For practical purposes, these toxins are not inactivated by normal cooking procedures.

Due to the fact that the enterotoxins are heat stable, detection of live *Staphylococcus aureus* in foods has only limited value in terms of assessing the potential of the food to cause staphylococcal food intoxication. For example, if a food is contaminated with a toxigenic strain of *S. aureus* and the organisms grow to 1 million cells, they will then release large amounts of enterotoxins (in micrograms) into the food. When the food is subsequently cooked, even though live *S. aureus* cannot be found in it, the food is still capable of causing a case of staphylococcal

intoxication due to the heat-stable toxins in the food. Several years ago in the United States, imported canned mushrooms caused a great deal of concern. No live *S. aureus* were found in the canned mushrooms, yet the preformed enterotoxins in the mushrooms caused many cases of food intoxication.

The value of monitoring live *S. aureus* is to ascertain the hygienic quality of the food and the potential of the live organisms to grow and produce the enterotoxins in foods. Detection of staphylococcal enterotoxins has been a subject of much research in the past 35 years. Monkeys, cats, and other animals have been used to detect toxins, but using these animals is not practical for routine testing. Immunological methods such as ELISA test, Latex agglutination tests, and gel diffusion tests are used to detect the toxin in foods. The commercial kits can detect enterotoxin A, B, C, D, and/or E. either singularly or in combination.

Fortunately, the organism is not a good competitor compared with other spoilage organisms (e.g., *Pseudomonas*) in raw foods such as ground beef and fish. However, in the absence of competitors, such as in salty food (e.g., ham) or processed foods (e.g., processed cheese), the organism can grow and produce the heat-stable toxins. They can produce enough toxins in 4 hours at room temperature to cause a problem. That is the reason why this intoxication is called picnic food poisoning because during a picnic, food may be left nonrefrigerated for hours before consumption by partygoers. It is, therefore, essential to use proper refrigeration (4°C) to prevent *S. aureus* from growing in the food or by keeping hot food hot (60°C). This advice is applicable to all subsequent discussions on food intoxication and infections.

Aspergillus

Aspergillus flavus and *A. parasiticus* are molds that can produce a group of carcinogenic toxins called aflatoxins. In 1960 in England, 100,000 turkeys died of unknown causes, and the disease was called Turkey X disease. After much work, the contaminant was found to have originated from peanut meal from Brazil. The organisms responsible for producing the toxic compounds were identified as *A. flavus*. Later, *A. parasiticus* was also found to be able to produce the toxin. Recently, *A. nomius* has also been added to the list of cultures producing the aflatoxin. The mold can grow between 7.5°C and 43°C, with optimal temperatures at 24° to 28°C. The minimal water activity for growth is 0.82 and the optimal is 0.99. The pH range for growth is from 2 to near 11. Production of the toxins generally parallels the growth of the organisms. Research data from the author's laboratory suggested that sporulation of the cultures seems to be a prerequisite for toxin production. In 1 to 3 days of growth, the organism can produce the toxins. The primary toxins are B_1, B_2, G_1, and G_2. B and G indicate that the toxins fluoresce blue or green under ultraviolet light, respectively. When cows consume B_1 and B_2 toxins, they can modify the toxins and excrete the toxins as M_1 and M_2 in milk.

Spores of these molds are ubiquitous; the organisms have been found to grow in rice, sorghum, peanut, corn, wheat, and soybean crops, as well as animal feed. Human food shown to support growth and toxin production of this mold include peanuts, peanut butter, pecans, beans, dried fruits, fish, and even cheese. Because the toxins are carcinogenic, they are under strict government scrutiny, since the Delaney Clause of 1958 prohibits the presence of carcinogenic compounds in U.S. foods. Currently, the allowed limit is 20 ppb for animal feed and all foods, except milk, which has an action level of 0.5 aflatoxin M_1. Although no direct food-related aflatoxin cases have been reported in the United States, there are concerns that aflatoxin can affect the immune systems of patients. Aflatoxin fatality cases were reported in Southeast Asia when people con-

sumed food heavily contaminated with molds. The toxins can be detected by animal tests using ducklings or chick embryo. Thin-layer chromatography and high-performance liquid chromatography can also be used to detect these toxins. Recently, monoclonal antibodies have been employed to detect these toxins with great rapidity (10 to 30 min) and sensitivity (1 ppb and lower). Attempts to detoxify aflatoxin by ozone, peroxides, and ammonia have met with limited success. Thus, the best preventive measure is not to allow the mold to contaminate the food and feed, and keep these commodities in a dry environment unfavorable for mold growth.

Exotoxins versus Endotoxins

It is necessary to differentiate these toxins before having a discussion on food-borne infections.

Exotoxins are toxins produced by an organism and later released into the environment. The cell remains alive and intact. Ingestion of these preformed toxins causes food-borne intoxication. These toxins are protein toxins, mainly produced by Gram-positive organisms. Because they are proteins, they can be neutralized by corresponding antibodies and detected by a variety of immunologic methods. These toxins are relatively heat sensitive (except the staphylococcal enterotoxins described earlier). These toxins also have a distinct pharmacology. Examples of exotoxins are staphylococcal enterotoxins (affecting the intestinal tracts) and botulinum neurotoxins (affecting the nervous system).

Endotoxins are part of the cell wall material of Gram-negative cells. Every Gram-negative bacterium examined has endotoxins. These are complex molecules containing protein, carbohydrate, and lipid. The protein moiety determines antigenicity, the carbohydrate moiety determines immunologic specificity, and the lipid moiety causes toxicity. Unlike exotoxins, antibodies will not neutralize toxicity because the toxic part is the lipid. All endotoxins have the same action and are released when the Gram-negative bacterium undergoes lysis. These endotoxins cause fever by acting as exogenous pyrogens. The exogenous pyrogen, when absorbed into the bloodstream, causes injury to the leukocytes, which in turn release an endogenous pyrogen. This endogenous pyrogen stimulates the thermoregulatory center of the brain at the hypothalamus and causes fever. Therefore, fever in a patient is indicative of a food-borne infection case.

Endotoxins can be detected by the limulus amebocyte lysate (LAL) test. In the presence of endotoxins, the LAL will form a gel. The reaction takes about 1 hour. Hospital materials should be pyrogen-free, and LAL is the standard test for pyrogens in hospital supplies and environment. Because endotoxins are released upon lysis of the cell, it is advisable in certain cases that antibiotics not be administered in mild food infection cases. Lysis of cells by antibiotics intended for other infections such as *Escherichia coli* O157:H7 may allow the release of other harmful toxins in the intestinal tract, causing a more severe infection case.

Bacterial Infection

Clostridium perfringens

Clostridium perfringens occupies an interesting position, since it is both a food-borne infection agent as well as a food-borne intoxication agent. On the one hand, the susceptible person has to ingest large numbers of viable *C. perfringens* before coming down with a food-poisoning case, and on the other hand, the organism produces an enterotoxin to cause the illness. In 2002 in the United States, there were 57 outbreaks and 2,772 cases, with no deaths reported. It is estimated, however, that 250,000 cases occur annually, with an average of 7.6 deaths per year at an

annual cost of $123 million to the U.S. economy. *C. perfringens* is a Gram-positive anaerobic spore-forming rod and produces at least 13 different toxins, which can cause diseases such as gas gangrene. One of the toxins is named *Clostridium perfringens* enterotoxin (CPE), which is released in the intestinal tract and causes infection/intoxication by this organism. Spores of this organism naturally distribute widely and can easily contaminate foods.

Most of the incidents of *C. perfringens* food poisoning involve meats prepared in large quantities one day and consumed the next day, after the food has been kept at lukewarm temperatures. In such conditions, most vegetative cells of competitors die off, while the spores of *C. perfringens* have a chance to survive, germinate, and grow into large numbers (about 10,000 to 1 million per gram). When ingested by a susceptible person, these will start to sporulate in the small intestine due to the favorable anaerobic environment there. The gene coded for sporulation also controls the release of an enterotoxin that is responsible for the diarrhea characteristics of *C. perfringens* food poisoning. It is noteworthy that *C. perfringens* does not sporulate in foods, and therefore, the CPE is not preformed in food to cause food-poisoning cases.

Symptoms occur between 8 and 20 hours after ingestion of a large number of viable *C. perfringens* and include acute abdominal pain, diarrhea, and nausea, with rare vomiting. The symptoms are milder than those caused by *Salmonella*. Detection of this organism is by anaerobic cultivation of food using differential anaerobic agar, such as tryptose sulfite cycloserine agar. *C. perfringens* forms black colonies in this agar medium. Immunologic methods such as reverse-passive agglutination assay and ELISA test have been developed to detect the CPE in food, culture fluid, and feces.

Recently, in the State of Hawaii, there has been interest in using *C. perfringens* as the indicator of fecal contamination in recreational water. Fung et al. (2007) developed the Fung Double Tube (FDT) system, which can detect live *C. perfringens* in the tubes about 5 hour after sampling the seawater. The generation time (time for doubling of a population of cells) of *C. perfringens* in ideal conditions, such as in the FDT, is as short as 7.1 min at 42°C. This is the fastest method known to obtain visible colony-forming units of any bacteria in an agar system. More recently, in 2009, by adding the phosphatase test to the agar system, the black presumptive *C. perfringens* colonies can be confirmed as *C. perfringens* in the FDT, due to the fluoresces surrounding the black colony. With more confirmation, this test will help authorities determine when to close beaches to protect the public from contamination by fecal microbes in recreational waters.

Salmonella

Salmonella is the classic example of food-borne infection. *Salmonella enteritidis* was isolated in 1884 and still is an important food-borne organism. In 2002, there were 357 outbreaks, with 32,610 cases and 13 deaths due to *Salmonella* reported in the United States. The organism is a Gram-negative, facultative anaerobic, non-spore-forming rod, motile by peritrichous flagella. It does not ferment lactose and sucrose but ferments dulcitol, mannitol, and glucose. There are exceptions to the general characteristics. For example, lactose-positive cultures have been found, and nonmotile species exist, such as *S. pullorum* and *S. gallinarum.* The organism is heat sensitive but can tolerate a variety of chemicals, such as brilliant green, sodium lauryl sulfite, selenite, and tetrathionate. These compounds have been used for the selective isolation of this organism from food and water. To confirm the isolate as *Salmonella*, one must perform serologic tests using polyvalent anti-O antiserum (against cell surface antigens) or

polyvalent anti-H antiserum (against flagella antigens).

This genus went through several revisions in classification and taxonomy of species and subspecies in the past 20 years, due to the advancement of genetic typing systems. Currently, there are two species, namely, *S. enterica* with six subspecies and 2,356 serovars, and *S. bongori* with 19 serovars. Each serovar is potentially pathogenic. In the literature, many scientists still use the traditional genus and species nomenclature, such as *S. typhimurium, S. typhosa,* etc. The current accurate way to present *Salmonella* in the literature is to use, for example, "*Salmonella enterica* serovar Typhimurium." Note that the word "Typhimurium" is NOT italicized. When used in a short version the proper way is to spell it as follows: *Salmonella* Typhimurium. This is different from the traditional way of spelling, which would be "*Salmonella typhimurium*" (italicized and not capitalized for the first letter).

Salmonella has been found in water, ice, milk, dairy products, shellfish, poultry and poultry meat products, eggs and egg products, animal feed, and pets. Human beings can be healthy carriers of this organism. It has been estimated that 4% of the general public carries this organism, with more females than males being healthy carriers.

There are actually three types of diseases caused by *Salmonell*a: enteric fever caused by *S.* Typhosa (typhoid fever), in which the organism, ingested along with food, finds its way into the bloodstream, disseminates to the kidney, and is excreted in the stools; septicemia caused by *S.* Cholerasuis, in which the organism causes blood poisoning; and gastroenteritis caused by *S.* Typhimurium and *S.* Enteritidis, a true food-borne infection. In the last case, large numbers of live *Salmonella* are ingested with food; in 1 to 3 days, they liberate the endotoxins, which cause localized violent irritation of the mucous membrane, with no invasion of the bloodstream and no distribution to other organs. Symptoms of salmonelosis occur 12 to 24 hours after ingestion of food containing 1 to 10 million *Salmonella* per gram and include nausea, vomiting, headache, chills, diarrhea, and fever. The illness lasts for 2 to 3 days. Most patients recover; however, death can occur in the very old, the very young, and those with compromised immune systems.

Since no *Salmonella* is allowed in cooked food for interstate commerce and international trade, the detection of *Salmonella* has been a subject of much research and development.

Detection of *Salmonella* by the classical method includes pre-enrichment of culture from food samples, enrichment or selective enrichment of the liquid culture, plating of liquid on selective agar to isolate cultures, biochemical tests of suspect colonies, and confirmation of isolates with typical biochemical profiles by serological tests. These procedures may take up to 5 days for completion. Recently, a variety of methods and procedures have been developed and implemented for the effective isolation, enumeration, detection, identification, and characterization of *Salmonella*. Improvement of pre-enrichment and enrichment procedures has been made by manipulating incubation temperature (using 42°C instead of 37°C), adding various stimulation compounds such as Oxyrase enzyme, and concentrating cells through immunomagnetic separation (Dynal system). A large number of biochemical diagnostic kits, such as API, MicroID, Enterotube, Biolog, and Vitek, have been developed and marketed to conveniently and automatically identify isolates. Manual and automated sandwich ELISA tests by EIA Assurance test, VIDAS, Tecra, etc., and lateral immunomigration tests kits by BioControl VIP system and Neogen have been used widely. In terms of genetic tests, DNA/RNA probes system by Genetrak, PCR test by Perkin-Elmer, Probelia, BAX system and ribotyping by Qualicon system, and

others are finding their ways into food microbiology laboratories.

Because *Salmonella* is heat sensitive, proper cooking will destroy the organism. Also, proper chilling, refrigeration, and good sanitation will minimize the problem. *Salmonella* remains one of the most important food pathogens in our food supply.

Shigella

Shigella is a Gram-negative, facultative anaerobic non-spore-forming rod quite often confused with *Salmonella* in the bacteriologic diagnostic process. It is nonmotile and hydrogen sulfide negative. The colonies are smaller than *Salmonella*. In terms of foodborne infection, *Shigella* is not as prevalent as *Salmonella* but this organism is very important in waterborne diseases, especially in tropical and subtropical countries where sanitation conditions are poor. In 2002, there were 43 outbreaks, 1,555 cases, and no deaths reported to the CDC. The organism is transmitted by water, food, humans, and animals. The four F's involved in the transmission of *Shigella* are food, fingers, feces, and flies. One to four days after ingestion of the organisms, there will be an inflammation of the walls of the large intestines and ileum. Invasion of the blood is rare. Bloody stool will occur, owing to superficial ulceration. The cell wall of *Shigella*, when lysed, will release endotoxins. In addition, *S. dysenteriae* produces an exotoxin that is a highly toxic neurotoxin. This toxin can be neutralized by a specific antibody. The mortality rate of shigellosis is higher than that of salmonellosis. Prevention of shigellosis can be achieved by sanitation, good hygiene, water treatment, prevention of contamination, detection of carriers, and isolation of patients from the general public.

Vibrio cholerae

Vibrio cholerae was worldwide a very important disease-causing organism in the late nineteenth century and early twentieth century. The organism is under control in many industrialized countries; however, it is still a very important waterborne disease in places with poor sanitary conditions. The classical work of John Snow in 1854 showed the transmission of *V. cholerae* through poorly designed water-delivery systems in London. His work led to the development of much improved water-delivery systems and water-treatment systems by public health official and environmental engineers in developed countries around the world. In the United States in 2002, there was one outbreak, two cases, and no deaths reported. The appearance of *V. cholerae* in industrialized countries often causes panic, since this organism has the potential to start a pandemic infection.

It is a Gram-negative, curved rod that looks like a comma under the microscope; thus, the original name of *V. comma.* No spore is formed. *V. cholerae* grows well in alkaline medium and is actively motile with a single polar flagellum.

The organism is endemic in India and Southeast Asia, and is spread by person-to-person contact, water, milk, food, and insects. The organism produces enterotoxins and endotoxins in the intestines and causes severe irritation to the mucous membranes, with resultant outflow of fluid and salts, and impairs the sodium pump of mammalian cells, thus causing severe diarrhea, dehydration, acidosis, shock, and even death. The mortality rate may be as high as 25% to 50%. The most effective therapy is replacement of water and electrolytes to correct severe dehydration and salt depletion.

Vibrio cholerae remains a dreaded communicable disease in many parts of the world, and much education and public health work needs to be done to reduce human suffering from this organism. Besides conventional biochemical tests, currently there are immunologic and DNA probes and PCR methods for rapid detection of this organism.

Vibrio parahemolyticus

Vibrio parahemolyticus is an organism that has caused many cases of food-borne disease in Japan for many years. This is because citizens in Japan like to consume raw or undercooked seafood that may be contaminated with the organism, especially in the summer months when the water is warm in the Northern Hemisphere. Most of the original reports and research work were in Japanese and not readily understandable or available to microbiologists in the West. U.S. scientists started working on the organism in earnest around 1969. In 1971, three outbreaks of this organism occurred in the United States. Since U.S. citizens do not regularly consume raw seafood, the sources of the illness were probably re-contamination of cooked foods. In 1990, there were 4 outbreaks and 21 cases reported. The fact that no outbreaks were reported in 1988, 1989, 1991, and 1992 indicates that this organism is not a source of common food-borne infections in the United States. In 2002, there were 5 outbreaks, 40 cases, and no deaths reported.

The organism is a Gram-negative, curved rod and is halophilic (salt loving), growing best in a 3% to 4% salt medium (but can grow in 8% salt also). The growth temperature range is 15° to 40°C, and pH range is 5 to 9.6. The organism is sensitive to streptomycin, tetracycline, chloramphenicol, and novobiocin, but resistant to polymyxin and colistin. The Kanagawa-positive strains hemolyze human blood. Environmental strains are negative for this test. The organism is distributed in fish and shellfish from seawater as well as from freshwater. Most of the outbreaks are recorded in the summer months when the water is warm in the Northern Hemisphere. Symptoms of the disease occur about 12 hours after ingestion of a large number of viable cells (10^5/g) and include abdominal pain, diarrhea, vomiting, mild chills, and headache. The symptoms are similar to those of salmonellosis but more severe. It has been noted that salmonellosis affects the abdomen of the patient, whereas *Vibrio parahemolyticus* infection affects the stomach of the patient. Detection of the organism is best achieved by good selective medium such as BTB-salt-Teepol agar. Infection by this organism is prevented by cooking seafood adequately.

Vibrio vulnificus

This organism can be considered an emerging pathogen. It causes more than 90% of all seafood related deaths in the United States. The organism is widespread in estuarine environments and has been isolated from waters around the world. Consumption of raw oysters contaminated with this organism may lead to septicemia and death. Also, the organism may invade people's wounds when they wade or work in contaminated water. The organism is a typical vibrio-shaped organism and is classified as biotype 1 and biotype 2. The organism grows well in common bacteriological agars such as MacConkey agar and blood agar. The incubation time of the illness is from 1 to 7 days. The disease involves fever, chills, nausea and to a lesser extend vomiting, abdominal pain, and diarrhea. Development of secondary lesions can be serious and may result in vasculitis and necrotizing fasciitis, necessitating surgical removal of tissues or even limb amputation. Since the organism is killed by common cooking practices, the problem is the consumption of raw seafood, especially raw oysters. People with liver damage and with immunocompromised conditions should definitely avoid eating raw seafood. There was one outbreak with two cases and one death reported in the United States in 1990. There was no report of an outbreak of this organism in 2002.

Bacillus cereus

Bacillus cereus and other *Bacillus* species have been implicated in food-borne diseases only in recent years, although these organisms have been suspected as agents of food-

borne illness for a long time. In 1991 in the United States, there were 5 outbreaks, 253 cases, and no deaths reported. In 2002, there were 14 outbreaks, 691 cases, and no deaths.

These are Gram-positive, aerobic, spore-forming rods occurring widely in nature and contaminating foods easily. Because of the general resistance of this organism's spores and the prolific biochemical activity of the vegetative cells, it can be considered one of the most important environmental bacterial contaminants of foods. Two distinct clinical symptoms are caused by this organism. The diarrheal syndrome occurs 12 to 24 hours after ingestion of large numbers (about 1 million) of viable *B. cereus* and includes abdominal pain, watery diarrhea, rectal tenesmus, and nausea without vomiting. The diarrheal enterotoxin is formed in the intestine of the host and causes the disease. The diarrheal syndrome is the result of consuming proteinaceous foods, such as pudding, milk and milk products, sauces, and vegetables. The emetic syndrome causes illness almost exclusively associated with cooked rice and noodles and is characterized by a rapid onset (1–5 h) with nausea, uncontrollable vomiting, and malaise. The toxin is preformed in the food by large numbers of *B. cereus* (1 to 10 million cells). Large numbers of viable *B. cereus* found in food indicates poor food handling and storage practices. To truly assess the food-borne illness potential, the toxins involved must be detected. Currently, no diagnostic kits are available for the detection of emetic toxin, but there are two kits available for diarrheal enterotoxin, one by OXOID, utilizing reverse passive latex agglutination tests, and another by Tecra, utilizing the ELISA format.

Other *Bacillus* suspected of causing food-borne diseases include *Bacillus licheniformis* and *Bacillus subtilis*, in which large numbers (10^5 to 10^6 organisms/gram of food) of these organisms are ingested by susceptible persons. It should be noted that *Bacillus subtilis var natto* is used to ferment a popular food item in Japan called Natto, in which the organisms produce polymer of glutamic acids as well as other flavor compounds.

Control of *Bacillus* food poisoning is complicated by the ubiquitous nature of this organism. The best measures are to prevent the spore from germinating and to prevent multiplication of vegetative cells in cooked and ready-to-eat foods. Freshly cooked food eaten hot immediately after cooking should not be a problem. However, slow reheating of previously cooked rice products should be treated with caution. Refrigeration of leftover cooked rice products is highly recommended as a preventive measure.

Campylobacter jejuni

Campylobacter jejuni, recognized as an emerging pathogen in the past 10 years, has been reported as the most common bacterial cause of gastrointestinal infection in humans, even surpassing rates of illness caused by *Salmonella* and *Shigell*a. In 1992 in the United States, there were 6 outbreaks, 138 cases, and 2 deaths reported. In 2002, there were 25 outbreaks, 539 cases and 1 death. *Campylobacter* was originally called *Vibrio fetus*, because it was first recognized as an agent of infertility and abortion in sheep and cattle. The organism is a member of the family *Spirillaceae* because of the physiologic and morphologic similarities to *Spirillum*.

The organism is a Gram-negative, slender, curved bacteria that is motile with a single polar flagellum. It neither ferments nor oxidizes carbohydrates, is oxidase positive, reduces nitrates but will not hydrolyze gelatin or urea, and is methyl red and Voges-Proskauer reaction negative. It will grow between 25° and 43°C. The organism is an obligate microaerophile that grows optimally in 5% oxygen. This attribute has been used for isolation of the organism by applying appropriate gas mixtures into the head space of cultivation media. Recently in the author's

laboratory, the enzyme Oxyrase was found to greatly stimulate the growth of this organism even in the absence of special gas mixtures, thus facilitating its rapid and convenient detection and isolation. The incubation time of *C. jejuni* food poisoning ranges from 2 to 5 days; the duration of the sickness may be up to 10 days. The patient will exhibit enteritis, fever, malaise, abdominal pain, and headache. The stools become liquid and foul smelling. Blood, bile, and mucus discharge may occur in serious cases. The organism has a worldwide distribution, with outbreaks related to milk, poultry, eggs, red meat, pork, and water reported. It has been isolated in 50% to almost 100% of poultry carcasses in several studies. Competitive exclusion protocols have been devised to prevent the attachment and growth of *C. jejuni* by inoculating large numbers of natural intestine microorganisms in newly hatched chicks. Detection of this organism is by suitable liquid and solid growth media designed for the organism and rapid tests involving ELISA, PCR, Ribotyping, etc. One complication in studying this organism is the presence of viable but nonculturable populations of *C. jejuni* in the environment.

Proper food-processing techniques (heating, cooling, chemical treatment of foods, etc.) will control this fragile organism. Its prevalence as a food-borne pathogen can be attributed to post-processing contaminations of food. Again, good sanitation and hygiene should reduce the incidence of this organism in our food supplies. Because of increased outbreaks and cases related to this organism, much research is being conducted worldwide to monitor the organism. *C. jejuni* may be the next major food-borne disease-causing organism to be faced by food microbiologists around the world.

Escherichia coli

Escherichia coli is one of the most common bacteria in our environment. Most people do not think of *E. coli* as a food-borne pathogen; however, recent research and information indicates that some strains of *E. coli* can indeed cause severe food-borne diseases. The sensational outbreak of *E. coli* O157 : H7 in 1993 in the United States, involving hundreds of people and resulting in four deaths, was caused by the consumption of undercooked hamburger served by a fast-food chain; it awakened the general public to the realization of the importance of food safety. Food industries, academic communities, regulatory agencies, and consumer groups have been actively working on solving the problem of *E. coli* O157 : H7 ever since that outbreak. Although much has been learned about this organism, far more needs to be done to determine its habitat, detection, and control.

E. coli is a Gram-negative, facultative anaerobic, non-spore-forming rod that occurs widely in nature as well as in the intestines of humans and animals. It is glucose- and lactose-positive and indole and methyl red positive but Voges-Proskauer and citrate negative. The most useful way to classify the species is by serotyping, using antibodies against O, H, and K antigens of various strains of *E. coli*.

Most *E. coli* isolated from the environment are not pathogenic. However, there are six classes of pathogenic and diarrheagenic *E. coli*. They are enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EaggEC), enteropathogenic (EPEC), and diffusely adherent (DAEC) *E. coli.*

EHEC or enterohemorrhagic *E.coli* was first identified as a human pathogen in 1982. The most important serotype is O157 : H7. Other serotypes in this group are O26 : H11, O103, O104, O111, and others. *E. coli* O157 : H7 causes the most concern worldwide because of its unusual cultural characteristics and pathogenicity. Unlike most *E. coli,* this serotype does not ferment sorbitol within 24 hours, does not possess beta-glucuronidase activity, and does not have the

ability to hydrolyze 4-methylumbelliferyl-beta-D-glucuronide (MUG), which is an important diagnostic characteristic of most other *E. coli* strains. Because of these differences in routine microbiological manipulations, *E. coli* O157:H7 has been excluded in the protocol for common *E. coli*. The organism produces one or more Shiga-like toxins (SLT; also known as verotoxin, VT) and it possesses an attaching and effacing gene (*eae* gene) and a large plasmid (60 MDA). The organism causes several illness, especially in children and immunocompromised patients. There are three manifestations of the disease: hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytogenic purpura (TTP). Symptoms of HC occur within 1 to 2 days after consuming contaminated foods. The initial symptoms are mild, nonbloody diarrhea followed by severe abdominal pain and a short fever or no fever. The watery diarrhea will last for 24 to 48 hours, followed by 4 to 10 days of bloody diarrhea, severe abdominal pain, and dehydration. Patients with HC may develop more severe life-threatening complications such as HUS or TTP. HUS symptoms are characterized by microangiopathic hemolytic anemia (pallor, intravascular destruction of red blood cells), thrombocytopenia (depressed platelet counts), and acute renal failure that may lead to death. TTP affects mostly adults and is a rare syndrome of *E. coli* O157:H7 infection. It causes neurological abnormalities such as nervous system deterioration, seizures, and strokes. Patients will often develop blood clots in the brain and may die. The infectious dose of *E. coli* O157:H7 is between 2 and 200 cells.

Adhesion of the organisms to the intestinal walls is important, but it does not enter the circulatory system. The organism colonizes the intestinal tract, where toxins are produced and then become active in the colon. For this reason, much research is being conducted to achieve competitive exclusion by nonpathogenic organisms attaching to the epitheleal cells before *E. coli* O157:H7 can have a chance to interact with them. Much research on this organism is being conducted around the world. In 2002, there were 84 outbreaks, 3,260 cases, and 8 deaths reported that were attributed to *Escherichia coli* (the serotype was not specified, but it was probably O157:H7).

Much is now known about the characteristics of this organism. It grows well at 37°C but poorly at 44–45°C, a temperature usually used to isolate *E. coli*. It can grow between 8–45°C and can survive in ground beef at −20°C for nine months. It can grow in neutral pH ranges of 5.5 to 7.5 but can also grow in pH 4.0 to 4.5 range, and more recent data indicated that it can survive in apple cider in the range of pH 3.6 to 4.0. The organism is heat sensitive. Proper cooking temperatures of 71°C will destroy the organism in foods. The organism is quite salt tolerant, with the ability to grow at 8% NaCl at 37°C; however, at a lower incubation temperature of 10°C, growth was inhibited to 4% to 6%. This organism grows well in water activity around 0.99, with a minimum at 0.95.

Outbreaks of *E. coli* O157:H7 have been reported from water, meat, poultry, dairy products, salad, apple cider, and even fermented meats and mayonnaise. Detection methods include conventional culture procedures designed specifically for *E. coli* O157:H7, a variety of diagnostic kits, serologic tests, ELISA. PCR, and Ribotyping. The aim is to accurately and rapidly screen for the presence or absence of the organisms in 25 grams of food. A 24-hour negative screening protocol is now available. Some commercial companies developed an 8-hour protocol. Research from Fung's laboratory perfected a 5.25-hour test to detect this organism using the Pathatrix system. This involved a short incubation period, followed by a circulating system to concentrate the target *E. coli* O157:H7 by immunomagnetic separation technology and complete the procedure by using a 25-minute ELISA test.

This method is now used extensively for large-scale detection of *E. coli* O157 : H7 and other pathogens. This organism will continue to be very important in food microbiology for the foreseeable future.

ETEC or enterotoxigenic *E. coli* are the major causes of infantile diarrhea in developing countries and are most frequently responsible for traveler's diarrhea. The serotypes involved include O8, O15, O20, O25, and others.

EIEC or enteroinvasive *E. coli* are strains that cause nonbloody diarrhea and dysentery by invading and multiplying within colonic epithelial cells. Serotypes include O28ac, O112, O124, and others.

EAggEC—enteroaggregative *E. coli*—cells affect infants and children with persistent diarrhea. They have the characteristic pattern of aggregative adherence on Hep-2 cells.

EPEC or enterotoxigenic *E. coli* has been defined as "diarrheagenic *E. coli,* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related to heat-liable enterotoxins (LT), heat-stable enterotoxins (ST), or to *Shigella*-like invasiveness. The serotypes included in EPEC are O55, O86, O111ab, O119, O125ac, o126, and others.

DAEC or diffusely adherent *E. coli* have been associated with diarrhea in children in Mexico and can produce mild diarrhea without blood or fecal leukocytes.

A comprehensive treatment of *E. coli* O157 : H7 and other *E. coli* strains, as well as many food-borne pathogens, can be found in the book by Doyle, Beuchat, and Montville (1997).

Prevention and control of pathogenic *E. coli* is best done by educating food-handlers, who should adhere to strict hygienic practices. Fecal and other waste materials from humans and animals should be decontaminated and not allowed to be in contact with water and food supplies.

Kalamaki, Price, and Fung (1997) summarized screening and identification test kits for *Escherichia coli* in Table 1 of their article. Similar tables for the detection of *Enterobacteriaceae, Campylobacter, Salmonella, Listeria,* Rotavirus, *Staphylococcus aureus, Vibrio cholerae,* and *V. vulnificus* are presented in the same publication. Due to these developments, it is possible now to have a negative screening of *Escherichia coli* 0157 : H7 in about one day. However, when a food sample shows a positive screening result, the conventional methods must be used to confirm the presence or absence of *E. coli* 0157 : H7.

From a food industry point of view, *E. coli* O157 : H7 has an even more important role in food safety and commerce because in 1994, this was the only microbe to be declared a "food adulterant" by officials of the U.S. government. This implies that if *E. coli* O157 : H7 is found in a batch of ground beef, the producer has violated the law. Thus, millions of pounds of ground beef have been recalled and destroyed in the past several years because of this ruling in the United States.

Yersinia enterocolitica

Yersinia enterocolitica is a Gram-negative, facultative anaerobic, non-spore-forming bacterium; it is sucrose-positive, rhamnose-negative, indole-positive, motile at 20°C but not at 37°C, and highly virulent to mice. Serotyping is very important in separating this organism from other closely related Gram-negative bacteria. Although *Y. enterocolitica* has an optimal growth temperature at around 32° to 34°C, it is often isolated on enteric agars at 22° to 25°C. It grows slowly in simple glucose-salts medium but grows much better with supplements such as methionine or cysteine and thiamine. One important aspect of this organism is that it can grow in refrigerated vacuum-packaged meat because it is a facultative anaerobe and is a

psychrotroph. After ingestion of large numbers of this organism, the susceptible person can develop fever, abdominal pain, and diarrhea, with nausea and vomiting occurring less frequently. More serious intestinal disorders include enteritis, terminal ileitis, and mesenteric lymphadenitis. Extraintestinal infections of *Y. enterocolitica* have been reported, including septicemia, arthritis, erythema nodosum, sarcoidosis, skin infection, and eye infection.

Foods suspected of being a source of yersiniosis in the United States include chocolate milk, milk powder, chow mein, tofu, and pasteurized milk. Pork products have also been suspected.

Isolation of this organism typically goes through an enrichment step using nutrient broth or Rappaport broth and then through a plating medium using an enteric agar (SS, XLD, DCL, etc). The CIN agar (cefsulodin-irgasan-novobiocin agar) is commercially available for the isolation of this pathogen; however many other organisms, such as *Salmonella* and *Serratia,* also grow on this agar. In the author's laboratory, a new agar named KV202 has been developed that allows *Salmonella* and *Serratia* colonies to be separated from *Yersinia* by the development of black colonies. Control of yersiniosis depends on the proper handling of raw and cooked food of all types, especially pork products, as well as water for food processing. There have been no reported outbreaks of *Yersinia enterocolitica* between 1988 and 1992, but in 2002 there were 2 outbreaks, 27 cases and 1 death reported.

Listeria monocytogenes

Listeria monocytogenes has developed into a very important food pathogen in the past 20 years from the standpoint of economic and public health impact. The organism is a small, short, Gram-positive non-spore-forming rod. It is motile by a characteristic tumbling motion or slightly rotating fashion. The organism grows on simple laboratory media in the pH range between 5 and 9. On solid agar, the colonies are translucent, dew-drop-like, and bluish when viewed by 45° incident transmitted light (Henry's illumination step). Biochemically, this organism can be confused with such organisms as *Lactobacillus, Brochothrix, Erysipelohrix*, and *Kurthia*. A variety of biochemical tests have been devised to separate *L. monocytogenes* from other *Listeria* species, such as *L. innocua*, *L. welshimeri*, and *L. murrayi*. Serotyping is also important in the identification of this organism, the most important ones being 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, and 4b. *Listeria* is a psychrotroph capable of growing at temperatures as low as 2.5°C and as high as 44°C. Because dairy products have been implicated in outbreaks of listeriosis, much research has been directed toward cheese and milk products. The organism has been found to survive the processing of cottage cheese, cheddar cheese, and Colby cheese. A question of great concern is whether *L. monocytogenes* can survive the current pasteurization temperature of milk (i.e., 63°C for 30 min or 72°C for 15 s). Data on this issue are still inconclusive, and research on this topic is still ongoing. It is important to note that at present, the time and temperature regulation for pasteurization of milk has not been affected by the possible heat resistance of *L. monocytogenes*.

The disease starts with infection of the intestine, though the infective dose is currently unknown. Patients may develop transitory flu-like symptoms such as malaise, diarrhea, and mild fever. In severe cases, virulent strains are capable of multiplying in macrophages and later producing septicemia. When this occurs, the bacteria can affect the central nervous system, the heart, the eyes, and may invade the fetus of pregnant women and result in abortion, stillbirth, or neonatal sepsis.

Several well-documented cases of listeriosis have been reported in Nova Scotia (1981),

Massachusetts (1983), and the most well-known one involving Mexican-style soft cheese in southern California (1985). Due to concerted effort by the food industry and government agencies, the outbreaks of *L. monocytogenes* seemed to have subsided for about 10 years. Between 1988 and 1992, only one outbreak involving two cases and one death was recorded for *L. monocytogenes,* and it appeared that the problem of *L. monocytogenes* was under control. However, in 1998 and 1999 the organism was found on surfaces and equipment of frankfurter production lines, air condition lines, hot dogs, lunch meat, and turkey breasts, causing many outbreaks and recalls. One company recalled 30 million pounds of ready-to-eat products due to *L. monocytogenes*. Another company recalled 15 million pounds of hot dogs and deli meat products due to an outbreak of *L. monocytogenes* that included 20 deaths—14 adults and 6 miscarriages/stillbirths—and at least 97 illnesses in 22 states. In 2002, there were 3 outbreaks with 100 cases and 2 deaths recorded. There is a resurgence of concern about this organism due to the pathogenic nature of the illness resulting in miscarriages and stillbirths.

L. monocytogenes has been isolated in a variety of commodities, including poultry carcasses, meat and chopped beef, dry sausages, milk and milk products, cheese, vegetables, and surface water. Control measures include eliminating the occurrence of the organism in raw-food materials, transporting vehicles, and food-processing plants (where it is especially important to control cross contamination of raw and finished products); practicing good general sanitation of the entire food-processing environment; regular monitoring for this organism in food-processing facilities; and preventing pregnant females from working in and around environments that have the possibility of exposure to *L. monocytogenes.* Because the organism is killed by heat and is susceptible to sanitizing agents, proper cooking of food and decontamination of food-preparation environment will also help reduce risks.

Much research has been devoted to the rapid isolation, enumeration, and identification of this organism. Many diagnostic kits, immunological systems, and genetic systems have been developed to rapidly screen for this organism in the food supply.

Currently, there is an important debate as to the regulation of allowed levels of *L. monocytogenes* in foods. In the United States, there is zero tolerance of *L. monocytogenes* in ready-to-eat (RTE) foods and even in the environment and processing equipment for manufacturing RTE. This is a very stringent rule. However, Canada and the European Union allow 100 L.m./g of certain foods to be further processed in certain conditions. Currently, there is a move to suggest the United States should follow the 100 L.m./g allowance, as it is almost impossible to enforce the zero-tolerance rule in current U.S. regulation. The debate goes on. In 2002, 3 outbreaks involving 100 cases and 2 deaths were reported.

Aeromonas hydrophila

Aeromonas hydrophla has been associated with food-borne infection, although the evidence is inconclusive. The organism is a facultative anaerobic, Gram-negative, motile rod. Biochemically, it is similar to *E. coli* and *Klebsiella*. The optimal temperature for growth is 28°C and the maximum is 42°C. Many strains can grow at 5°C, which is a temperature usually considered adequate to prevent growth of food-borne pathogens.

Diseases caused by *A. hydrophila* include gastroenteritis (cholera-like illness and dysentery-like illness) and extra-intestinal infections such as septicemia and meningitis. This organism has been isolated from fish, shrimp, crabs, scallops, oysters, red meats, poultry, raw milk, vacuum-packaged pork and beef, and even bottled mineral water.

Because the organism is a psychrotroph, cold storage is not an adequate preventive measure. Proper heating of food offers sufficient protection against this organism. Consumption of undercooked food or raw food such as raw shellfish is discouraged.

In 2002, no outbreak of this organism was reported to the CDC.

Plesiomonas shigelloides

Plesiomonas shigelloides has been a suspect in food-borne disease cases. The organism is Gram-negative, facultative anaerobic, catalase negative, and fermentative. It is oxidase positive, which can be used to differentiate it from other bacteria in the family *Enterobacteriaceae*, since the latter is oxidase-negative. The organism also resembles *Shigella* but can be differentiated from *Shigella* by being motile. It is capable of producing many diseases, ranging from enteritis to meningitis.

Gastroenteritis by *P. shigellosides* is characterized by diarrhea, abdominal pain, nausea, chills, fever, headache, and vomiting after an incubation time of 1 to 2 days. Symptoms last for a week or longer. All reported foods involved with cases of gastroenteritis were from aquatic origin (salted fish, crabs, and oysters). The organism can be isolated from a variety of sources, including humans, birds, fish, reptiles, and crustaceans. The true nature of this organism as a food-borne agent is not fully known because the organism has not been well studied to date. In 2002, no outbreak of this organism was reported to the CDC.

Miscellaneous Bacterial Food-Borne Pathogens

Many other microbes are suspected of being food-borne pathogens. However, they are not currently labeled as true food-borne pathogens, owing to a lack of reports on these organisms, as well as a lack of isolation methods and research. Many of these organisms may be identified as food-borne pathogens in the future. Among these organisms are the Gram-negative bacteria *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Klebsiell*a, *Hafnia*, *Kluyvera*, *Proteus*, *Providencia*, *Morganella*, *Serrati*a, *Vibrios,* and *Pseudomonas*, and the Gram-positive bacteria *Corynebacterium*, *Streptococcus*, and other species of *Bacillus* and *Clostridium*. Miscellaneous organisms include *Brucella*, *Mycobacterium* (T, B), *Coxiella burnetii* (Q-fever), *Leptospirosis*, *Erysipela*s, and *Tularemia*.

Food-Borne Viruses

Food-borne viruses are much less studied by food microbiologists than are bacteria and fungi, owing to the difficulty of cultivating these entities, as conventional bacteriologic media will not allow these particles to grow. There are, no doubt, many food-borne outbreaks and cases caused by a variety of viruses, but scientists in many cases are not able to identify the sources of the infection. Viruses that have been incriminated in food-borne diseases include hepatitis A virus (oysters, clams, doughnuts, sandwiches, and salad), Norwalk virus (oysters), polio virus (milk and oysters), ECHO virus (oysters), enteroviruses (oysters), and coxsackievirus (oysters). Much more research needs to be done in the field of food virology to help reduce the incidents of food-borne diseases caused by viruses. There were 56 total viral outbreaks, 4,066 cases, and no deaths reported in 2002.

Protozoa and Related Organisms

Protozoans such as *Cryptosporidium, Cyclospora, Toxoplasma, Giardiasis, Entamoeba, Balantidium,* and others can also cause human food-borne diseases. The most sensational outbreak was the one involving *Cryptosporidium parvum,* which affected

400,000 people and caused several deaths in Milwaukee in 1993. *Cyclospora cayetanensis* from imported fruits was also in the news for causing a food-borne outbreak. These organisms have complex life cycles and are studied by specialists in this area. Recently, an organism named *Pfiesteria piscicida* was responsible for killing a million fish on the eastern shores of the United States. The organism has 24 life stages, ranging from a cyst stage to a toxic zoospore phase to an amoeba stage. People in contact with water infected with this organism complained of vomiting and liver problems, but no conclusive data are available on the pathogenicity of this organism to humans.

Nonmicrobial Food-Borne Disease Agents

Consumption of food containing other living organisms can directly and indirectly cause food-borne diseases as well. Among nonmicrobial food-borne disease agents are scombroid fish (associated with high levels of histamine), cestodes (flatworms such as *Taenia saginata*, *T. solium*, and *Diphyllobothrium latum*), nematodes (hookworms such as *Trichinella spiralis*), trematodes (flukes such as *Clonorchis sinensis*), shellfish (indirect toxin from the dinoflagellate *Gonyaulax catenella*), ciguatera (from eating fish such as barracudas, groupers, and sea basses that feed on toxic algae), and other poisonous fish (such as puffer fish and moray eel).

Summary

Food safety is everybody's responsibility. Scientists are charged with identifying the agents causing food-borne infections and intoxications; studying the mechanisms of intoxication and infection; and working on the isolation, enumeration, characterization, and identification of the causative agents and on their control by developing intervention strategies and preservation methods. The food industry uses this basic knowledge and applies it to good manufacturing practices to produce wholesome, nutritious, and safe foods by utilizing modern equipment, systems, processing techniques, and distribution systems. Government agents are charged with the responsibility of monitoring the safety of food supplies and enforcing regulations to ensure the production, distribution, and sale of wholesome foods. The consumer must also be educated in the handling of raw and cooked food at the point of purchase, as well as preparation of the food and final consumption. All parties are responsible for the food safety of all involved.

The delightful book *Safe Eating* by Acheson and Levinson (1998) detailed the problems involved in food safety and offered solutions to protect consumers in laymen's terms that nevertheless provide much scientific information about the entire issue of food safety and consumer protection. It is a book worth reading and studying by consumers concerned about food safety.

The book *Food Microbiology: Fundaments and Frontiers* by Doyle and Beuchet (2007) should be studied for an in-depth understanding of the subject of food microbiology and safety. Updated information on rapid Methods and automation in microbiology are also provided in a CD edited by Fung (2009a).

There is no doubt that microorganisms and their toxins and byproducts can be hazardous to our food supplies. Much more work on this topic needs to be done in the near and far future.

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Chapter 29

Assessment of Genetically Modified Organisms (GMO) in Meat Products by PCR

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Introduction

A genetically modified organism (GMO) is any organism transformed by using the recombinant DNA technology or that has incorporated a gene sequence into its genome. That sequence can be of the same species as happened with the first commercially available GMO in 1995, the Flav Savr™ tomato, or a sequence derived from a different species than that of the recipient, which is called *sensu stricto* transgene. However, both terms are used nowadays without distinction. GMO have been developed from the three domains: archaea, bacteria, and eukaryote, but only a very few species are commercially approved. In Spain, there are 45 maize varieties approved for cultivation. There are important genetically modified (GM) microorganisms used for fermenting foods like cheese or beverages such as wine or beer, as well as transgenic animals and plants developed for medical or research purposes. Currently, no GM animal has been authorized for human consumption, and therefore, GM animals have not been introduced into the food chain.

The study and analysis of GMOs used for foodstuff production involves regulatory establishments, research institutes, analytical laboratories, farmers, and consumers, making the GMO area a notorious issue with profound social and economical effects (Rodríguez-Lázaro et al. 2007; Marmiroli et al. 2008). Since the first genetically modified crop was approved for commercialization in 1995, there has been an exponential increase in cultivated GMOs (up to 125 million hectares in 2008, with an annual increase of 9.4%), in 25 countries (15 developing and 10 industrial countries worldwide), involving more than 13.3 million of farmers (James 2008). In addition, the tendency is to increase not just the cultivated area, but also the number of different traits and plant species transformed. To date, cultivated GM crops mainly include four plant species: soybean (53%), maize (30%), cotton (12%), and canola (5%). The most common dominant traits introduced are herbicide tolerance (63%) and insect resistance (15%), with tacked double or triple traits occupying a larger area (22%). However, other crops are being developed to improve other aspects such as nutritional value and environmental stress resistance, as well as for textile or biofuel use (EFSA GMO Panel 2008).

The lack of information and confidence within society's food chain regarding the food safety of the novel foods produced has meant that the public perception of GMOs has been controversial, particularly in Europe (Frewer et al. 2004; Michelini et al. 2008) and to a lesser extent, in North America (Dale 1999), where public information programs were carried out by major grain biotech producers in the early 1980s (Ahmed 2002). With the intention of alleviating negative consumer perception, some governmental, regulatory agencies have established

compulsory labeling requirements. This policy has been introduced to carry out commercial regulations for the use of GMOs in the food chain and guarantee consumers' rights to information for making an informed choice.

The European Union (EU) made labeling mandatory since 1997 in food and feed products containing, consisting of, or produced from GMOs in a proportion higher than 0.9% of authorized GMO, 0.5% for nonauthorized GMOs, and compulsory at any level if the GMO presence could not be demonstrated to be adventitious or technically unavoidable (European Commission 2003a, b). In addition, all GM additives and GM flavorings have to be labeled according to Regulation (EC) 50/2000 (European Commission 2000). Prepackaged products containing GMO delivered to the final consumer or to mass caterers are required to state on the label "This product contains genetically modified organisms," and in non-prepackaged products, the words must appear in the display of the product (European Commission 2003a, b). Norway and Switzerland, which are not members of the European Union, also demand the labeling of GMOs in their food. Competent authorities of different countries (Australia, New Zealand, Brazil, Chile, China, Croatia, Ecuador, El Salvador, Indonesia, Japan, Mauritius, Mexico, Russia, Saudi Arabia, South Africa, South Korea, Sri Lanka, Taiwan, Ukraine, Thailand, and Vietnam) have also established a labeling policy for products containing detectable ingredients produced from GMOs, and in other countries, such as the United States or Canada, voluntary labeling of bioengineered foods is recommended (Marmiroli et al. 2008).

Model studies to detect GMOs in animal products have been carried out, showing that analysis of milk, muscle, or blood cannot serve as an indicator of the use of GMOs for feeding animals (Poms et al. 2003; Nemeth et al. 2004; Bertheau et al. 2009), and a few articles have been published regarding the detection of GMOs in meat products. For example, Taski-Ajdukovic et al. (2008) analyzed 50 processed meat products containing soybean and found 12 positive within a 35S promoter. In order to fulfil regulations and to ensure consumers' rights to information, as well as to introduce new insights for performing traceability and coexistent GMO studies (Aarts et al. 2002), several analytical approaches have been developed, mostly based on molecular methodologies that rely on the detection of either protein or DNA. Protein-based methods employ western blots, enzyme-linked immunosorbent assays (ELISA), or lateral flow strips, while DNA-based methods use traditional, hybridization techniques such as southern blotting, qualitative- and quantitative-PCR, or new hybridization techniques such as microarrays.

This chapter summarizes the current available DNA-based methodologies for the detection, identification, and quantification of GMOs, as well as their uses, limitations, and key issues in implementation.

DNA-Based Methodologies for GMO Analysis

The current methodologies for GMO analysis are based on the detection of nucleic acids (DNA by PCR, or RNA by RT-PCR or NASBA). DNA is a ubiquitous molecule in living organisms with chemical properties that confer resistance to the harsh treatments performed by food industries, and therefore, the analytical methods based on its detection are nowadays the methodologies of choice in GMO analysis. Consequently, the analytical strategy is based on the detection and identification of the introduced DNA (the analytical result is the presence/absence of a given GMO), and/or its quantification (the analytical result is the exact GMO percentage). However, prior to handling and processing any sample in the laboratory, a critical aspect that must be carefully addressed in any meth-

odological approach focused on food safety, especially in GMO analysis, is the selection of a sound and rational sampling strategy (Michelini et al. 2008). The sample taken for analysis must be statistically representative of the original population or batch. This issue is of particular importance for validation studies where protocol performance is assessed. Intimately related with this is the selection of the correct weight or volume that must be processed from each sample. Generally, a portion about 0.1 to 1 g from the received sample is considered sufficient for processing (Pietsch et al. 1997; Zimmermann et al. 1998a, b, c; van Duijn et al. 2002).

The DNA-based methods, especially those based on its amplification, rely on different analytical steps: the DNA extraction, the DNA amplification by PCR, and finally, the detection of the specific amplification products. In the case of the use of real-time PCR-based methods, the two later steps can be combined into a single one, as the amplification and detection occurs simultaneously in a single analytical step.

DNA Extraction from Food Matrices

The first step of PCR-based methods in food analysis relies on a careful DNA-extraction procedure, since components of food samples and nucleic acid extraction reagents can reduce or even block the PCR amplification, and DNA may be degraded. Food components that can act as PCR inhibitors are proteins, fatty acids, and other secondary compounds such as polyphenols. Consequently, adequate nucleic acid purification is crucial to convert food samples into samples amenable for amplification by adaptation of extraction procedures to each food matrix. The first stage is homogenization to reduce the sample particles to an appropriate size by grinding in homogenizers, such as blenders, stomacher, polytron, ultra-turrax, mills, and mortars, which reduce considerably the sampling error (Begg et al. 2007). However, particle size, composition, or heat and pressure processing might induce distortions in the results of GMO quantification due to their influence on DNA degradation (Moreano et al. 2005; Hird et al. 2006). Then, cell membrane lysis by enzymatic activity and/or mechanical disruption is performed, mostly in the presence of detergents (guanidinium isothiocyanate, SDS, CTAB) and chelating agents (EDTA); cleanup steps come next, using organic solvents (chloroform, phenol); and finally, DNA is separated and concentrated by alcohol/salt precipitation (ethanol or isopropanol), affinity, or ion/exchange purification columns (Hernandez et al. 2005). One of the most commonly used procedures for DNA extraction in GMO analysis is the CTAB-based protocol (Rogers and Bendich 1985) or its variations (Meyer and Jaccaud 1997). Binding and elution from silica has also become the procedure of choice for most nucleic acid extraction procedures (Smith et al. 2003). In addition, many different commercial kits and automated procedures for food samples are currently available (Marmiroli et al. 2008), although the automation is restricted nowadays to a limited number of food samples (Hahnen et al. 2002). Several papers compare different extraction methods (Zimmermann et al. 1998a; Peano et al. 2004).

After DNA extraction, quantity and quality must be evaluated by means of UV spectrophotometry at 260 nm; by fluorimetry using fluorescent ds-DNA-specific dyes; or by visualization under UV light of DNA separated in agarose gels stained with ethidium bromide. Before DNA estimation, RNA must be carefully eliminated in order to avoid an overestimation of yield. For this purpose a straightforward treatment with RNAase A is recommended. Then, it is simple to correlate the amount of DNA (ng) with copy number by using the C-value (Arumuganathan and Earle 1991; Bennett and Leitch 2003). Purity of DNA can be evaluated by assessing the degree of degradation using agarose

electrophoresis, by means of the ratio of UV spectrophotometry at 260/280 nm (desirably close to 1.8) and 260/230 (above 1.8–2.2); and by the determination of the presence of PCR inhibitors by spiking a control sample.

PCR Detection

Polymerase chain reaction (PCR) is a simple, versatile, sensitive, specific, and reproducible technique (Saiki et al. 1988). It is an *in vitro* exponential amplification of a DNA fragment, and its principle is similar to the mechanism of DNA replication. The double-stranded DNA is first denatured, and the two strands of single-stranded DNA are duplicated using PCR primers that specifically anneal to these strands and are elongated through the activity of DNA polymerase. This is repeated during generally 30 to 50 cycles along the reaction. Usually, the analytical result obtained by PCR is the determination of the amplified products at the end point of the reaction, especially when only qualitative data is required. There are several types.

Nested PCR

As conventional PCR based on the agarose gel electrophoresis is generally less sensitive, a modification, the nested PCR, which dramatically increases the sensitivity and specificity of DNA amplification, can be used. It consists of two rounds of PCRs, where the primers of the second PCR bind to an internal region of the first amplified DNA product. Thus, the larger fragment produced by the first round of PCR is used as the template for the second round. The specificity is particularly enhanced because this technique helps to eliminate any spurious nonspecific amplification product. However, the high risk of cross contamination is the main drawback, and great care must be taken when performing such PCRs. Several GMO methods based on nested PCR are available in the bibliography (see Table 29.1).

Quantitative PCR

In certain circumstances, such as the determination of percentage of transgenic ingredients in a food sample, quantitative data are required, which cannot be provided using conventional PCR. This has been addressed, especially using the real-time (RTi-) PCR that allows the quantification of the initial amount of the template by tracking the reaction cycle-by-cycle. Another quantitative approach, competitive PCR, can be used when the PCR product from the sample is compared with internal standards of known concentrations. It coamplifies the target sequence and one competitor sequence (so-called internal standard) in a single reaction using conventional PCR. Several methods have been published using this approach for GMO quantification (Studer et al. 1998; Hardegger et al. 1999; Zimmermann et al. 2000).

Real-Time PCR

The principal characteristic of the RTi-PCR is that it allows the real-time monitorization of the synthesis of new amplicons throughout the PCR via the fluorescence emitted, which is proportional to the amount of new PCR products generated (Heid et al. 1996). Therefore, the RTi-PCR results consist of amplification curves that can be used to quantify the initial amounts of template DNA molecules with high accuracy over a wide range of concentrations (Schmittgen et al. 2000). Other advantages are the closed-tube format, which reduces the risks of carryover contamination, the fast and simple performance, the wide dynamic range of quantification (higher than six orders of magnitude), and the significantly higher reliability of the results compared with conventional PCR.

There are two different strategies for the detection of the new amplicon being gener-

Table 29.1. GMO specific PCR methods for GMO analysis. cPCR: conventional PCR; RTi-PCR: real-time PCR; dc-PCR: double competitive PCR; qc-PCR: quantitative competitive PCR. Modified from Hernandez et al. (2005)

GMO	Target sequence	Technique	Reference
Maize event 176	*cry*IA(b), *bla, bar*, P-35S/*bar, ivr1*	cPCR	Ehlers et al., 1997
	*cry*IA(b)	nested PCR	Studer et al., 1997
	*cry*IA(b) and eukaryotic genomic region	cPCR	Hupfer et al., 1997
	P-CDPK/*cry*IA(b)	nested PCR	Hupfer et al., 1998
Soybean GTS40-3-2	P-35S/CP4-*EPSPS*, P-35S/CTP-*EPSPS, le1*	nested PCR	Meyer & Jaccaud, 1997
	P-35S/CTP-EPSPS, CTP-EPSPS/CP4-EPSPS	nested PCR	Köppel et al., 1997
	P-35S, T-*nos*	cPCR	van Duijn et al., 2002
	CP4-EPSPS/T-*nos*	cPCR	Hörtner 1997
	P-35S/CTP-EPSPS	dc-PCR	Wurz and Willmund, 1997
	P-35S/CTP-EPSPS	cPCR (validation)	Anonymous, 1998
	P-35S/CTP-EPSPS and lectin	RTi-PCR	Wurz et al., 1999
	Junction	RTi-PCR	Berdal and Holst-Jensen, 2001
	Junction	RTi-PCR	Terry and Harris, 2001
	Junction	RTi-PCR	Taverniers et al., 2001
	EPSPS	RTi-PCR	Terry et al., 2002
	EPSPS, le1	RTi-PCR (validation)	Hird et al., 2003
	EPSPS, CaMV, Nos ter, Even specific, lectin	RTi-PCR	Corbisier et al., 2005
	Juntion, le1, soybean genome/P35S	RTi-PCR	Huang and Pan, 2005
Maize event 176 and soybean GTS40-3-2	*cry*IA, *EPSPS*	qc-PCR	Studer, et al., 1998
	*cry*IA(b), CP4-EPSPS, zeine, lectin	RTi-PCR	Vaïtilingom et al., 1999
	P-35S/peru-r1, *cry*IA, *le1, ivr*	cPCR (validation)	Jankiewicz et al., 1999
	P-35S, T-*nos, npt*II, CTP, *cry*IA(b), *lectin, ivr*	cPCR	Vollenhofer et al., 1999
	Lectin, ivr, EPSPS, *cry*IA(b)	cPCR	Tengel et al., 2001
	P-35S and T-*nos*	cPCR	Mao et al., 2002
	Lectin, lipid transfer protein (*ltp*) maize y P-35S	RTi-PCR	Alary et al., 2002
Maize MON810	P-35S/*HSP*70 intron	nested PCR	Zimmermann et al., 1998c
	5′ junction	RTi-PCR	Holck et al., 2002
	3′ junction	RTi-PCR	Hernandez et al., 2003a
Maize MON863	zsSSIIb,p35S, TNOS, junction	RTi-PCR	Lee et al., 2006
Maize Bt11	*cry*IA(b)	cPCR	Ahl Goy 1998
	*cry*IA(b)	qc-PCR	Zimmerman et al., 2000
	3′ junction and 5′ junction	RTi-PCR	Rønning et al., 2003
Maize T25	Sintet. *pat*, P-35S/*bla*	cPCR	Höchst et al., 1998
Maize GA21	Junction	RTi-PCR	Hernandez et al., 2004a
Maize CBH-351	*cry*9C	cPCR and RTi-PCR	Matsuoka et al., 2001
	Junction A, Junction B, *cry*9c	cPCR	Windels et al., 2003
Maize NK-603	Junction 5′	RTi-PCR	Nielsen et al., 2004
Rapeseed Liberty Link	*PEPC*	cPCR	Weiblinger et al., 1999

(continued)

Table 29.1. GMO specific PCR methods for GMO analysis. cPCR: conventional PCR; RTi-PCR: real-time PCR; dc-PCR: double competitive PCR; qc-PCR: quantitative competitive PCR. Modified from Hernandez et al. (2005) *(cont.)*

GMO	Target sequence	Technique	Reference
Rapeseed Falcon6/Ac, HCN10, Liberator6/ Ac, HCN28, HCN92	*EPSPS, pat*, P-35S y *PEPC*	RTi-PCR	Zeitler et al., 2002
Potato B33	*npt*II, gbss-as; B33, T-DNA	cPCR	Hassan-Hauser et al., 1998
Potato B33-INV	aphIV	cPCR	Anonymous et al., 1997
Potato NewLeaf Plus	PLRV-rep gene	cPCR	Hernandez et al., 2004b
Potato NL Russet Burbank and NL Superior	P-35S and *cry*3A	cPCR	Mel'nychuk et al., 2002
Tomato FlavrSavr™	*npt*II, d-P-35S/ polygalacturonase	cPCR	Meyer 1995a, b
Tomato Zeneca	T-*nos*, polygalacturonase	cPCR	Busch et al., 1999
Maize Bt176, MON810, Bt11, GA21 and T25) and soybean Roundup Ready	Different junctions	RTi-PCR five GMO	Kuribara et al., 2002
Maize Bt176, MON810, Bt11, GA21 and soybean Roundup Ready	Cp4EPSP/NOS terminator, 35SCamV/BAR, 35ScaMV/ adh, 35ScaMV/hsp70, actin promoter/CTP	RTi-PCR five GMO	Peano et al., 2005
Maize Bt176, Bt11, GA21, and soybean GT73	Different junctions	RTi-PCR four GMO	Taverniers et al., 2005
Maize Bt176, MON810, Bt11, and soybean Roundup Ready	Different junctions	RTi-PCR four GMO	Greiner et al., 2005
Maize Roundup ready and soybean Roundup ready	CTP/EPSPS	RTi-PCR	Lerat et al., 2005

ated: a "nonspecific strategy" independent of the target sequence (e.g., through fluorescent dyes that have special fluorescent properties when bound to double stranded (ds) DNA), and a "sequence-specific strategy" by sequence-specific fluorescent oligonucleotide probes. The latter strategy also allows the simultaneous detection of multiple targets, using multiple probes labeled with different reporter dyes (Bernard et al. 1998; Wittwer et al. 2001; Dupont et al. 2002). This is of special interest in GMO analysis, since a wide diversity of transgenic events is introduced in the market.

Several fluorescent dsDNA-specific dyes have been used in RTi-PCR methods. The first used was ethidium bromide (Higuchi et al. 1993; Le Pecq and Paoletti 1996; Wittwer et al. 1997), and subsequently, other intercalating dyes have also been introduced, such as YO-PRO-1 (Ishiguro et al. 1995; Tseng et al. 1997) and SYBR Green I (Hernandez et al. 2003c). They are cost-effective, as it is not necessary to add a target-specific fluorescent

probe or specific DNA polymerases. However, the specificity is determined entirely by the primers, as in conventional PCR, and thus the risk of amplifying nonspecific PCR products increases (Simpson et al. 2000). It is possible to verify the correct production of a given PCR product by means of plotting fluorescence as a function of temperature to generate a melting curve of the amplicon at the end point (Ririe et al. 1997). Another nonspecific detection system is the Sunrise primers (AmpliFluor™ system) developed by Intergen Co. It uses a universal, energy-transfer hairpin primer (UniPrimer™) that emits a fluorescent signal when unfolded during its incorporation into an amplification product (Nazarenko et al. 1997).

The "sequence-specific fluorescent probes" can be classified into two major groups: hydrolysis and hybridization probes. Both types consist of an oligonucleotide, homologous to the internal region of the amplicon, that is double-labeled, with a fluorophore or reporter dye (donor of fluorescence) at the 5′-end and a quenching moiety (acceptor of fluorescence) at the 3′-end. The distance between the fluorophores is a key factor in generating sequence-specific signals (Förster 1948; Clegg 1992), and a change in the distance between them is used to produce the RTi-PCR signals. The hydrolysis probes such as TaqMan® probes and TaqMan® MGB probes are cleaved by the 5′-3′ exonuclease activity of several DNA polymerases during the primers' elongation phase (Holland et al. 1991), yielding a real-time, measurable fluorescence emission directly proportional to the concentration of the target sequence. In contrast, hybridization probes are not hydrolyzed during PCR. The fluorescence is generated by a change in its secondary structure during the hybridization phase, which results in an increase of the distance that separates the reporter fluorophore from the quencher moiety. The most relevant hybridization probes are those containing hairpins such as Molecular Beacons, Scorpion primers, and FRET hybridization probes. Among the different types of probes currently available in the market, the TaqMan probes are nowadays the probes of choice.

The fluorescence is determined cycle-by-cycle by the RTi-PCR platform, and the result is an amplification plot. A typical amplification curve presents three different phases. The initiation phase occurs during the first PCR cycles when the emitted fluorescence cannot be distinguished from the baseline. During the exponential or log phase there is an exponential increase in fluorescence, and finally, during the plateau phase, the reagents are exhausted, and no increase in fluorescence is observed. Quantification is only possible, therefore, at the beginning of the exponential phase when the reaction is totally efficient and all the reagents are available. The most important parameter in the RTi-PCR is the threshold cycle (C_T value) (Higuchi et al. 1992), which is used for the quantification of the sample. It corresponds to the cycle at which a statistically significant increase in amplification-associated florescence is first detected, and is inversely correlated to the concentration of DNA present in the original sample (Walker 2002). The initial DNA concentration of the sample can then be determined by interpolation of the resulting C_T value in a linear standard curve of serially diluted, known-amount standards.

Other DNA Techniques

Other modifications and techniques based on DNA detection are continuously appearing in the scientific literature (Garcia-Canas et al. 2002; Burns et al. 2003; Feriotto et al. 2003; Rudi et al. 2003; Glynou et al. 2004; Obeid et al. 2004; Fantozzi et al. 2008; Morisset et al. 2008). Recently, a novel DNA-based technology has been developed, the NASBA Implemented Microarray Analysis (NAIMA) (Morisset et al. 2008). In this technology, a cRNA product is obtained from a consecu-

tive first primer extension reaction, using tailed primers, and a subsequent transcription-based amplification, using universal primers. The NAIMA product is directly ligated to fluorescent dyes labeled 3DNA dendrimers, allowing signal amplification and hybridized without further purification on an oligonucleotide probe-based microarray for multiplex detection. Another ingenious hybrid protocol has been published aimed at the simultaneous quantification of multiple nucleic acid targets (Rudi et al. 2003). This approach, named MQDA-PCR, is based on two-step multiplex amplification of target sequences, followed by sequence-specific labeling of the probes to be used for DNA-array hybridization. The approach has been tested using diluted mixtures of certified GMO material, as well as with commercial food samples with similar success. Fantozzi et al. (2008) have developed a screening protocol for the detection of the regulatory sequence p35S and the specific event epsps. It is based on the Luminex xMAP technology, and two different sets of fluorescent beads are cross-linked to the specific oligonucleotide probes previously amplified and labeled by polymerase chain reaction (PCR) in the presence of a biotinylated nucleotide.

However, an important aspect that must be considered prior to routine use of these techniques in food analysis is the validation of their performance through use by the scientific community as has occurred with the real-time PCR methods.

Strategies for Detection, Identification, and Quantification of GMOs

Different analytical strategies have been exploited in GMO analysis: (1) screening methodology that commonly detects regulatory sequences or marker genes, and which aims to detect the presence or absence of GM-material; (2) GMO identification via the identification of specific genes, detection of junction regions of DNA from different origins, and flanking regions of the introduced construction, which aims to determine which GMO is present in the sample, and therefore, if it is authorized or not; and finally, (3) GMO-quantification that targets the specific gene, or the border sequences, and accurately quantifies the percentage of GMO in the food product as a necessary step for labeling if required by the norm in force (Hübner et al. 1999). In any case, DNA from the plant species should be analyzed by using an endogenous gene in the amplification reactions that can be used as a reference to normalize the GMO content.

Analysis of Regulatory Sequences and Marker Genes

Screening methodologies exploit the detection of common elements present in GMOs, such as marker genes or regulatory sequences (i.e., promoters and terminators). Most commercially approved GMOs have been transformed using constructs containing sequences from the Cauliflower Mosaic Virus (CaMV, i.e., 35S promoter [P-35S] and/or 35S terminator [T-35S]) or from *Agrobacterium tumefaciens* (i.e., nopaline synthetase terminator [T-*nos*]). Screening methodologies have also traditionally used selectable marker genes that encode proteins that confer herbicide or antibiotic resistance (Draper and Scout 1991; Flavell et al. 1992; Kok et al. 1994; MacCormick et al. 1998). The most accepted marker gene has been *npt*II, encoding resistance to aminoglicosidic antibiotics (neomicin/kanamicin). The *bla* gene, encoding ampicilin resistance, and the *bar* gene, encoding phosphinothricin tolerance, have also been widely employed (D'Halluin et al. 1992). Therefore, most of the screening methods are based on the detection of P-35S, T-*nos*, and *bla* and *npt*II genes. Another strategy is the use of regions from cloning vectors regularly used for transformation (i.e., plasmid sequences derived from *pBR322*

Table 29.2. PCR screening methods for GMO analysis. cPCR: conventional PCR; RTi-PCR: real-time PCR Modified from Hernandez et al. (2005)

Target sequence	Technique	Reference
T-*nos*	cPCR	Depicker et al., 1982
*npt*II	cPCR	Beck et al., 1982
T-*ocs*	cPCR	DeGreeve et al., 1983
pat	cPCR	Töpfer et al., 1987
T-CaMV	cPCR	Töpfer et al., 1987
*npt*II, P-35S, T-*nos*	cPCR	Pietsch et al., 1997
P-35S, T-*nos*	cPCR	Brodman et al., 1997
P-35S, T-*nos*	cPCR	Lipp et al., 1999
P-35S, T-*nos, npt*II, *lec* and *ivr*	cPCR	Vollenhofer et al., 1999
P-35S, T-*nos*	qc-PCR	Hardegger et al., 1999
P-35S, T-35S, P-*nos*, T-*nos,* P-*FMV35S, npt*II	Multiplex-PCR coupled with oligonucleotide microarray	Xu et al., 2006
P-35S	cPCR (validation)	Weighardt et al., 2004
P-35S, zeine	RTi-PCR duplex	Höhne et al., 2002
bar	RTi-PCR	Lipp et al., 2001

such as *pUC19*) as targets of screening methodologies. Table 29.2 summarizes the available screening methods published for GMO analysis.

In addition, virus-infected plants or samples contaminated with plant material carrying the virus could lead to false positive results. To overcome this problem, PCR systems are available that specifically detect the virus coat protein of all CaMV isolates, allowing the differentiation of infected plants and positive GMO samples (Cankar et al. 2005).

Identification of Endogenous Reference Genes

In a food sample, only a certain percentage may contain the GM ingredient. In order to identify the total amount of DNA corresponding to that particular GMO, endogenous reference controls that define 100% of each ingredient must be used. These are based on genes present in both transgenic and nontransgenic varieties of the same plant species. They must not present genetic modifications (a SNP was detected in *ADH1* gene affecting quantification; Broothaerts et al. 2008), and must show species-specificity, stability among different varieties of the same species, and preferably, must be present in the genome in a single copy. Table 29.3 summarizes the endogenous reference control genes currently available.

Analysis of Transgenes and Flanking Regions

Different strategies based on PCR amplification of the introduced transgene have been defined (Holst-Jensen et al. 2003): trait-specific methods, where transgene-specific sequence are used for amplification (Vaïtilingom et al. 1999; Zeitler et al. 2002); construct or plasmid-specific methods, with the amplification of a fragment containing two introduced regions from different origins (Kuribara et al. 2002; Hernandez et al. 2004a); and event-specific methods, which amplify the border region between the introduced sequence and the plant genomic DNA (e.g., for Roundup Ready soybean: Berdal and Holst-Jensen 2001; Taverniers et al. 2001; Terry and Harris 2001), for transgenic maize lines, such as MON810 (Holck et al. 2002; Hernandez et al. 2003a), CBH-351 (Windels et al. 2003), Bt11 (Zimmermann et al. 2000), and NK603 (Nielsen et al. 2004). Table 29.1 summarizes the published GMO analytical systems based on PCR.

Table 29.3. PCR methods for analysis of endogenous reference genes. cPCR: conventional PCR. RTi-PCR: real-time PCR. Modified from Hernandez et al. (2005)

Plant species	Endogenous reference genes	Technique	Reference
Eggplant	β-fructosidase	RTi-PCR	Chaouchi et al., 2008
Maize	Zeine	cPCR	Studer et al., 1997
	Invertase (*ivr1*)	cPCR	Ehlers et al., 1997
	Zeine	RTi-PCR	Vaïtilingom et al., 1999
	High mobility group protein (*hmg*)	cPCR	Zimmermann et al., 1998a
	Adh1, hmga, ivr1, zeine	RTi-PCR	Hernandez et al., 2004b
Pepper	β-fructosidase	RTi-PCR	Chaouchi et al., 2008
Potato	Sucrose-synthase	cPCR	Akiyama et al., 2002
	Metallo-carboxypeptidase (*pci*)	RTi-PCR	Hernandez et al., 2003b
	β-fructosidase	RTi-PCR	Chaouchi et al., 2008
Rapeseed	Acetyl CoA carboxylase (*acc1*)	RTi-PCR	Hernandez et al., 2001
	Acetyl CoA carboxylase (*acc1*)	RTi-PCR	Schmidt and Rott, 2006
Rice	Sucrose phosphate synthase	RTi-PCR	Ding et al., 2004
Soybean	Lectin (*le1)*	cPCR	Meyer et al., 1996
	Heat-shock protein (*HSP*)	cPCR	Krech, 1997
	Lectin (*le1)*	cPCR	Wurz et al., 1998
Tomato	Metallo-carboxypeptidase (*mpci*)	RTi-PCR	Hernandez et al., 2003b
	LAT52	RTi-PCR	Yang et al., 2005
	β-fructosidase	RTi-PCR	Chaouchi et al., 2008
Wheat	25S–18S ARNr	cPCR	Allmann et al., 1993
	Low Molecular Weight glutenin	RTi-PCR	Terzi et al., 2003
	Waxy-D1	RTi-PCR	Ida et al., 2005

Commonly used techniques for plant transformation introduce a randomized and unknown copy number of the construct into the host plant, and it may be difficult to accurately determine the copy number of the integrated sequence as well as the location in the nuclear genome. If the detection method targets a certain part of the gene construct, exact absolute quantification becomes uncertain if the inserted copy number is not known. Consequently, the ideal DNA target sequence for GMO quantification is a sequence found only once in a stable and known copy number per genome. For this reason, several works describe the characterization of junction regions between the host plant genome and the transgene (Zimmermann et al. 2000; Holck et al. 2002; Hernandez et al. 2003a; Windels et al. 2003; Nielsen et al. 2004; Taverniers et al. 2004) that are unique for a single transformation event (event-specific). Only one copy of these junctions is present in the genome of the di- or polyploid transformant, as commercialized GMO lines are generally heterozygous (Berdal and Holst-Jensen 2001).

Relative and Absolute Quantification

Absolute quantification is the determination of the amount or copy number of the target DNA sequence in the analyzed sample, while relative quantification is the determination of GMO percentage referred to the endogenous reference gene. Relative quantification is achieved by the ratio of two quantification values: the GMO-specific gene versus the endogenous reference control gene. The ratio is expressed in percentage of genome/genome (g/g%) or of weight/weight (w/w%). A critical point for the relative quantification is that PCR efficiencies of each PCR system must be comparable. Another analytical strategy is the use of the $2^{-\Delta\Delta CT}$ analysis (Livak and

Schmittgen 2001); recently, for example, a validated quantitative RTi-PCR method for MON 810 has been assessed using this approach (Aguilera et al. 2009).

The lowest serial dilution allows the determination of absolute detection and quantification limits of the method, while a correct determination of the practical quantification limits requires a thorough statistical examination of the sampling procedure when preparing the serial dilutions, based on binomial distributions and Monte-Carlo simulations (Hernandez et al. 2003a). Finally, careful analysis and interpretation of data from the real-time PCR method have to be performed properly, with the use of the correct calculation and interpretation of correlation coefficients and regression techniques, especially when traces are analyzed (Burns et al. 2004).

Multiplex PCR

The number of commercialized GMOs is constantly increasing in the market; therefore, routine methodologies for GMO detection adapted to multiple analyses are convenient. Multiplex PCR requires several primers that lead to amplification of unique DNA regions under a single reaction (Atlas and Bej 1994; Elnifro et al. 2000; Wittwer et al. 2001). This approach is cheaper and less labor intensive, saving time and effort. However, the use of many PCR primers in a single tube can cause some problems, such as the increased formation of misprimed PCR products or "primer dimers," and the amplification discrimination of longer DNA fragments (Higuchi et al. 1992; Atlas and Bej 1994).

Application of Current Methodologies to GMO Analysis

The ultimate purpose of the development of any method for GMO analysis is its practical application in food analysis. Therefore, relevant issues such as the use of certified material and the validation and implementation of useful methodologies in food-industrial processes must be carefully considered. The Community Reference Laboratory for GM Food and Feed of the Joint Research Centre (JRC) of the European Commission has defined minimum performance requirements for analytical methods of GMO testing (http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requir_Analyt_methods_131008.pdf).

Use of Certified Reference Materials

The use of certified reference materials (CRM) is needed in RTi-PCR systems for assessment of GMO quantification. The reference materials used are either GM raw materials (i.e., flour where DNA must be purified) or plasmids (that contain the required targets) (Taverniers et al. 2001, 2004; Kuribara et al. 2002). Ideally, the reference material should simulate the sample under study as much as possible, although this is not always feasible due to the wide range of food matrices (Yates 1999). Furthermore, DNA content per mass must be considered because it fluctuates among different cultivars of the same species due to the variation of the DNA content of endosperm, embryo, and teguments of kernels (Trifa and Zhang 2004). Currently, only high-quality DNA samples purified from certified raw materials or plasmids are being used as reference controls to determine the ratio of transgenic DNA to total. It is still a matter of debate whether genomic DNA extracted from certified raw materials better qualifies as a standard compared with the use of plasmids containing the target sequences. While the genomic certified material better mimics the target of detection in the real sample, it is sometimes difficult to obtain and is expensive. The use of plasmids as standards has been recently introduced (Hernandez et al. 2003a; Taverniers et al. 2004, 2005; Toyota et al. 2006), with the advantages of being

cost-effective, reliable, and having a wider linear range of detection compared with genomic reference material

A new strategy for construction of reference material has been devised (Roth et al. 2008). This technique is based on the genome amplification by multiple displacement amplification (MDA). It is based on the use of a specific DNA polymerase, *phi29* DNA polymerase, and random hexamer primers for the replication of genomic DNA in an isothermal reaction at 30°C, leading to the synthesis of large amounts of DNA with fragments >70 kb in size.

Validation of Analytical Methods

Methods for the detection, identification, and quantification of GMOs have become widely used by enforcement laboratories, and the number of published systems has increased considerably in recent years. Nowadays, a wide range of methods is available, which can generate confusion for final users. In order to determine the suitability of each method for providing reliable analytical data, every method should be validated (Anklam et al. 2002). The concept of validation implies that the application of a given method must provide similar analytical results in different laboratories using different reagents and operators. For this reason, the validation process must involve several laboratories, which have to be coordinated by a principal one, usually different from the laboratory that developed the method. In Europe, there are institutes that coordinate GMO validation studies, such as the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) in Germany, or the Community Reference Laboratory (CRL) through the Biotechnology and GMOs Unit of the Joint Research Centre (JRC) in Italy (http://gmo-crl.jrc.it). The Gipsa Grain Inspection, Packers and Stockyards Administration (GIPSA) of the U.S. Department of Agriculture (USDA) and FAPAS®- FEPAS®-GeMMa (Food Analysis Performance Assessment Scheme, Central Science Laboratory, York, UK) promote rounds of quantitative proficiency testing and qualitative (presence/absence) detection, in which any food laboratory is encouraged to participate to test its performance.

A common set of criteria for performance of GMO detection methods should be evaluated (Bertheau et al. 2002; Bellocchi et al. 2008; Žel et al. 2008). Recommendations for the validation of quantitative PCR methods are presented by Hübner and coworkers (2001), and currently there is an international standard that defines the PCR performance parameters for GMO analysis (ISO 2006).

Implementation of Methodologies in the Food and Feed Chains

GMO quality-control programs are increasingly applied throughout food-chain production under the framework of legislative control measures. Thus, the availability of reliable, rapid, and accepted test systems to detect the presence or absence, or even the degree of contamination of GMO, becomes increasingly important for the agricultural and food industry. However, the implementation of GMO detection methodologies in the food and animal feed chains is not a simple issue. For a realistic implementation in food laboratories, several important aspects, such as the sampling procedure and the use of adequate reference materials for controls and standards, as well as the selection of the analytical methods, must be seriously considered. In addition, in the GMO analysis scenario, two main limitations are still present: (1) the important restrictions for access to protected transgene sequences, and (2) the scarce availability of GM certified material usually provided by the biotech companies only under strict confidentiality agreements. In this context, nowadays, certified reference material (CRM) is only available for the following GMOs: GTS40-3-2,

356043 and 305423 soybean, Bt11 event 176, CBH-351, GA21, NK-603, MON810 MON863xMON810, 1507, 3272, MIR604, 59122 maize lines, EH92-527-1 potato, 281-24-236 x 3006-210-23 cotton seed, and H7-1 sugar beet (http://irmm.jrc.ec.europa.eu/html/reference_materials_catalogue/catalogue/RM_Catalogue.pdf).

Finally, the selection of the analytical method to be used must consider three premises: (1) the scientific knowledge in the field; (2) the principal performance features of the available methodologies (e.g., specificity, sensitivity, accuracy, and precision); and (3) practical aspects, such as cost per analysis, time to achieve conclusive results, and ease of sample handling and processing (Auer 2003). The CRL publish validated methods for detection of authorized GMOs (http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm).

During recent years, molecular approaches have significantly contributed to the field of GMO detection. However there are several limitations that prevent the total implementation of these methodologies into food laboratories. The major inconvenience is the cost of the required devices and reagents, and the need for qualified personnel. Even though the demand for RTi-PCR instruments has constantly decreased in recent years with a parallel decline in their cost, the expense-per-analysis is still too high to be practical for routine analysis in food laboratories. Other potential problems facing the implementation of molecular-based methodologies include the risk of contamination and the difficulties in the extraction process of certain types of food sample. As instrumental techniques, the molecular-based methods have a tendency to produce false-negative and -positive results. The main cause of false-positive results is the accidental contamination of the samples or the reagents with positive samples (cross contamination) and with amplification products and plasmid clones (carry-over contamination). This is a central issue for any GMO-detection protocol based on highly sensitive PCR methodologies.

High Throughput Detection.

The methodology for reliable GMO detection, based on real-time PCR, is rather well established under laboratory conditions. This validated technology needs to be compliant with EU mandatory rules governing the labeling of food products with over 0.9% of authorized GMO (European Commission 2003b). However, two practical limitations offer hurdles to its adjustment to the expected growing requirements: the number of GMO authorizations is expected to increase in the future (Golden Rice will be available for farmers in 2011), and subsequently, the number of agronomical traits and transgene events should parallel such an increment; and the economical cost for GM food analysis, based on real-time PCR methods, is still far from being affordable due to the large number of samples to be analyzed in the future.

A promising approach that could bypass both limitations is the application of microarray technology, which enables the simultaneous detection of a large number of different targets (Elenis et al. 2008). The development of colorimetric-based detection methods in the microarray system would also help reduce the cost of expensive current fluorescent methodologies for detection. In spite of its great potential to become the standard GMO detection methodology, microarray technology suffers a severe restriction related to the needs of efficient quantification as required for GMO labeling, which may hamper its apparent superiority. The possible solution to this drawback is to perform quantitative amplification and detection after hybridization on the chip, which could be achieved with available techniques.

Some microarray-based methods have already been developed. Germini et al. (2005) developed a PNA microarray for the detection of four GM maizes, one GM soybean,

and two endogenous controls, the zein gene for maize and lectin gene for soybean. A previous step of multiplex PCR was used, and one primer of each set was labeled. The detection limit was below the EU recommendation (0.25%) for each GMO. Xu et al. (2006) designed three different microarrays, the first for the most used regulatory sequences including the 35S promoter, 35S terminator, *nos* promoter, *nos* terminator, *npt*II terminator, and the FMV 35S promoter, while the second microarray treated specific gene inserts (soybean, cotton, and rapeseed), and the third one was for endogenous controls. A previous step of multiplex PCR was done in which the amplified fragments were labeled with Cy5-dCTP. The detection ranged from 0.5% (soybean) to 1% (maize). The same research group has also developed a multiplex PCR-microarray for the detection of GM soybean and six GM maizes. Amplified fragments were labeled with Cy5-dCTP; the limit of detection was similar to the other microarray (0.5–1%) (Xu et al. 2007). Leimanis et al. developed (2006, 2008) a PCR-Microarray for the detection of five plant species and three screening sequences (35S promoter, T-*nos*, and the *npt*II gene) that has been validated (Leimanis et al. 2008). The detection was based on streptavidin-conjugated gold nanoparticles, and the limit of detection was below 0.3%. Schmidt et al. (2008) have developed a rapid multiplex PCR-microarray method for screening GM canola. It contains probes that are construct-specific to the 12 approved GM canola lines in Canada, regulatory elements (CaMV 35S promoter, T-*nos*, and *npt*II), a canola-specific endogenous gene, and endogenous genes from heterologous crops. The limit of detection was determined to range from 0.1% to 0.5%. In addition, a real-time PCR array has been developed for universal detection and identification of unapproved GMOs that consists of 30 primer-probe sets distributed in a 96-well plate for detection of GM lines, recombinant DNA segments, endogenous reference genes, and donor organisms (Mano et al. 2009). There are also other platforms reported for high-throughput detection based on multiplex assays on the basis of SNPlex technology (Applied Biosystems), which allows the simultaneous detection of up to 79 SNPs in two panels containing 47 and 48 probes, respectively (Chaouachi et al. 2008).

While the quantification obstacles for the microarray approaches are surmounted, its practical application in GMO analysis will probably be restricted to an initial step for detection and identification that may be combined with subsequent specific quantitative approaches with validated real-time PCR procedures.

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Chapter 30

HACCP: Hazard Analysis Critical Control Point

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Food Safety and Hazard Analysis Critical Control Point

Food safety is one of the concerns and objectives of the current policy of several developing and developed countries. It aims to improve the life quality (increasing quality and years of healthy life, and eliminating health disparities) of their citizens and is considered to be one of the challenging goals for the twenty-first century (COM 2000; USDHHS 2000; WHO 2004; PAHO 2008). Food safety has increasingly become a current issue, owing to changing consumer eating habits, changing products and production practices, changing population (aging of the population and the increased number of immunocompromized individuals), and increased food infections. In fact, the global incidence of food-borne diseases is difficult to estimate, but it has been reported that in 2005 alone, 1.8 million people died from diarrhoeal diseases (WHO 2007). The priority is to reduce food-borne diseases.

In addition, in a global market, food safety is a priority for food trade and is an economic issue. Food trade growth and food marketing changes lead to exposure to new risks and greater potential consequences of food-borne illness outbreaks. Consumers in industrialized countries have become more aware of the potential food safety hazards through media reports and increased availability of scientific knowledge. The challenge is to develop integrated effective food safety systems, essential to maintain consumer confidence in the food system, and to provide a sound regulatory foundation for national and international trade in food, in order to support economic development.

In the past few years, there has been some reinforcement for these efforts from new legislation and regulations by international food safety authorities and national and international governments (examples being the Codex Alimentarius standards [FAO/WHO 2005], the General Food Law [EC 2002/178], and all legal documents based on the General Food Law [Trienekens and Zuurbier 2008]), where there is a definite move away from the old end-of-line product inspection approach to a new quality-assurance approach where the suppliers or food operators in the chain assume responsibility for safety. The General Food Law (EC 2002/178) provided a solid base upon which further important food safety rules, such as the so-called "Hygiene Package" (EC 2004/852; EC 2004/853; EC 2004/854), were then built at the EU level. The industrialized nations are adopting a common approach to food safety regulation.

Elements of this common approach include use of Hazard Analysis Critical Control Point (HACCP) methodology, farm-to-table risk assessment, and greater use of policies to create incentives for safety. In several countries, new food safety regulatory agencies have been created with the express purpose of integrating and focusing expertise from both agriculture and public health (EC 2007). These common trends should lead to

greater fundamental agreement about food safety standards.

Hazard analysis and critical control points are considered worldwide to be essential to an effective and rational proactive methodology, which must be integrated into every step of the food chain, from primary production to final consumption, in order to assure food safety. "Food safety from farm to fork" is an expression frequently used and stated in the White Paper (COM 2000), reinforcing the integration of all participants and interactive communication between them. This communication represents an essential key for the clarification and the accomplishment of safety objectives through suppliers and direct consumers.

Before being enforced by law and praised by global free trade, HACCP was, and is, recognized by food operators as the most reliable system to assure food safety (Scott et al. 2009). The terms of reference for food safety include biological, chemical, and physical agents that may be present in a food and are hazards likely to cause illness or injury to consumers if not put under control (Mortimore and Wallace 1997).

The risk (probability for a hazard to occur) of food-borne illness associated with meat products is very high (CDC 2008; EFSA 2009). Meat products head the list of food most frequently associated with safety hazards and human illness. This risk could be reduced to the maximum extent possible only by ensuring that appropriate and feasible preventive and corrective measures are taken at each stage of the production process where safety hazards occur, for which a possible strategy is the implementation of HACCP systems in every establishment. This strategy has become mandatory in several countries (the final rule HACCP, FSIS/USDA 1996; EC 2004/852; EC 2004/853). However, there is no single technological or regulatory solution to the problem of food-borne illness. Continuous efforts are required by industry, other food chain interveners, and governments to improve methods for identifying and preventing hazards and to minimize the risk of illness.

Preparing Development and Implementation of an HACCP Plan

The development and implementation of an HACCP system in a meat-processing establishment or other food plant is a major task and depends on the commitment and motivation of the upper administration; workforce acceptance and understanding of the importance of each individual activity to assure safety is also fundamental. Development and implementation of a HACCP system supported only by middle management or technical personnel is an impossible mission.

A team leader should be designated who has organizational and communication skills, familiarity with plant processes, and absolute authority to track the program in the company. The team leader's work starts by organizing a team of workers who have been previously selected and trained in hazard food analysis and HACCP principles. The team should include operational staff who know all the particularities of the practical tasks; a theoretical team must be avoided. HACCP team personnel should have competence in different areas, such as quality, commodities purchasing, production, engineering, distribution, microbiology, toxicology, and auditing. Most of the meat industries, large or small units, need help to construct and implement the plan because all the abovementioned competences are difficult to put together and might not be available. Expert advice should be obtained from outside sources, such as trade and industry associations, independent experts, regulatory authorities, and assisted HACCP training, with the workforce guided by sector-specific operative manuals. However, consultants could be used to provide advice rather than write

the whole plan. If consultants provide the plan with minimal food business operator input, the team can have difficulty during audits, since they are not identified with the plan.

A strategy approach to HACCP program development must be previously defined and decided; the approach could be defined either by product or by process, as an individual plan or one by sector (sectorial) (Mortimore 2001).

The initial steps that the HACCP program will include are:

1. The description of all products that are produced in the plant and the means of distribution. This is particularly important to product formulation and screening of potential abuse through distribution or by consumers.
2. Identification of projected uses and consumers of the products. According to the diversity of the meat products, consumption can take place after culinary operations (cooking, frying, or grilling) or they can be presented as ready to eat. It is also vital to identify if the product will be consumed by segments of the population who are at increased risk, such as infants, the elderly, and the immunocompromised.
3. Construction of a flow diagram for each process, providing a brief description of all operative steps involved, from the reception of raw meat and other materials to finished product distribution. It may seem like a simple process to draw up a flow diagram, but most processors seem to miss some of the process steps, particularly significant delays, or routine variations (e.g., the handling of part-filled cartons at the end of a production shift or a product waiting for cooking or cooling steps). Inputs and outputs to normal flows should also be described, as these can produce their own hazards (e.g., ingredients, specific risk material disposal). The flow diagram must be verified in the plant to confirm its accuracy.
4. Prepare a HACCP plan with a hazard analysis based on stated terms of reference, while assessing the critical control points (CCPs) to put them under control. The HACCP plan must be coupled to the operating instructions and updated with it.

The construction and implementation of this system is a big, expensive task, the success and financial return of which implies both the support and full involvement of upper management as was stated above. HACCP implementation problems associated primarily with plant size come up consistently in the literature. The smaller operators have the greatest difficulty with HACCP implementation. On the industry side, the transition to HACCP is most challenging for small and very small plants, most of which do not have the technical and other resources that large plants have (Stafko 2008).

HACCP requirements should take into account the principles contained in the Codex Alimentarius. They should provide sufficient flexibility to be applicable in all situations, including small businesses. In particular, it is necessary to recognize that in certain food businesses, it is not easy to identify those critical control points that make implementation of the plan difficult and also that, in some cases, good hygienic practices are essential to assure safety. Similarly, the requirement of establishing "critical limits" does not imply that it is necessary to fix a numerical limit in every case. In addition, the requirement of retaining documents needs to be flexible, in order to avoid undue burdens for very small businesses (EC 2004/852). However, flexibility should not compromise food hygiene and safety objectives.

HACCP Principles

Food business operators shall put in place, implement, and maintain a permanent procedure or procedures based on the seven HACCP principles.

Principle 1

Perform a hazard analysis based on terms of reference. The terms of reference for food safety include biological, chemical, or physical agents that may be present in a food and are hazards likely to cause illness or injury to consumers if not put under control (Mortimore and Wallace 1997). Hazard analysis consists of two distinct parts: the first is hazard identification and the second is hazard evaluation (risk assessment of that hazard at each process step until the point at which the hazard is controlled). If the correct significant hazards aren't identified, then the HACCP plan that is developed cannot possibly be valid. This is where a "whole-chain" approach can help with defining the hazards. Operators should use a formal approach to hazard analysis, such as a matrix, to ensure a more disciplined approach to the process (FAO 1997; Mortimore and Wallace 1997).

Principle 2

Identify adequate critical control points (CCPs) in materials and process steps to control the hazards.

Principle 3

Define critical limits for each CCP. The critical limits are associated with the preventive measures that control hazards and are measurable. Deviation tolerance must be defined.

Principle 4

Establish monitoring requirements to warrant fulfilment of procedures at each stated CCP.

Principle 5

Create corrective actions to be immediately applied to restore control when a deviation from the outlined limits occurs at a CCP.

Principle 6

Set up verification procedures to test compliance of the plan with the HACCP system.

Principle 7

Produce documents and establish records of operations, including procedures for monitoring, corrective actions, and verification to provide an effective demonstration of the system as it works.

The final element for HACCP development is to update the plan when changes in the process or new legal or trade requirements are introduced.

The HACCP plan is specific to a certain product or process and enterprise. Nevertheless, in many cases, several product lines are so similar that they can be grouped together in generic models. Due to the diversity of meat products that can be produced with similar process steps, one HACCP plan can cover a process that is used for a number of similar products with different commercial codes (e.g., Toulouse sausage, barbecue sausage, fresh sausage, longanissa, merguez pork, chipolata, white wine sausage). It is not necessary to have a separate HACCP plan for each product if the hazard analysis shows that the products share the same potential hazards, risks, CCPs, and critical limits (Tompkin 1996).

The use of a generic HACCP model to develop specific HACCP plans always needs creative adaptation and tuning. In this case, system validation is always needed for each specific plan.

To accomplish with success the implementation of HACCP plans, some prerequirements need to be fulfilled. These are

mandatory for food safety. In small food workshops, they can per se be the foundation of so-called light HACCP. It may be assumed *in extremis* that a well-trained person with access to guidance is able to implement in-house HACCP methods.

On the Pathway to a Generic HACCP Model for Processed Meat Products

Pre-Requirements

HACCP plans must be supported by comprehensive prerequisite programs. They are the groundwork for successful HACCP plan implementation. The operator should establish, implement, and maintain a cluster of procedures to control the introduction of hazards through the environment and cross-contamination. These practical procedures or processes, known as good hygiene practices (GHP) and good manufacturing practices (GMP) programs, are critical for small meat- and poultry-processing plants, and are required to fulfil a HACCP plan for meat products. GHP and GMP return the processing environment to its original condition (disinfection or sanitation programs); keep building and equipment in efficient operation (maintenance program); control employees' security and hygiene; control cross-contamination during manufacture (usually related to people, surfaces, the air, and the segregation of raw and processed products); provide potable water; control pests; control chemicals; and calibrate equipment (FDA 2005; Raspor 2008). The definition of raw material specifications and its agreement with suppliers is one of the fundamental pre-requirements for prevention of hazards that should be introduced in a meat-processing plant. The traceability of meat and any other ingredient used in a meat product must be established and guaranteed at all stages of production and distribution. Training for all meat product handlers should be routinely provided and updated with regard to good-hygiene practices and fundamental processing steps; understanding of their role as part of a safety system is fundamental.

General GHP and GMP codes for different food process categories have already been provided by regulatory food standard organizations (FAO/WHO 2005a). However, enterprises must define and elaborate their own GHP and GMP codes as operative guides. If not, the HACCP plans developed will either try to control too many issues through the use of critical limits or just will not control them at all.

Where possible, these prerequisite programs should have definite outcomes specified, in order to verify and validate operations (e.g., *Enterobacteriaceae* counts and total counts for food contact surfaces after sanitation has been completed)(Gonzalez-Miret et al. 2001). Also, a checklist designed to evaluate pre-requirements should be included in audits for their verification.

According to data from a checklist designed to evaluate pre-requirements for GHP and GMP presented in the Tradisausage project (2006), approximately 80% of the small meat-processing units (regarding essentially traditional products) in Southern Europe (including France, Greece, Italy, Spain, Portugal, and Slovakia) have the main pre-requirements essential to implement the HACCP system. However, they have to overcome the problem relating to documented evidence and validation of GHP (Fraqueza et al. 2007).

The application of a validated Code of Hygiene practice will be effective to reduce the risk of hazards and is fundamental for subsequent implementation of a HACCP-based approach.

Product Definition and Intended Uses

A great diversity of meat products with singular organoleptic characteristics are found in the meat industries (large or small) in dif-

ferent countries. To achieve the best profitability, a meat-processing industry usually tries to get the best valorization from all pieces of a pork carcass and other ingredients by producing a diversity of meat products that could be classified in different groups, according to the particular technology used, as fresh, cooked (cured or meat emulsions or finely comminuted meat), fermented/dry/smoked, and cured/dry meat products (Raken 2000). Regarding these different products, different HACCP plans specific to a certain product, process, and enterprise need to be developed. Even so, in many cases, several product lines are so similar that they can be grouped together in generic models. A generic HACCP model for fermented meat sausages has been presented in a previous work (Fraqueza et al. 2007); so to avoid repetition, generic models of HACCP plans for a fresh meat product (fresh sausage) and for a cooked meat product (cooked ham) will be presented as examples here.

There is a great diversity of fresh sausages, with different recipes and different denominations in different countries. Nevertheless, these sausages have in common a sequence of process steps. Thus, fresh sausages (Table 30.1) are the end product of ground pork meat mixed with fat, salt, preservatives (sulphite), and seasonings (pepper, garlic, or other spices) and stuffed into natural or collagen casings. These sausages are marketed without the assurance of a lethal heat treatment. Other types of meat such as poultry, beef, or lamb can be used with similar seasoning and processing.

Cooked ham is an international meat product consumed by many people and very popular among children and young people. This product (Table 30.1) is made from leg or shoulder deboned meat, injected with a brine (prepared with cold water, salt, flavors, polyphosphates, and nitrite) and cooked for a specific time period to assure completed protein coagulation and color development.

It is important for an HACCP team to know what kind of product or products they are actually dealing with to develop their HACCP plans (Table 30.1), and how and by whom they will be consumed (Mortimore and Wallace 1997).

Technology: Process Description and Flow Diagram

Before initiating the design of an HACCP plan, a technological flow diagram for each product, depicting all pertinent manufacturing steps from the reception of raw materials to final product, from receiving to shipping, should be outlined by the HACCP team. This diagram is a simple schematic picture of the process used in the plant to produce the product. It does not need to be complex.

The best way to make sure that your specific flow diagram is accurate (correct and complete) is to verify it carefully by the HACCP team walking through the plant and making sure all the steps in the process are included in the flow diagram (Blumberg 2008). The flow diagram will be used for hazard analysis after being validated on the premises.

The technology of process description must comply with all specified good hygiene and manufacturing practices for the elaboration of a specific fresh sausage (Fig. 30.1) or cooked ham (Fig. 30.2), as defined in the pre-requirements.

Hazards Identification and Analysis

A hazard is defined as a biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control (FAO 1997).

A hazard analysis is conducted to develop a list of hazards that may be reasonably expected to occur at each step of the food process and, in the present case, in a meat product process from the reception of all

Table 30.1. Product definition and intended use for fresh pork sausages and cooked ham in pieces

Product names	Fresh sausage, Toulouse sausage, barbecue sausage, longanissa, merguez pork, chipolata, white wine sausage	Cooked Ham
Product composition (depends on the recipes of different countries)	Pork lean meat and fat trimmings, water, salt, sugar, dextrose, lactose, condiments (pepper, nutmeg, ginger, garlic, coriander, other spices), additives (sulphite, ascorbic or citric acid, carminic acid (cochineal) or Ponceau 4R, polyphosphates, monosodium L-glutamate), casings	Pork leg or shoulder, water, sugar, salt, nitrite, ascorbate, phosphates, flavours, vegetable protein hydrolyzed, yeast extracts, lactose, starches, hydrocolloids (carrageenans), dry blood powder, smoke extracts
Product characteristics	Size and weight: 10-15 cm, 1.5–2 cm Ø, pH: 5–6.5 aw ≥ 0.98	Size and weight: 4–6 kg pH: 6.0 aw ≥ 0.98
How it is to be used	After culinary operation T ≥ 68°C: cooked, fried, grilled	Ready to eat
Packaging	Aerobic package or under modified atmosphere package	Vacuum package
Shelf life	7 days	6 months
Places/premises of commercialization or marketing	Hypermarkets and butcher's shops, but also in restaurants, retail dealers	Hypermarkets, and butcher's shops, but also in restaurants, retail dealers
Storage conditions	Under refrigeration	Under refrigeration
Labeling instructions	According to legal labeling requirements	According to legal labeling requirements
Commercialisation or marketing conditions	Keep in a refrigerated place	Keep in a refrigerated place

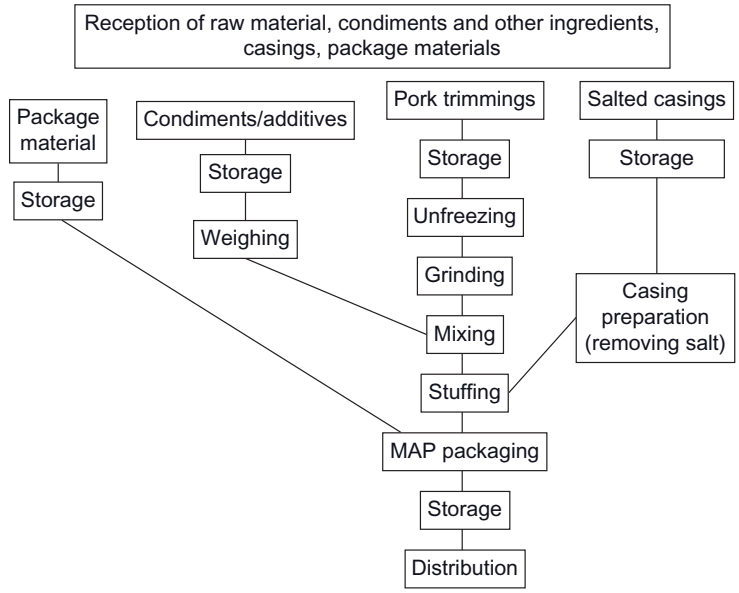

Figure 30.1. General flow diagram of a fresh sausage.

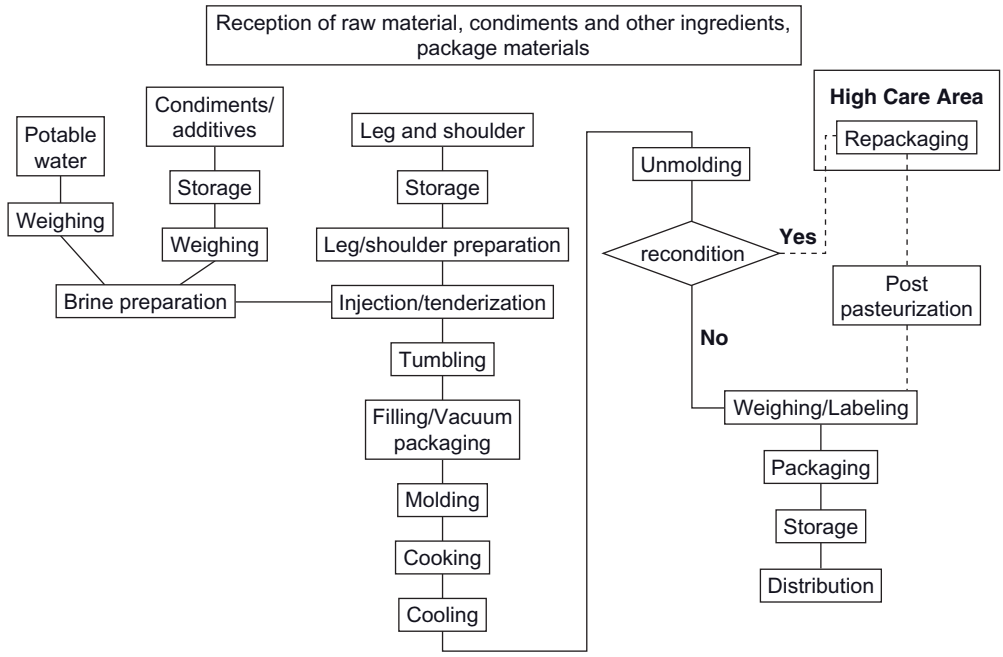

Figure 30.2. General flow diagram of a cooked ham.

ingredients, storage, etc., to the end product and its distribution.

In Table 30.2, potential biological, chemical, and physical hazards are shown for incoming materials common to several meat products, with the process for fresh sausage and cooked ham taken as examples for this presentation. Table 30.3 presents hazards identified in the processing steps for fresh sausage, while Table 30.4 shows those identified for the cooked ham process. The hazard list is usually based on experimental and epidemiological historic data and literature references.

This task must then consider what control measures, if any, exist that can be applied to each hazard. Finally, the hazards associated with each step should be listed along with the measures necessary to control the hazards (Tables 30.5 and 30.6). More than one control measure may be required to control a specific hazard, and more than one hazard may be controlled by a specified control measure. After considering the probability of occurrence and severity of a hazard, its risk must be assessed, which then determines which hazards are to be addressed in the HACCP plan.

The justification for why a hazard is or is not reasonably likely to occur based on epidemiological data or referenced works is important and can also be viewed as a form of validation (Scott 2005).

The microbial hazards in meat-processing industries depend on the prevalence of pathogens in raw meat and premises, taking into consideration their specific microbial ecology (Tompkin 2002). The occurrence of potential microbial hazards (*E. coli*, *Salmonella* spp., *S. aureus*, *Listeria monocytogenes, Campylobacter spp.*) associated with meat products has been reported (Doyle and Erickson 2006; CDC 2008; EFSA 2009). These pathogens can be present in meat-processing environments (machines, cutting tables, knives), and inadequate hygiene prac-

Table 30.2. List of potential biological, chemical and physical hazards to be addressed in incoming materials for meat products such as fresh sausages and cooked ham (Oiye and Muroki, 2002; Roy *et al.*, 2003; Pearce *et al.,* 2004; FDA/CFSAN, 2009; CDC, 2008 and 2009; Bonardi *et al*., 2003; Doyle and Erickson, 2006; Toldrá and Reig, 2007; Mc Dowell *et al*., 2007; Fredriksson-ahomaa et al., 2007; Nørrung & Buncic, 2008; EFSA, 2009)

		Potential Hazards Identification		
		Biological (bacteria, parasites, virus)	Chemical	Physical
Incoming materials	Raw meat: pork	Non-sporulating bacteria: *E. coli, Salmonella* spp., *S. aureus, Listeria monocytogenes, Campylobacter* spp, *Yersinea enterocolitica*. Sporulating bacteria: *C. botulinum, C. perfringens* Parasites: *Trichinella; Cysticercus cellulosae, Toxoplasma gondii*	Antibiotic/drug residues. Hormones. Pesticides. Biogenic amines of microbial origin. Dioxines. Micotoxines	Bone, plastic, wood, metal particles
	Spices	*B. cereus, C. botulinum, Salmonella* spp., *Listeria monocytogenes*	Non-food chemical. Pesticides Micotoxines	Plastic, sand and wood particles, stones
	Additives		Non-food chemical. Polycyclic aromatic hydrocarbons (PAH), heavy metals	Metal, sand and soil particles, stones
	Salt	*E. coli, Salmonella* spp., *Vibrio* spp., Staphylococcus aureus; *Clostridia*	Non-food chemical.	Metal, sand and soil particles, stones
	Natural Casings salt	*E. coli, Salmonella* spp., *Listeria monocytogenes, Clostridia*	Micotoxines	
	Water	Not potable, *E. coli, Salmonella* spp., *Vibrio* spp., *Clostridia, Cryptosporidium*, virus (Hepatitis A and E virus, Rotavirus, Noravirus)	Non-food chemical	
	Packages		Chemical migration of non food grade packaging material or improper printed labels, inaccurate labeling by supplier	

tices may result in a loss of control, thereby creating a hazard (Mexaopoulos et al. 2003). Practices pre-required by HACCP methodology, coupled with monitoring, will prevent introduction of microbial hazards in the meat-processing system or will control their presence in the environment.

According to several authors, some toxic chemical compounds may be present on raw meats or generated in certain types of meat processing (Kan and Meijer 2007; Toldrá and Reig 2007; Demeyer et al. 2008). The use of veterinary drugs in animals for therapeutic or prophylactic reasons must be under the strict control of a responsible veterinarian. The probability of veterinary drug contaminants in raw meat, however, is not excluded, due to bad management of animal production practices or illegal practices. Programs for monitoring drug residue in live animals

Table 30.3. List of potential biological, chemical and physical hazards to be addressed in fresh sausage processing steps

		Potential Hazards Identification		
		Biological (bacteria, parasites, virus)	Chemical	Physical
Fresh Sausage Process Steps	Reception	Reception of noncompliant material with legal or predefined requirements Growth of pathogens on frozen meat due to time/temperature abuse	Non food grade packaging material or improper printed labels	Reception of noncompliant material with legal or predefined requirements
	Storage	Growth of pathogens on meat due to time/temperature abuse Bacterial contamination of spices during storage, growth of fungi producers of micotoxines due to humidity abuse	Micotoxines	
	Unfrozen: pork trimmings	Contamination and growth of pathogens due to time/temperature abuse		
	Grinding	Contamination and growth of pathogens due to time/temperature abuse Contamination due to poor sanitizing of equipment	Detergent, disinfectant and lubricant residue	Bone and metal particles falling into meat during grinding
	Casings desalting	Growth of pathogens due to time/temperature abuse		
	Weighing ingredients and additives		Excess of nitrites/nitrates or other additives due to weighing errors	
	Mixing	Growth of pathogens due to time/temperature abuse	Detergent, disinfectant and lubricant residue	
	Stuffing	Contamination with pathogens by poor hygiene practices	Detergent, disinfectant and lubricant residue	Casing bursts, bone particles, metal clips, cotton string falling into meat during stuffing.
	MAP Packaging and labeling	Pathogen bacterial contamination by environment or handling during packaging Growth of pathogens by improper package sealing or gases concentration	Allergies to an ingredient due to wrong labelling of a product or lack of advice on allergenic substances	Metal clips or fragments, bone particles falling into the packages during packaging
	Storage end product	Growth of pathogens due to improper time/temperature/humidity		
	Distribution	Pathogenic bacterial contamination through damaged packages Growth of pathogens due to improper time/temperature/humidity	Non food chemical residue cross-contamination through damaged packages	Plastic, wood, metal particles in damaged packages

Table 30.4. List of potential biological, chemical and physical hazards to be addressed in cooked ham processing steps

Cooked Ham Process Steps	Potential Hazards Identification		
	Biological (bacteria, parasites, virus)	Chemical	Physical
Reception	Reception of noncompliant material with legal or predefined requirements Growth of pathogens on fresh meat due to time/temperature abuse	Non food grade packaging material or improper printed labels	Reception of noncompliant material with legal or predefined requirements
Storage	Growth of pathogens on meat due to time/temperature abuse		
Pork leg or shoulder deboning and preparation	Contamination and growth of pathogens due to time/temperature abuse		Bone and metal particles falling into meat during cutting
Weighing ingredients and additives		Excess of nitrites, phosphates or other additives due to weighing errors	
Brine preparation	Contamination and growth of pathogens due to time/temperature abuse		Foreign material falling into the pickle solution
Injection/tenderization	Contamination and growth of pathogens due to time/temperature abuse, pathogens biofilms	Excess of nitrite or other additives (overaddition) Detergent, disinfectant and lubricant residue	Bone and metal particles Broken needles Metal fragments from damaged inadequately maintained equipment
Tumbling	Growth of pathogens due to time/temperature abuse		Metal fragments from damaged inadequately maintained equipment
Filling/vacuum package	Bacterial pathogens growth due to time/temperature abuse Contamination with pathogens by poor hygiene practices	Detergent, disinfectant and lubricant residue	Package bursts, bone particles, metal clips, falling into meat during filling. Metal fragments from damaged inadequately maintained equipment
Molding	Contamination with pathogens by rupture of vacuum bag and poor hygiene practices		

(continued)

Table 30.4. List of potential biological, chemical and physical hazards to be addressed in cooked ham processing steps. *(cont.)*

		Potential Hazards Identification		
		Biological (bacteria, parasites, virus)	Chemical	Physical
Cooked Ham Process Steps	Cooking + Cooling	Survival of pathogens due to inadequate temperature or cooking time Spores of *C. perfringens* sporulation & growth due to inadequate chilling rate		
	Unmolding	Bacterial contamination from poor handling of bags with contamination of the outside of the cook & strip bag Cross-contamination with pathogens (e.g., *Salmonella* sp., *L. monocytogenes, S. aureus, etc.*) by employee inadequate handling/unclean equipment		
	*Repackaging/High Care area	Cross-contamination with pathogens (e.g., *Salmonella* sp., *L. monocytogenes, S. aureus, etc.*) by employee inadequate handling/unclean equipment		
	*Postpasteurization	Survival of pathogens due to inadequate temperature/time of pasteurization		
	Weighing and labeling	Pathogen bacterial contamination by environment or handling during packaging Growth of pathogens by improper package sealing or gas concentration and to improper coding (best before)	Allergies to an ingredient due to wrong labeling of a product or lack of advice on allergenic substances	
	Packaging	Growth of pathogens due to improper time/temperature/		
	Storage end product	Growth of pathogens due to improper time/temperature/ humidity		
	Distribution	Pathogenic bacterial contamination through damaged packages Growth of pathogens due to improper time/temperature/ humidity	Non food chemical residues cross-contamination through damaged packages	Plastic, wood, metal particles in damaged packages

Table 30.5. Preventive measures to control potential hazards identified in the production of fresh sausages

Process steps	Hazard Identification	Preventive measure
Reception:		
Frozen Pork trimming	Biological Chemical Physical	Suppliers selected and homologated and/or certified Correct temperature of delivery (<–12°C) Visual control
Other ingredients		Suppliers selected homologated and/or certified Visual control of shelf life dates according to specified requirements
Water		According to specified and legal requirements of potability
Package materials		Suppliers selected homologated and/or certified, according to specified legal requirements
Storage	Biological	GHP and GMP Corrected temperature and relative humidity
Unfrozen Pork trimming	Biological	GHP and GMP Corrected temperature and relative humidity
Casing desalting	Biological	GHP, GMP Corrected time/temperature
Grinding	Biological Chemical Physical	GHP, GMP Preventive equipment maintenance Use of nontoxic food compatible cleaning compounds
Weighing of ingredients and additives	Chemical	Adequate weighing control, safe operating practices according to additive instructions and legal requirements, calibration of scales
Mixing	Biological Chemical	GHP, correct time/temperature, preventive equipment maintenance, use of nontoxic food compatible cleaning compounds
Stuffing	Biological Chemical Physical	GHP, GMP, correct time/temperature, use of non toxic food compatible cleaning compounds, preventive equipment maintenance, correct stuffing pressure machine Visual check
Packaging (MAP) and labeling	Biological Chemical Physical	GHP, GMP, preventive equipment maintenance, control of sealing and CO_2/O_2 concentration into packages Label monitoring by photoelectric cell or visual check, metal detector
Storage end product	Biological	GHP, GMP Correct time/temperature/humidity
Distribution	Biological Chemical Physical	GHP, GMP Correct time/temperature/humidity

and food products have been implemented in several countries, thereby contributing to better supervision and diminishing the occurrence of residues in meat (EC Directive 96/23/EC; FSIS 2007). Also, emerging antibiotic-resistant pathogen occurrence could be consequently diminished (Doyle and Erickson 2006).

The use of nitrite is common in cooked cured products, in order to develop the

Table 30.6. Preventive measures to control potential hazards identified in the production of cooked ham cooked in the bag

Process steps	Hazard Identification	Preventive measure
Reception:		
Raw Meat	Biological Chemical Physical	Suppliers selected and homologated and/or certified Correct temperature of delivery (<7°C) Visual control
Other ingredients		Suppliers selected homologated and/or certified Visual control of shelf life dates according to specified requirements
Water		According to specified and legal requirements of potability
Package materials		Suppliers selected homologated and/or certified, according to specified legal requirements
Storage	Biological	GHP and GMP Corrected temperature and relative humidity
Pork leg or shoulder deboning	Physical Biological	GHP and GMP Visual control Corrected temperature and relative humidity
Weighing of ingredients and additives	Chemical	Adequate weighing control, safe operating practices according to additive instructions and legal requirements, calibration of scales
Brine preparation	Biological Physical	GHP, GMP, correct temperature of brine inferior to 2°C, Visual check
Injection/tenderization	Biological Chemical Physical	GHP, GMP, Correct time/temperature, preventive equipment maintenance (injection pressure must be set), weight control after injection = weight before injection plus "X"% injection, use of nontoxic food compatible cleaning compounds
Tumbling	Biological Physical	Correct time/temperature, preventive equipment maintenance, use of nontoxic food compatible cleaning compounds
Filling/vacuum package	Biological Chemical Physical	GHP, GMP, correct time/temperature, use of nontoxic food compatible cleaning compounds, preventive equipment maintenance, correct filling pressure machine Control of sealing and vacuum packages Visual check, metal detector
Molding	Biological	GHP, GMP
Cooking + Cooling	Biological Biological	Correct time/temperature Potable Water Correct time/temperature Rapid cooling to temperatures ≤ 28°C as soon as possible and chill to 2 ± 2°C in 12 hours or less Held cooling ≈ 24 h/4°C
Unmolding	Biological	GHP, GMP Control of sealing and vacuum packages Visual check Recondition*
*Repackaging/High Care area	Biological	GHP, GMP, preventive equipment maintenance, correct stuffing pressure machine Control of sealing and vacuum packages, Visual check
*Post pasteurization	Biological	Correct time/temperature
Weighing and labeling	Biological Chemical	GHP, GMP, preventive equipment maintenance, Label monitoring by photoelectric cell or visual check
Packaging	Biological	GHP, GMP Correct time/temperature
Storage end product	Biological	GHP, GMP Correct time/temperature/humidity
Distribution	Biological Chemical Physical	GHP, GMP Correct time/temperature/humidity

attractive rose color. However, if an overdose of this additive occurs, it can cause hazardous situations, such as the formation of carcinogenic nitrosamines (Toldrá and Reig 2007). The sulphite used as a preservative in fresh sausages is also referred to as an allergen substance. Other ingredients frequently used in meat processing, such as milk, wheat, soy, egg, lupin, and pea protein, can provide hidden exposure to allergic people (Björkstén et al. 2008).

Contaminants from the environment could occur in meat used for processing. Dioxins, organophosphorous and organocloride compounds, mycotoxins, and heavy metals are mentioned. These contaminants can be present in animal feed or in the ingredients of the feed (Kan and Meijer 2007). With the integration of HACCP-based methodologies and Good Practices in animal production, it is important to reduce the occurrence of these hazards.

Epidemiological data is unknown for chemical residues of products used in sanitation and maintenance programs in meat products or raw meat (Alaña et al. 1996). The GHP and GMP implemented prevent their occurrence, so they are not considered to be an actual risk.

There is hardly any data regarding the assessment of physical hazards (bone, glass, plastic, and metal fragments) occurring in meat products. In spite of the low frequency of occurrence, there are high social repercussions with economic losses to the producer when this type of hazard is detected.

Critical Control Points Identification

A critical control point (CCP) is defined as a step in the flow diagram of the meat product process at which control measures can be applied. A CCP is essential to prevent or eliminate a hazard or reduce it to an acceptable level (FAO 1997). Complete and accurate determination of CCPs is fundamental to the control of food safety hazards. If a hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the process should be modified at that step, or at any earlier or later step, to include a control measure. Therefore, the added step would be the CCP in such a case.

In general, the CCP consists of two basic elements: the control system itself that implements the control measures, and the monitoring system. Control systems are usually practices/procedures that, when not done correctly, are the leading causes of food-borne illness outbreaks (i.e., hazards for consumers' health). Examples of CCPs may include thermal processing, chilling, chemical residue and metal control, and product formulation control. Effective monitoring systems of these practices that control hazards are crucial to the safety of the product.

CCPs can be found by using the HACCP team's knowledge of meat products for only real and likely hazards and where preventive measures are available for their control. A tool provided by FAO/WHO (2005) can be used for structured thinking and to ensure a consistent approach to CCP finding in the process steps: the Decision Tree (Fig. 30.3).

The same reasoning can be used for raw materials. Table 30.7 summarizes CCP identification in fresh sausage processing steps, using the CCP Decision Tree. The same brainstorming was undertaken for cooked ham (Table 30.8). For the purpose of the Decision Tree reasoning, steps where hazards are controlled by GHP and GMP are considered Control Point-GHP/GMP.

Critical Limits

Critical limits are specified for controlling the preventive measures at each CCP identified, which will define if a product is safe or unsafe. A critical limit is a maximum and/or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce to

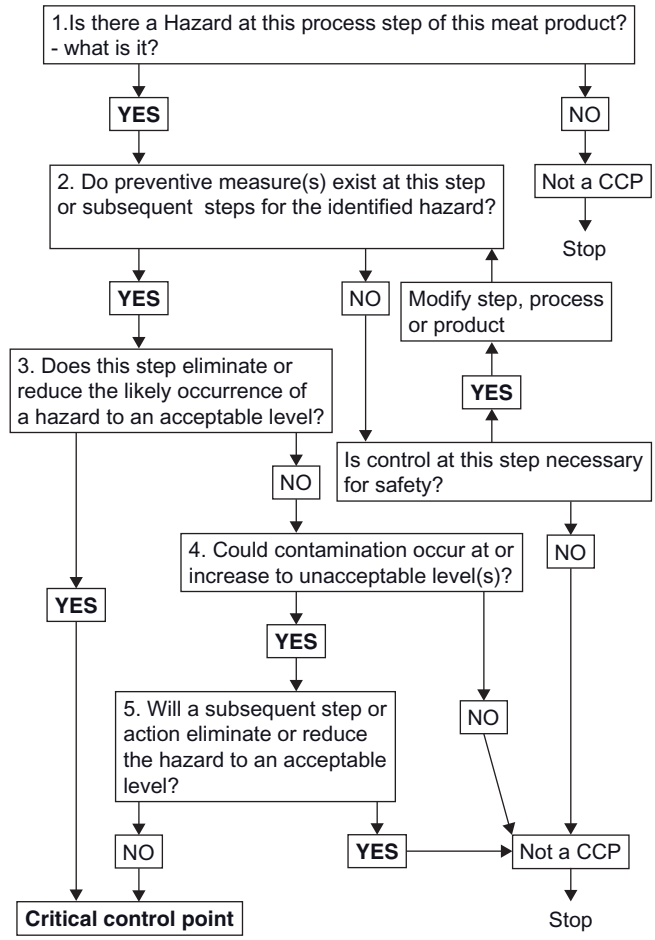

Figure 30.3. CCP *Decision Tree* for establishing critical control points, adapted from FAO/WHO (2005).

an acceptable level the occurrence of a food-safety hazard.

In meat products' processing steps, there are external or intrinsic factors, such as temperature, time, pH, moisture or a_w, salt concentration, additives concentration, and acidity, that can be measured (quantitative parameters) and routinely monitored according to a fixed schedule. The maximum level of tolerance at a CCP will be defined as the critical limit. These critical limits are established based on published data (scientific literature, in-house and supplier specifications, regulatory guidelines), experimental data, expert advice, and mathematical modeling (Mortimore and Wallace 1997). In fact, these critical limits must be established not only for monitoring actions (measurements and sensorial quotable observations) but also for verification procedures. Validation of critical limits is essential in an industry, confirming that control measures at critical points are capable of controlling the identified hazards (Scott 2005). So-called target limits are often established to act before a deviation CCP occurs.

According to Hoornstra et al. (2001), quantitative risk assessment is a powerful

Table 30.7. Critical control points identification in the production of fresh sausages

Process steps	Is there a Hazard at this process step of fresh sausages? —what is it?	Do preventive measure(s) exist at this step or subsequent steps for the identified hazard?	Does this step eliminate or reduce the likely occurrence of a hazard to an acceptable level?	Could contamination occur at or increase to unacceptable level(s)?	Will a subsequent step or action eliminate or reduce the hazard to an acceptable level?	Final answer
Reception	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
	Yes-Ph.	Yes	No	No	—	Not a CCP
Storage	Yes-Bi.	Yes	No	No	—	Not a CCP
Unfrozen pork meat trimmings	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
Casing desalting	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
Grinding	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
	Yes-Ch.	Yes	No	Yes	Yes	Not a CCP
	Yes-Ph.	Yes	No	Yes	Yes	Not a CCP
Weighing of ingredients and additives	Yes-Ch.	Yes	Yes	—	—	Yes a CCP
Mixing	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
Stuffing	Yes-Ph.	Yes	No	Yes	Yes	Not a CCP
	Yes-Bi.	Yes	No	No	—	Not a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
MAP Packaging	Yes-Bi.	Yes	No	No	—	Not a CCP
	Yes-Ph.	Yes	No	Yes	No	Yes a CCP
Labeling	Yes-Ch.	Yes	Yes	—	—	Yes a CCP
Storage end product	Yes-Bi.	Yes	Yes	Yes	No	Yes a CCP
Distribution	Yes-Bi.	Yes	No	Yes	No	Yes a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
	Yes-Ph.	Yes	No	No	—	Not a CCP

Bi. = biological; Ch. = chemical; Ph. = physical

Table 30.8. Critical control points identification in the production of cooked ham

Process steps	Is there a Hazard at this process step of fermented sausages? —what is it?	Do preventive measure(s) exist at this step or subsequent steps for the identified hazard?	Does this step eliminate or reduce the likely occurrence of a hazard to an acceptable level?	Could contamination occur at or increase to unacceptable level(s)?	Will a subsequent step or action eliminate or reduce the hazard to an acceptable level?	Final answer
Reception	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
	Yes-Ph.	Yes	No	No	—	Not a CCP
Storage	Yes-Bi.	Yes	yes	Yes	Yes	Not a CCP
Deboning of pork leg or shoulder	Yes-Ph.	Yes	No	Yes	Yes	Not a CCP
	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
Weighing of ingredients and additives	Yes-Ch.	Yes	Yes	—	—	Yes a CCP
Brine preparation	Yes-Ph.	Yes	No	Yes	Yes	Not a CCP
	Yes-Bi.	Yes	No			
Injection/	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
Tenderization	Yes-Ch.	Yes	No	No	No	Not a CCP
	Yes-Ph.	Yes	No	Yes	Yes	Not a CCP
Tumbling	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
	Yes-Ph.	Yes	No	No	—	Not a CCP
Filling/Packaging	Yes-Ph.	Yes	No	Yes	No	Yes CCP
	Yes-Bi.	Yes	No	No	—	Not a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
Molding	Yes-Bi.	Yes	No	No	—	Not a CCP
Thermal treatment: Cooking + Cooling	Yes-Bi.	Yes	Yes	—	—	Yes a CCP
Unmolding	Yes-Bi.	Yes	Yes	Yes	Yes	Not a CCP
*Repackaging/High Care area	Yes-Bi.	Yes	No	Yes	Yes	Not a CCP
*Postpasteurization	Yes-Bi.	Yes	Yes	—	—	Yes a CCP
Weighing and labelling	Yes-Ch.	Yes	Yes	—	—	Yes a CCP
Packaging	Yes-Bi.	Yes	No	No	—	Not a CCP
Storage end product	Yes-Bi.	Yes	Yes	Yes	No	Yes a CCP
Distribution	Yes-Bi.	Yes	No	Yes	No	Yes a CCP
	Yes-Ch.	Yes	No	No	—	Not a CCP
	Yes-Ph.	Yes	No	No	—	Not a CCP

Bi. = biological; Ch. = chemical; Ph. = physical

tool that could be used by food companies for critical limits' validation at critical control points.

In Europe, food microbial criteria used for verification actions at CCPs were recently discussed and defined by the European Commission (EC 1441/2007). There were specified criteria for the evaluation of products and process hygiene and safety. It is assumed, as a starting point, that values below the fixed criteria limit do not result in significant health effects and those above that limit lead to an increased probability of an adverse health effect. All legal requirements are criteria used as monitoring or verification action of control measures at CCPs.

The HACCP Control Chart

Accomplishing the practical application of the seven HACCP principles leads to the creation of a control chart, which is the main document in each specific HACCP plan (Tables 30.9 and 30.10). This document, which is elaborated according to the plan conception and development, contains any essential detail of actions to be carried out in relation to the process steps where CCPs have been identified.

Monitoring Actions

Factor measurements or sensorial quotable observations at a CCP are monitoring actions able to detect if the process is operating within the critical limits (Mortimore and Wallace 1997). Methods of analysis used in monitoring must produce rapid answers to understand if there is loss of control at a CCP and to set and run the stated corrective action.

Monitoring procedure could be a continuous on-line measurement where critical data are continuously recorded, or a discontinuous off-line monitoring system. There are disadvantages related to discontinuous off-line measurements because sampling size and frequency may not be fully representative of the batch or could create information gaps about the process.

Objective methods that provide a rapid answer to control the fresh-sausage or cooked-ham process according to the CCPs established (Tables 30.9 and 30.10) are the measurement of temperature, air circulation, time, residual CO_2/O_2 concentration, and relative humidity; the sealing package test; and metal detectors. Scheduled visual inspection can also be used for monitoring some preventive measures at CCPs, despite their being criticized because the impartiality and accuracy of human sensorial evaluation are influenced by various factors. However, this subjectivity can be reduced and avoided with training. Records of monitoring measurements are essential to provide a pool of data that the process is under control. Over a period of time and after the application of statistical methods and critical analysis, these records will contribute to the establishment of new criteria and new safety objectives, thereby improving the implemented system.

Corrective Actions

When there is a deviation from critical limits at a CCP following a monitoring action, it is mandatory to act quickly and take corrective actions.

There are different types and levels of corrective actions, including the adjustment of the process to bring it back under control and the amount of product that can be noncompliant with hygiene and safety requirements. Corrective actions can include the correction of temperature and/or time of a cooking step or the segregation of suspect product, holding it during the time needed to obtain advice from the HACCP team or outside experts, and performing analysis to assess safety. All this information will lead to different decisions: rejection and destruction of product, product reworking, or product release. The analysis of cause regarding the

Table 30.9. HACCP control chart for fresh sausages

Process steps	Preventive Action	**CCP**/CP	Critical limits	Monitoring		Corrective actions	Verification
				Procedure	Frequency		
Reception of raw materials (meat trimmings, casings, ingredients/ additives)	Suppliers selected and homologated and/or certified: agreed specification (maximum acceptable levels) Visual check ("Best Before" dates) Temperature of meat (<–12°C)	CP-GHP/ GMP	Presence of certificates or stamp of homologation, legal limits "Best Before" date Temperature of meat (<–12°C)	Visual check of certificates (or stamp of homologation) and "Best Before" date, check ingredients Measurement of meat temperature	100% 20% 20% of batch	Reject batch; Contact supplier Change supplier	Raw materials microbial and chemical analysis according to planned sampling Suppliers audits Calibration of thermometers Check temperature against calibrated thermometer Data record analysis
Storage of raw materials	Temperature of frozen room Temperature and relative humidity of refrigeration room Effective stock rotation, time of storage (first in first out)	CP-GHP/ GMP	Frozen room temp. (<–12°C); Refrigeration room temp. 2 ± 2°C RH = 85 ± 5%; "Best before" Date	Measurements of frozen room temperature, refrigeration room temperature and relative humidity Visual check of labels to ensure stock rotation	On line 20%	Correction of temperature and relative humidity Segregation of meat and evaluation of its hygiene and safety Reject product at end of shelf life	Calibration of thermometer and hygrometer
Unfrozen pork trimmings	Temperature and relative humidity of refrigeration room	CP-GHP/ GMP	Refrigeration room temp. 2 ± 2°C RH = 85 ± 5%;	Measurements of refrigeration room temperature and relative humidity	On line	Correction of temperature and relative humidity Segregation of meat and evaluation of its hygiene and safety	Calibration of thermometer and hygrometer
Casing desalting	Time of desalting GMP	CP-GHP/ GMP	Time of desalting < 2 h	Measurement of time	100%	Reject batch Training operators	Data record monitoring analysis

Weighing of ingredients and additives	Safe and accurate weight practice of each ingredient in each batch GHP/GMP	CCP	Additives weight according to legal standards Salt concentration ≤ 1.5%	Weight visual check, control of each ingredient/ Additive, and record	100%	Correction of weight, segregation of batch Recalibration of scales Correction of sausage formula	Chemical additives analysis Calibration of scales Dates record monitoring analysis
MAP Packaging	Preventive maintenance of equipment, effective metal detection, visual inspection Control of selling and CO_2/O_2 concentration in packages GHP/GMP	**CCP**	0% metal particles (ferric particles ≥ 2.5 mm; non ferric particles ≥ 3.5 mm, stainless steel particles ≥ 5 mm); 0% bones 0% fail of package seal, CO_2 residual head space package ≥ 20%; Legal limits for MAP packaging	Visual check Metal detection, check, Measure of residual CO_2/O_2 concentration in packages Visual check of package Selling test	On line	Reject product Notify maintenance, Operators training Stop line, replace package and notify maintenance	Calibration of metal detector, Maintenance equipment plan Calibration of residual CO_2/O_2 concentration equipment analysis Maintenance equipment plan, Data record monitoring analysis
Labeling	Control of label with photoelectric cell or visual check	CCP	0% absence of label	Visual or photoelectric cell labeling check	On line	Label product or replace label	Maintenance equipment plan, data record monitoring analysis
Storage end product	Time/temperature control GHP/GMP	CCP	Room Temp. = 2 ± 2°C "Best before" date	Measurement of room temperature and storage time	On line or periodically scheduled	Correction of temperature Segregation of end product and evaluation of its hygiene and safety Reject product at end of shelf life	Calibration of thermometer Check temperature
Distribution	Temperature control, GHP, GMP	CCP	Temp. 2 ± 2°C or other specified for MAP packaged sausages	Measurement of vehicle temperature	On line	Correct temperature Training drivers	Calibration of thermometer Check temperature Audit records of car temperature

CP-GHP/GMP—Control point of GHP and GMP

Table 30.10. HACCP control chart for cooked ham

Process steps	Preventive Action	**CCP**/CP	Critical limits	Monitoring		Corrective actions	Verification
				Procedure	Frequency		
Reception of raw materials (carcasses/ meat cuts, casings, ingredients/ additives)	Suppliers selected and homologated and/or certified: agreed specification (maximum acceptable levels) Visual check ("Best Before" dates) Temperature of meat (4 ± 2°C)	CP-GHP/ GMP	Presence of certificates or stamp of homologation, legal limits "Best Before" date Temperature of meat (4 ± 2°C)	Visual check of certificates (or stamp of homologation) and "Best Before" date, check ingredients Measurement of meat temperature	100% 20% 20% of batch	Reject batch; Contact supplier Change supplier	Raw materials microbial and chemical analysis according to planned sampling Suppliers audits Calibration of thermometers Check temperature against calibrated thermometer Data record analysis
Storage of raw materials	Temperature and relative humidity of room refrigeration Effective stock rotation, time of storage (first in/first out)	CP-GHP/ GMP	Room temp. 2 ± 2°C; RH = 85 ± 5%; time of meat storage = 5 ± 2 days "Best before" date	Measurement of room temperature and relative humidity Visual check of labels to ensure stock rotation	On line 20%	Correction of temperature and relative humidity Segregation of meat and evaluation of its hygiene and safety Reject product at end of shelf life	Calibration of thermometer and hygrometer
Weighing of ingredients and additives	Safe and accurate weight practice of each ingredient in each batch GHP/GMP	CCP	Additives weight according to legal standards Salt concentration ≤ 1.8%	Weight visual check control of each ingredient/ additive and record	100%	Correction of weight, segregation of batch Recalibration of scales Correction of sausage formula	Chemical additives analysis Calibration of scales Dates record monitoring analysis

Filling/vacuum packaging	Preventive maintenance of equipment, effective metal detection, visual inspection Control of selling and vacuum GHP/GMP	**CCP**	0% metal particles (ferric particles ≥ 2.5 mm; non ferric particles ≥ 3.5 mm, stainless steel particles ≥ 5 mm); 0% bones 0% fail of package seal, 0% fail of vacuum	Metal detection, check, visual check Visual check of vacuum package Selling test	On line On line	Reject product Notify maintenance, Operators training Stop line, replace package and notify maintenance	Calibration of metal detector, Maintenance equipment plan Data record monitoring analysis
Cooking	Correct time/ temperature	CCP	Internal Temp = 68–70°C/20 min. Temp = 70°C Time ≈ hours x kg of meat Or other according to specific technology	Measurement of temperature and time	On line	Correction of temperature and time. Reprocess. Notify maintenance Reject product	Calibration of thermometer Maintenance equipment plan Data record monitoring analysis
Cooling	Potable cooled water, GHP, GMP Correct time/ temperature Rapid cooling to temperatures ≤ 28°C as soon as possible and hold to cool 24 h/0 ± 1°C until internal temperature of 2°C	CCP	Rapid cooling Time < 4 h Rapid cooling Internal Temp ≤ 28°C Chill to < 2 ± 2°C in refrigeration room at 0 ± 1°C/24 h) Or other according to specific technology	Measurement of temperature and time	On line	Reprocess. Notify maintenance Segregation of product and evaluation of its hygiene and safety Reject product	Calibration of thermometer Maintenance equipment plan Data record monitoring analysis
Unmolding	GHP, GMP Control of sealing and vacuum packages Visual check Recondition*	CP-GHP/ GMP	0% fail of package seal, 0% fail of vacuum	Visual check	100%	Recondition* Notify maintenance Operators training	Maintenance equipment plan Data record monitoring analysis

(continued)

Table 30.10. HACCP control chart for cooked ham *(cont.)*

Process steps	Preventive Action	**CCP**/CP	Critical limits	Monitoring		Corrective actions	Verification
				Procedure	Frequency		
*Repackaging	GHP, GMP, preventive equipment maintenance, correct stuffing pressure machine Control of sealing and vacuum packages, Visual check,	CP-GHP/ GMP	0% fail of package seal, 0% fail of vacuum	Visual check	100%	Notify maintenance Operators training	Maintenance equipment plan, Data record monitoring analysis
*Post pasteurization	Potable water, GHP, GMP Correct time/ temperature	CCP	Water Temp = 90°C Time = 1.5 min. Or other according to specific technology	Measurement of temperature and time	On line	Reprocess Notify maintenance Operators training	Maintenance equipment plan, Data record monitoring analysis
Weighing/ Labeling	Control of label with photoelectric cell or visual check	CCP	0% absence of label	Visual or photoelectric cell labeling check	On line	Label product or replace label	Maintenance equipment plan, data record monitoring analysis
Storage end product	Time/ temperature control GHP/GMP	CCP	Room Temp. = 2 ± 2°C "Best before" date	Measurement of room temperature and storage time	On line or periodically scheduled	Correction of temperature Segregation of end product and evaluation of its hygiene and safety Reject product at end of shelf life	Calibration of thermometer Check temperature
Distribution	Temperature control, GHP, GMP	CCP	Temp. 2 ± 2°C or other specified for Cooked Ham	Measurement of car temperature	On line	Correct temperature Training drivers	Calibration of thermometer Check temperature Audit records of car temperature

CP-GHP/GMP—Control point of GHP and GMP

deviation from critical limits at a CCP is crucial in order to implement corrective actions that will prevent further deviations. Running records of all actions must be kept.

More examples of corrective actions can be seen in Tables 30.9 and 30.10 in relation to fresh sausages and cooked ham processes.

Verification

The HACCP system must provide evidence that will assure product safety. The system must be tested periodically to ensure that appropriate control measures and related monitoring procedures at critical control points are working effectively, according to the plan.

Verification can be carried out by different procedures (other than those used in monitoring) that guarantee the effectiveness of the HACCP plan implementation.

Tables 30.9 and 30.10 present possible verification actions related to an HACCP plan for fresh sausages and cooked ham.

Suppliers' or distribution operators' safety systems (GHP/GMP and HACCP) should be regularly audited if control points are associated with them.

Verification actions include checking procedures to assure CCPs are under control, examining metrology certificates for all equipment used for measurements, and inspecting CCPs' monitoring records and corrective actions records. The check of GHP/GMP control points and training records, examination of data from microbial analysis performed on environmental plant samples (equipment, air, water), and investigation of clients/consumers' complaints are also mentioned as verification procedures.

Sampling of raw materials and end products for physicochemical, sensorial, or microbiological analysis, the examination of the sampling plan, and the results of any analyses performed are also examples of verification.

Microbial analysis of processed meat end products, namely the quantification of certain microbial groups that are indicators of process hygiene and also the detection/quantification of pathogens, provides data to evaluate if the system is achieving the safety aims. The HACCP team must be careful with the selection of each analytical parameter, taking into account any new safety information about the product or when it was implicated as a disease vehicle, in order to avoid wasting time and money and to improve verification efficiency.

All planned verification actions must be stated in the HACCP system; however, others can be taken without any previous communication. Reports and records from verification actions must be written.

Records of HACCP Plan Data and Other Extraordinary Actions

All data produced by the HACCP plan must be kept on file for easy consultation by the team, auditors, and government food inspection authority agents to provide evidence of appropriate safety control. This is extremely valuable in order to prove existing effective HACCP practices and due diligence for court trials.

Also, all records that can provide evidence that preventive measures are effective to eliminate or reduce hazards to an acceptable level in the HACCP plan at CCPs, GHP/GMP, or other preventive measures are used for HACCP plan validation. Revalidation will be required when there are failures or when new information becomes available that suggests the HACCP plan may be inadequate, or when significant changes occur in an operation (Scott 2005).

All data from records must be treated and analyzed afterward to improve the HACCP plan.

HACCP Plan Updating

In meat-processing industries, as in other industrial activities, there are constant changes over the years regarding products and new products produced, the environment, people, and expected hazards. It is important that the effects of changes are evaluated and effectively introduced into the food-safety management system. Making advances in the meat products business and introducing production innovation without matching these to the safety system will be catastrophic.

Internal and external audit reports and records of corrective actions and client complaints provide output for an HACCP plan revision. HACCP plan requirements must be revised when any modification in production or equipment occurs.

Revision will contribute to the constant improvement of the plan, with the main responsibility being held by the industry itself. Also, regulatory agencies in many countries will provide useful additional verification when assessing the HACCP system, thereby contributing their input to improving the system.

Recognition of a Food Safety Management System Implemented in a Food Industry

Compliance of a food-safety management system, with a specific reference, such as ISO/FDIS 22000:2005 or other normative references, given by an independent and qualified institution, certifies the system and confers recognition to clients and consumers that that producer is able to control food safety hazards.

External audits, to check agreement with the standard of all data recorded and direct measurements produced in a HACCP working system, are performed by experienced, trained audit teams.

All procedures for safety-management system certification imply costs that small companies cannot afford. However, the stated standard can be used by small, developed meat industries to evaluate their own safety-management system.

Conclusions

The main purpose of generic HACCP plans is to assist with the creation and implementation of a specific HACCP-based food safety management system by any business whose dimensions (small or less-developed companies) do not allow it to have the critical mass necessary to undertake by itself the objective development of a HACCP system adapted to its own reality.

In fact, following the administration's agreement and commitment to food safety, the main difficulty involved with implementing a HACCP-based food safety management system in a food company is a lack of knowledge or understanding of HACCP and a real desire to do it.

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Chapter 31

Quality Assurance

Friedrich-Karl Lücke

Introduction

According to the ISO 9000:2005 standard, quality assurance (QA) is "a set of activities intended to establish confidence that quality requirements will be met." In contrast, quality control (QC) is "a set of activities intended to ensure that quality requirements are actually being met." Both QA and QC are parts of quality management (QM). This chapter discusses how meat processors should make sure that the consumer receives foods that meet his/her expectations in terms of safety and quality. From the ISO definition of quality ("The set of characteristics of an entity that give that entity the ability to satisfy expressed and implicit needs"), it follows that the manufacturers must also assure the "process quality" their customers expect. Criteria for assessment of process quality include environmental issues (less input of energy and water, less output of waste and climate-relevant trace gases), animal welfare, social aspects (regional food production, social responsibility of food businesses to their suppliers, their employees, and society), and the use (e.g., according to *kosher* or *halal* rules) or avoidance of certain processing methods (e.g., ionizing irradiation, gene technology). Since different processes may not always lead to differences distinguishable by physical, chemical, microbiological, or sensory analyses, an effective control of material flow throughout the supply chain of these "identity-preserved products" (Vasconcellos 2004) is important.

With relevance to quality assurance in the meat sector, there is a wealth of official regulations, guidelines (e.g., the Code of Hygienic Practice for Meat issued by the Codex Alimentarius Commission [2005]), standards (e.g., ISO norms 9001 and 22000), and specifications developed by industrial and retail organizations as the driving force (e.g., Codes for Good Hygienic Practice, notified and registered with the European Commission according to article 8 of Regulation [EC] 852/2004; standards benchmarked by the Global Food Safety Initiative). Most of these documents focus on meat safety and specify, among other things, that the processor must implement and maintain a system of food-safety assurance. However, the purpose of many regulations and standards is also to protect the consumer from being misled (in particular, by inadequate labeling on weight, composition, shelf life, nutritive value, origin, and production method) and to ensure fair competition. Obviously, it does not make sense that a meat processor installs different systems to assure safety, traceability, compliance with other standards, and sensory quality. Rather, the documents for controlling a given process step should cover all criteria of product quality.

For information on the implementation and maintenance of HACCP in meat processing, the reader is referred to official documents (e.g., the Generic HACCP models issued by the U.S. Food Safety and Inspection Service [USDA-FSIS 1999a]) or the relevant literature (Brown 2000a, b; Sheridan 2000;

see also the chapter on HACCP in this volume).

This chapter provides the key elements of quality assurance plans specific for the processing of meat (primal cuts and trimmings) into products that are either ready to cook or ready to eat. Measures to assure the quality of the slaughtering and butchering processes and the resulting (primal) meat cuts are not considered here. Moreover, the chapter will not review current legislation in different countries. Rather, it gives general information, combined with details based on (and biased toward) products, regulations, and standards important to meat processors in Germany. These documents include, among others, the regulations of the European Union on food safety (in particular, Regulation [EC] nos. 178/2002, 852/2004 and 853/2004), and the requirements specified in the International Food Standard (IFS, a standard accredited by the Global Food Safety Initiative) and in the guidelines for meat processors given by the German "Qualität und Sicherheit" program (2008).

Organization of Quality Assurance Activities

Quality assurance, being an essential part of total quality management, is a task for an organization's executive management (Vasconcellos 2004). Hence, QA activities cannot be "outsourced," and the QA officer must report directly to the executive management. Documents must be integrated into the "quality manual" of the organization.

An appropriate documentation is essential for an effective and efficient quality-assurance system. The purpose of process plans is to ascertain that processes work reliably, even though the persons responsible for them may change. Moreover, they are necessary to provide evidence that the management has delegated responsibility in an appropriate manner. Basically, the better trained and experienced the workers are, the less paperwork is necessary. The purpose of records on the processes is to provide evidence of appropriate process control (e.g., compliance with "due diligence" as defined in product liability legislation). Statistical process control and records such as the Shewhart control charts also serve as tools to identify problems and initiate corrective actions before critical limits are exceeded and the batch may have to be rejected.

On the other hand, over-documentation and problems related to information flow and distribution of documents are a major cause of staff frustration and of noncompliance with the system. In particular, over-documentation prevents effective use of documents (by increasing the time required for searching), increases the workload necessary for updating, and reduces flexibility. Hence, each document should be checked as to whether it is really necessary or whether its purpose could also be reached by other means (training and education, using other existing documents, etc.). Moreover, many documents are not concise enough, and their style and layout is not suitable for use by its target group.

To avoid confusion, it is also essential that documents are paginated (page x of y) and that every page of a document has a header or footer indicating the title, the author, the approving person, and the date and number of the version.

It is also important to make sure that everyone has exactly the documents available he/she needs, and that any previous versions of the document are removed.

Appropriate computer software may be very helpful to organize documentation and records, provided that it is easy to use by all employees, with a minimum of training. In particular, it should allow easy construction of organigrams, flow sheets, and standard operation procedures. All documents relevant to a given entry in these diagrams (e.g., to a specific process step) should be accessible after one or two "mouse clicks."

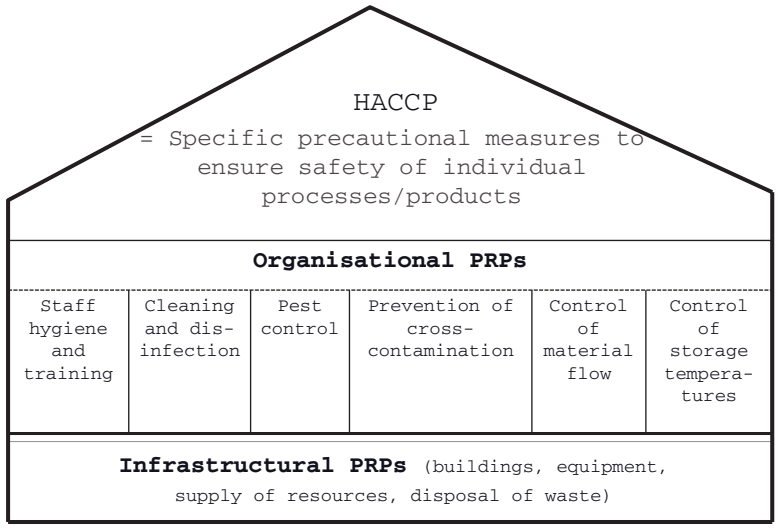

Figure 31.1. The "House of Hygiene." (Modified from Untermann and Dura 1996.)

Assurance of Basic Hygiene

"Basic hygiene" or "good hygienic practice" means a set of hygienic measures that are not specific to individual products or processes. In the ISO 22000 standard, these measures are designated as "prerequisite programmes" (PRP) and subdivided into "infrastructural PRPs" and "organizational PRPs." The PRPs and their relationship to HACCP are visualized in the "House of Hygiene" (Untermann and Dura 1996; see Fig. 31.1).

Quality Assurance Plans for Purchase, Selection, and Storage of Raw Materials

The principles for suppliers' approval, purchase of raw materials, and incoming goods' checks are specified by various standards, such as the International Food Standard (IFS; see Table 31.1). The quality of meat products is strongly affected by the quality of the raw materials. Moreover, the price of meat contributes markedly to the total production costs. The microbiological quality is particularly important if the manufacturing process (or the heat treatment before consumption) does not reliably reduce the load of non-spore-forming pathogenic bacteria (e.g., salmonellae or pathogenic strains of *Escherichia coli*) to an acceptable level. This is the case with raw (spreadable) fermented sausages, for example: if the manufacturing process of raw fermented sausages does not guarantee a 5-log reduction of salmonellae and *E. coli* O157:H7, U.S. suppliers must provide certificates indicating that the meat has been sampled and tested negatively for these pathogens (Anonymous 1999; USDA-FSIS 1999b).

Particularly for meat products with a long shelf life, fat quality is critical, since high levels of polyunsaturated fatty acid will lead to early rancidity. The water-binding capacity of the meat should be sufficiently high for the manufacture of cooked sausages, and sufficiently low for the manufacture of raw cured/salted meats and fermented sausages.

Clear information on the origin of the raw material is important. First, several risks (e.g., contamination by chemical residues; specified risk materials such as central nervous tissue from ruminants) must be con-

Table 31.1. Requirements of the International Food Standard (version 5, 2007) for the approval of suppliers and checking of incoming raw materials

Number	Requirement
4.2.2 ("knock-out criterion")	Specifications shall be available and in place for all raw materials (…). The specifications shall be up to date, unambiguous, available and always in conformance with legal requirements
4.4.1	Purchased products and services shall conform to current specifications and contractual agreements
4.4.2	There shall be records to identify which product is sourced from which supplier
4.4.3	There shall be a procedure for approval and monitoring of suppliers (internal and external), outsourced production or part of it
4.4.4	The approval and monitoring procedure shall contain clear assessment criteria such as: audits, certificates of analysis, supplier reliability and complaints, as well as required performance standards based on a hazard analysis
4.4.5	The results of suppliers' assessment shall be reviewed regularly. There shall be records of the reviews and of the actions taken as a consequence of assessment
4.4.6	Risk-based check of purchased products and services on the base of the existing specifications. The schedule of these checks shall take into account the product requirements, supplier status and the impact of raw materials on the finished product
4.11.1	Raw materials, semi-processed and finished products, as well as packaging, shall be checked against the specifications on receipt and in accordance with determined inspection plan. All results shall be documented

trolled at the level of primary production or slaughter and cannot be eliminated by the processing plant upon receipt or later. Hence, in the aftermath of the BSE crisis and other meat-related scandals, regulations were issued to ensure traceability. Moreover, material flow must be carefully controlled to preserve the identity of the meat if its origin or statements like "organic," "kosher," "no GMO in feed" are put on the label or into the specifications.

Table 31.2 gives a list of possible specifications for meat and fat.

Specifications should also be in place for nonmeat ingredients and additives. In addition to suitable chemical and microbiological parameters, these should also address components that have to be labeled on the final product. Such components may include allergens (such as celery or mustard seeds), glutamate, or constituents isolated from genetically modified organisms (e.g., from soybeans). Specifications for casings and packaging material should include data on permeability to gases and moisture, technological properties (e.g., stability against mechanical impacts), and (in the case of foils) migration of monomers into the product.

Specifications (e.g., of microbiological and chemical parameters) should always be based on scientific evidence and current legislation and standards, and should always contain a reference to the analytical method to be applied. All this is essential for a fair cooperation between all partners within the meat supply chain.

Norms like ISO 9001:2008 and the IFS require a careful selection of suppliers on the basis of clearly documented assessment criteria "such as: audits, certificates of analysis, supplier reliability and complaints, as well as required performance standards based on a hazard analysis" (IFS 2007). Purchasing raw materials from reliable suppliers with a functional quality-management system (verified by customer or third-party audits) reduces the costs of testing and dealing with complaints, and is, therefore, usually more economical than accepting the cheapest offers.

At receipt of raw materials, standards require that checks are carried out to reduce the risk that goods not complying with the specifications (and/or legal standards) enter

Table 31.2. A list of possible specifications for lean meat and fat purchased for further processing, and suggestions for checks to be carried out at delivery

Category	Specification	Checks on incoming material
Basic data	Animal species	Accompanying documents; inspection
	Type of cut/origin of trimmings	
	Weight	Re-weighing
Origin	Country	Accompanying documents
	Region	
	Abattoir/butchering plant	
	Supplier	
Compliance with legal requirements	No residues above permitted levels	
	Compliance with microbiological standards, e. g. safety and performance standards according to Regulation (EC) 1441/2007	
Other	pH, e.g. ≤5.8 for manufacture of fermented and/or dried products, ≤6.2 for manufacture of cooked products	pH measurement or inspection
	pH above 5.8 one hour after slaughter (to indicate absence of PSE [pale, soft, exudative] deviation	Accompanying documents; visual examination
	pH below 5.8–6.0 24–36 hours after slaughter (to indicate absence of "dark cutting beef" or "dark firm dry pork" deviations)	
	Fat content	
	Fatty acid composition, e.g. polyunsaturated fatty acid	
	Content of connective tissue/collagen	Visual examination
	Odor/flavor: free of off-odors, absence of spoilage indicators)	Sensory examination; reflectometry
	Appearance of lean meat (color and/or myoglobin content)	
	Appearance of fat (color, texture, no rancid smell)	
	Residual shelf life	Accompanying documents
	Transport and storage temperature	Inspection; temperature measurement
	Packaging material	Inspection of type & integrity
Special features	Organic/conventional	Accompanying documents
	Feeding regime (e. g. fats and oils in pig diet; absence of GMO in feed)	
	Decontamination treatment (if permitted)	
	Kosher/halal	

the processing plant and to monitor the performance of the supplier. Methods suitable for these checks should obviously give results instantly or within less than one hour. According to IFS (2007), "the schedule of these checks shall take into account the product requirements, supplier status and the impact of raw materials on the finished product." A typical checklist for lean meat and fat should comprise (see also Table 31.2):

- the accompanying documents on origin, weight, etc.
- certificates on compliance with legal requirements and standards, etc.
- temperatures (for chilled lean meat and fat: below 4°C in core, below 7°C on surface; frozen material: below −15°C)
- temperature history (as registered by data loggers or similar equipment)

- temperature and cleanliness within the transport vehicle
- integrity of containers (if applicable)
- overall appearance of the lean meat and fat (absence of sensory deviations)

Experienced employees are capable of assessing the suitability of the raw material by inspection only (e.g., by checking the color, odor, surface properties, and texture of the tissues, parameters that correlate with water-binding capacity, pH_1 and pH_{24} H_{24} values of lean meat, and with melting point and PUFA levels in fat, respectively). To avoid unnecessary paperwork, it is advisable to stamp and fill in a "miniature checklist" on the delivery note. This list should refer to the appropriate plan (work instruction) and contain the date, batch, inspection results, and, if applicable, corrective measures taken.

Laboratory examination of samples of raw materials is important to verify and monitor the functioning of the quality-assurance system of the suppliers, thereby enabling their assessment and selection. For example, it may be advisable to test incoming carcasses for the microbiological performance parameters (total viable counts, *Enterobacteriaceae*, salmonellae) indicated in Regulation (EC) 2073/2005.

It should be clear from the above discussion that purchase of raw materials is a complex process that should involve responsible staff from the processing, purchase, and quality-assurance department. It is essential that experienced, qualified staff perform checks of incoming material, as well as sort it according to its intended use, because many parameters can only be assessed through grading by "subjective" methods (sensory examination) rather than "objective" measurements. A standard operation procedure (SOP) should be in place to organize the purchase and receipt of raw materials.

After receipt, the goods should be stored at appropriate temperatures. For lean meat and fat, the temperature must never exceed 7°C, whereas poultry and prepared meats, edible offal (e.g., liver), and minced meat must be stored below 4°C, 3°C and 2°C, respectively (see Regulation [EC] 853/2004, Appendix III). Hence, cold rooms are usually adjusted to 0° to 2°C. A device registering temperature over time should be installed in cold rooms.

Quality Assurance Plans for Production: General Aspects

Quality-assurance plans for production may be either "horizontal" (i.e., applicable to all processes) or "vertical" (i.e., process- and/or product-specific). Typical horizontal documents are those on basic hygiene that address factory environment, buildings, facilities and equipment, personnel hygiene, cleaning and disinfection, pest monitoring and control, waste disposal, temperature controls in storage or processing rooms, separation between "clean" and "unclean" processes, and staff training (see earlier in this chapter and Fig. 31.1). Process-specific plans may include:

- recipes for individual products
- detailed flow charts, with links to work instructions, critical limits, and relevant standards
- HACCP documents (see previous chapter)
- forms for production plans
- monitoring plans (including forms for recording results and instructions on control of measurement devices)
- a plan for corrective actions to be taken if a process gets out of control

Monitoring of processes is also required by standards such as ISO 9001:2008. Parameters to be recorded may include output

Table 31.3. Example for the structure of a process documentation: (1) Preparation of sausage mixture

Input documents: Work instruction and links		Process	Output documents: Records
Type and preparation of equipment		Preparation of sausage mixture	Signed check list
Selection of ingredients: Specifications for	Lean meat		Weighing protocol
	Fat		
	Salt		
	Curing agents		
	Spices		
	Water		
	Other ...		
Recipe			
Instruction for comminution and mixing	Sequence and timing of ingredient addition		
	Target particle size and degree of mixing		Final temperature
	Batch identification		Batch no.

Table 31.4. Example for the structure of a process documentation: (2) Heat processing

Input documents: Work instruction and links		Process	Output documents: Records
Equipment	Type and preparation	Heat processing of sausages in casings	Signed check list
	Calibration of monitoring devices (thermometer, timer)		Calibration protocol
Sausages to be heated	Diameter		No. of batch of mixture
	Initial temperature		
Critical values for heating process	Target F value (70°C, z = 10)		Time-temperature protocol
	Adjustments of heating device (time, temperature)		
	Core temperature to be reached before cooling		
	Cooling rate/method		
Smoke application			
Relative humidity in chamber			
Batch identification			Batch no.

per hour, deficiency rates, capacity utilization, and downtime of equipment.

To reduce paperwork, it may be advisable to develop documents specific to unit operations (Vasconcellos 2004). Tables 31.3 and 31.4 give examples for the structure of documents relevant to the unit operations "preparation of sausage mix" and "heating."

Quality-Assurance Plans for Different Processes and Product Groups

Meat products can be divided into different product groups, as done earlier in this volume. The following part of this chapter specifically addresses these product groups.

Retail Cuts, Minced Meat, and Ready-to-Cook Fresh Meats

There is a growing market for "convenience" meat preparations, which not only include minced meat but also meats pretreated with spices and marinades in order to supply an oven-ready product to both caterers and private households. For quality assurance, however, it is important to note that any mechanical treatment is likely to transfer microorganisms from the surface into the interior, and that spices and marinades have little if any effect on the shelf life of the meat. Instead, they are likely to mask microbial spoilage, fat deterioration, and the intrinsic properties of the raw material. Moreover, in some countries, minced meat may be eaten without prior heating (as in Germany) or as "rare-cooked" hamburgers (as in the United States, despite all efforts to discourage this practice). Taken together, this means that the selection of suppliers, the choice and checks of raw material, the basic hygiene during preparation and handling, the maintenance of the cold chain (below 4°C, for minced meat below 2°C) and labeling giving clear "consume-by" dates, and storage and cooking instructions are the essential elements of quality assurance.

Heat-Processed Meat Products

Some critical values for the manufacture of cooked ham and shoulder, and of heat-processed sausages and pâtés are given in Tables 31.5 and 31.6, respectively. Most products are filled into casings and molds, heated to core temperatures between 68° and 80°C, and subsequently handled and sliced. Hence, their shelf life is limited to a few weeks under refrigeration, and they are usually spoiled by recontaminant bacteria. For products with extended shelf life, even at ambient temperatures, additional factors are important. For cooked dried sausages, these include formulation (water, fat, salt) and water loss during processing to make sure that the water activity is below 0.95. Sausages and hams, as well as uncured/unsalted meat, may also be cooked in hermetically sealed containers. For such meats, the integrity of the containers (seams, seals), the heat treatment (F value, pressure), and the cooling conditions (cooling rates, water quality) must be precisely controlled. With shelf-stable canned cured meats, the formulation is also important to ascertain the correct combination between water activity, pH, curing agents, and heat treatment. It must also be considered that products heated in hermetically sealed containers are often stored under insufficient refrigeration. Therefore, the need for chill storage should be indicated very clearly on the label, and the product should be sufficiently stable during temperature abuse.

Table 31.5. Examples for critical values for the manufacture of cooked ham and shoulder (Lücke and Troeger 2007; Müller 2007)

Process step	Critical values
Raw material	pH below 6.2; temperature below 7°C
Brine injection and mechanical treatment	– Brine concentration adjusted to reach target water activity and nitrite content – Temperature during brine injection below 12°C – Temperature in curing room and during mechanical treatment: below 5°C
Heat treatment	Core temperature above 68°C and/or F_{70} above 30 (z = 10)
Storage	below 5°C

Fermented Sausages

Critical values for the manufacture of fermented sausages have been summarized by Lücke (2007), and details on quality control and HACCP systems for these products have been published by Fraqueza et al. (2007) and Toldrá et al. (2007). Table 31.7 gives an example for a product common in Germany.

Table 31.6. Examples for critical values for the manufacture of heat-processed sausages (modified from Lücke and Troeger 2007; Lautenschläger and Troeger 2007; Fischer and Hilmes 2007)

Process step	Critical values for	
	Brühwurst[1]	Kochwurst[2]
Selection of raw material (lean meat)	pH value between 5.6 and 6.2; temperature below 7°C	
Selection of liver, blood, rinds and blood (if applicable)	Temperature below 2°C	
Pre-heating of lean meat and fat	Not applicable	Temperature above 65°C
Comminution	Final temperature below 15°C	Final temperature 35–40°C
Filling	Adjustment of filling equipment device to avoid air inclusion	
Heat treatment	Core temperature above 70°C; F_{70} (z = 10°) above 30	Core temperature above 75°C; F_{70} (z = 10°) above 40
Chilling	To below 7°C within 12 hours	
Storage	below 5°C	

[1] Bologna- or frankfurter-type sausages.
[2] Liver sausages and pâtés; sausages containing blood and/or rinds (blood sausages, brawns etc.).

Particular attention should be paid to the quality and pretreatment of the raw material (lean meat and fat), because a high load of undesirable microorganisms, polyunsaturated fatty acids, and derived peroxides markedly affect the safety and quality of the final product. Extended chill storage results in poor microbiological quality, and extended frozen storage results in fat deterioration. To inhibit fat deterioration and to favor drying of the sausages, it is also important that temperatures during comminution are kept low (below 0°C in the cutter, below 5°C in the meat grinder).

The exact dosage of nonmeat ingredients, such as salt, nitrite, ascorbate, sugars, starter cultures, and spices, is also critical for the quality and safety of the final product, and must be strictly controlled and recorded. The same applies for the casings. These must comply with specifications on microbiological quality, permeability to moisture, mechanical behavior (stability, shrinkage during drying), and adherence to the sausage

Table 31.7. Examples for critical values for the manufacture of a typical German semi-dry fermented sausage (modified from Lücke, 2007; Stiebing 2007)

Process step	Critical values
Selection of lean meat	pH value between 5.6 and 6.2; temperature below 2°C
Selection of fat	frozen, white, firm, less than 15% polyunsaturated fatty acids
Weighing of ingredients	– Curing salt 2.5–3%, equivalent to 100–150 mg sodium nitrite/kg – fermentable sugar 0.5–1% – active starter cultures (lactic acid bacteria, catalase-positive cocci) – ascorbate 0.05% – spices
Comminution and mixing (in cutter)	Final temperature below 0°C
Filling	Adjustment of filling equipment device to avoid air inclusion
Fermentation	20–25°C, 2–3 days, until pH below 5.3
Smoking	charring temperature below 700°C
Drying	12–15°C, 70–80% relative humidity, until water activity below 0.93
Storage	below 15°C

mix. Filling is also critical for product quality, because any holes remaining may be starting points for rancidity, discolorations, and growth of undesired microorganisms.

The microbiological quality and safety depends very much on the correct fermentation and drying/aging conditions (temperature, relative humidity, air velocity), which should be clearly specified and monitored. To protect the surface from undesired oxidative or microbiological changes, most sausages are either smoked after fermentation or surface inoculated with selected mold (sometimes also yeast) strains. To obtain the desired aroma and to minimize the levels of toxic residues, the smoking process should be well controlled by specifying and recording the type of the wood (or liquid-smoke preparations), the charring temperature, and the time and temperature during smoking. Mold starters should be able to rapidly colonize the surface after completion of lactic fermentation.

Process records should include, among other items, weighing protocols, and the time course for temperature, relative humidity, and weight loss. Continuous measurement of pH during fermentation is difficult, but it should be done at regular intervals, to verify that process parameters (activity of starters, sugar addition, fermentation conditions) are adjusted correctly.

Raw Dry Hams

Fraqueza et al. (2007), Lücke (2007), and Toldrá et al. (2007) have compiled data on the safe manufacture of high-quality, raw dry ham. Table 31.8 gives an example for a product common in Germany.

For raw hams, it is even more important to specify the origin and the quality of the raw material. For premium quality, the pigs' feeding regime, breed, and age at slaughter are important and must be specified and controlled. This is only possible within a supply chain with fair cooperation between all partners. At delivery, cuts must be inspected carefully for integrity and absence of any signs of spoilage and fat deterioration. High pH meat has a high water-binding capacity and needs very long salting times; hence, the pH of the ham before salting should generally not exceed 5.8. Only in certain pork muscles, may pH values up to 6.0 be tolerated.

The presence of spoilage or food-poisoning bacteria (including nonproteolytic strains of *Clostridium botulinum*) in the interior of the ham can be minimized by appropriate

Table 31.8. Examples for critical values for the manufacture of a typical German raw dry ham (modified from Lücke, 2007; Lautenschläger 2007)

Process step	Critical values
Selection of lean meat	pH value (lean meat) between 5.6 and 6.2; fatty tissue white, firm, less than 15% polyunsaturated fatty acids; temperature below 2°C; defined size, geometry and integrity of the cut
Weighing of ingredients	– Salt 40–50 g/kg meat – Curing agents, input level sufficiently low to comply with maximum levels for nitrite and nitrate as specified by law
Dry salting Salt equilibration	Temperature below 5°C, relative humidity above 80%, until water activity in core below 0.96
Preparation for smoking	Temperature below 18°C, no longer than 1 day
Smoking	Temperature below 22°C; charring temperature below 700°C
Aging	Temperature 12–15°C, 70–80% relative humidity, until water activity below 0.93
Storage	below 15°C

slaughtering and butchering hygiene, but it cannot be completely ruled out. Hence, the need for salting and salt equilibration; these processes must be carried out at 5°C or below and continued until the target water activity of 0.96 is reached in all parts of the ham.

The levels of salt must be specified and carefully controlled, in order to inhibit growth of undesired microorganisms while avoiding over-salting, especially of the surface layers. The same applies to the levels of curing agents (nitrite, nitrate) in the salt, which should be high enough for the desired sensory properties but low enough to comply with the maximum residual levels specified by official regulations. Appropriate work instructions should specify the composition of the salt (in particular, the levels of nitrite and/or nitrate in the salt), the salt content of the brine (if applicable), the amount of salt and/or brine to be added per kg of meat, the size of the cut (maximum distance from the surface to the geometric center), the temperature (≤5°C), and the minimal time for salting and salt equilibration. Records should include weighing and time-temperature protocols. Also, the results of visual inspections and/or measurements of the final salt content or a_w value in the core should be recorded, because the salt diffusion rate may vary between individual cuts.

After salting and salt equilibration, it is important to avoid undesired changes at the surface of the hams. For quality and safety of the product, extensive washing should be avoided, and the surface should be dried at low temperatures. Smoking should be controlled as in the production of fermented sausages (see above).

In particular, Mediterranean-type raw hams are usually subjected to extensive aging, to leave enough time for enzymatic processes, which makes the hams tender and tasty. Aging normally takes place at temperatures above 15°C. The hams are stable at ambient temperatures if the water activity is below 0.90. Aging time, temperature, and relative humidity, as well as the intended and measured weight loss, should be specified and recorded.

Bacon

Bacon differs from other raw cured meats in many aspects:

- Other cuts are used for curing (e.g., whole pork sides or bellies rather than ham).
- Curing is by brine injection.
- Maturing lasts only a few days.
- The product is sometimes heat-treated (hot smoked) after curing and almost always cooked before consumption.

For details, see earlier chapters of this volume. For the quality and safety of the product, the quality of the raw material (especially the fat quality), the composition of the curing brine, the proper maintenance of the multineedle injectors (to ascertain even distribution of the brine and to avoid introduction of physical hazards), and the times and temperatures during curing and further processing are essential.

Quality Assurance during Packaging, Storage, Distribution

The climate in the slicing and packaging room should be adjusted so as to avoid moisture condensation and the undesired surface growth of microorganisms. As a rule, the relative humidity in the slicing and packaging room should be below 60%. The temperature depends on the type of product and the time the product is held in the packaging room. To avoid contamination of the product by psychrotrophic spoilage bacteria and by listeriae, hygienic design of the slicing and packaging machinery is essential, and an appropriate cleaning and disinfection plan for it should be in place.

To prevent mold growth and to delay oxidative deterioration, the residual oxygen level in the package should be kept below 1%

(preferably below 0.5%). This is achieved by packaging under vacuum or under a modified atmosphere containing about 70% N_2 and 30% CO_2. Moreover, the oxygen permeability of the packaging material should be below 25 ml m^{-2} d^{-1}, or even lower if a shelf life of more than 1 month is desired (Stiebing 1992). However, mold-ripened, raw dry products should be packaged in material sufficiently permeable to oxygen.

The integrity of the packages should be checked by testing samples for leakage and/or inspecting their seams. Appropriate labeling is important, not only to inform the consumer but also for tracking the batch "downstream." If problems occur, the batch concerned may be recalled specifically, and the damage to the processor is limited.

High-throughput slicing and packaging lines often include a check weigher and a metal detector. Both should be regularly checked for proper performance.

The necessary storage temperature depends on the type of products. Retail cuts, minced meat, and ready-to-cook fresh meats should be stored below 2°C. Cooked perishable products should be stored at 5°C, preferably at 2°C, to extend shelf life. Most fermented sausages and hams are stored at 10° to 15°C; undried fermented sausages may require a storage temperature at 7°C or below, whereas products dried to lower water activities (e.g., sausages with a_w below 0.90) or commercially sterile (canned) products may be stored at ambient temperatures (≤25°C). Illumination in display cabinets may be detrimental to fat quality, and the light intensity should be adjusted to below 600 lux.

End Product Specifications and Testing

A detailed description of all products is part of the HACCP procedure and is also required by various standards. They shall be "up to date, unambiguous, available and always in conformance with legal requirements. … The recipe mentioned in the customer finished product specification shall be complied with. … There shall be a procedure for the amendment and approval of specifications" (IFS 2007). A typical specification for the final products should address:

- compliance with legal requirements and standards
- pH and water activity (or weight loss), where appropriate (in particular with fermented and/or dried products)
- selected sensory properties (such as color, firmness)
- macronutrients (protein, moisture, fat, carbohydrates, collagen, ash), with tolerances and methods of analysis
- micronutrients (where appropriate)
- packaging material
- instructions to the customer (intended use, shelf life, storage conditions, etc)

Systematic testing of whether the final products meet their specifications is also required by various standards (e.g., IFS 2007) and is useful to verify that the production process was under control, but end-product testing can never replace control measures and process monitoring. Sampling plans should be risk based, and the parameters used for end-product testing should be fit for purpose (i.e., provide a maximum of information with minimum input); standards referring to the absence of pathogens (salmonellae, *Listeria monocytogenes*) from the final products are, for example, defined in the Regulation (EC) 2073/2005 on microbiological criteria for foodstuffs. A product quarantine until test results are available may be useful if the product is sufficiently stable and the time for analysis is sufficiently short.

Concluding Remarks

A functional quality-assurance system provides many benefits to the meat processor. It

reduces the risk of supplying meat products to the customer that are either unsafe or not compliant with other standards or with the expectations of the customers. A well-organized documentation is also important to demonstrate "due diligence" in cases when unjustified statements and claims are made by customers or authorities. Last, establishment of a quality-assurance system requires analysis of existing processes, which again may lead to discovery and elimination of "idle" or "fail" processes, thus making the business more efficient and proficient. However, there are various factors impeding the acceptance of quality assurance, particularly in small- and medium-sized enterprises. These include:

- the wealth of sometimes competing auditing schemes (see, e.g., Van der Spiegel et al. 2003) addressing the same issues with only minor differences.
- audits by auditors who, due to lack of training and experience, tend to follow a schematic rather than a risk-based approach (see, e.g., Vandendriessche 2008).
- the trend among consultants to let all issues appear more complicated than they actually are.

It is to be hoped that these obstacles can be eliminated in the future, so that small- and medium-sized enterprises—which form a large part of the food industry and are essential for product diversity and quality—are not put at a disadvantage.

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Index