Table 11.1 **Protein Composition of Poultry Skeletal Muscle**

I.	Myofibrillar proteins (55% of total protein)
	Contractile proteins
	Examples: myosin, actin
	Regulatory proteins
	Examples: tropomyosin, troponin
	Cytoskeletal proteins
	Examples: titan, nebulin
II.	Sarcoplasmic proteins (35% of total protein)
	Glycolytic enzymes
	Mitochondrial/oxidative enzymes
	Lysosomal enzymes
	Myoglobin and other heme proteins
III.	Stroma proteins (3–5% of total protein)
	Collagen
	Elastin
	Reticulin

required in raw poultry products. Water binding, fat binding, and gelation are some of the important functional properties in cooked meat products. Proteins are often required to be multifunctional. That is, each protein is expected to exhibit more than one functional property either simultaneously or sequentially during processing.

This chapter will begin with a brief description of muscle ultrastructure and an introduction to the major protein fractions of muscle. A short discussion of the role of proteins in comminuted and formed products is presented next, followed by a more in-depth look at the major functional properties of muscle proteins. This chapter concludes with a brief look at the role of model systems in protein functional property research.

Muscle proteins

Poultry meat is comprised of about 20 to 23% protein. Muscle proteins are divided into three categories based primarily on their solubility properties: myofibrillar, sarcoplasmic, and stroma (Table 11.1).

Myofibrillar proteins

The myofibrillar or salt-soluble proteins comprise about 50 to 56% of the total skeletal muscle protein and are insoluble in water, but most are soluble at salt concentrations above 1%. This group is comprised of about 20 distinct proteins organized within a myofibril of an intact muscle. Myofibrils extend the length of a muscle fiber or cell and are surrounded by the sarcoplasm (Figure 11.2). A single muscle fiber may contain 1000 to 2000 myofibrils. The repeating contractile unit of a myofibril is the sarcomere. Myofibrillar protein can be divided into three groups based on their function: (1) contractile proteins, which are responsible for muscle contraction, (2) regulatory proteins, involved in regulation and control of contraction, and (3) cytoskeletal proteins that support and maintain the structural integrity of the myofibril. For more information on skeletal muscle ultrastructure, the reader should refer to one of the numerous reviews on the subject.[1–5]

Myosin is the predominant protein in the thick filament of the sarcomere and comprises about 50 to 55% of the total myofibrillar protein. At physiological ionic strength and pH, myosin molecules aggregate spontaneously to form the thick filaments. Myosin is a

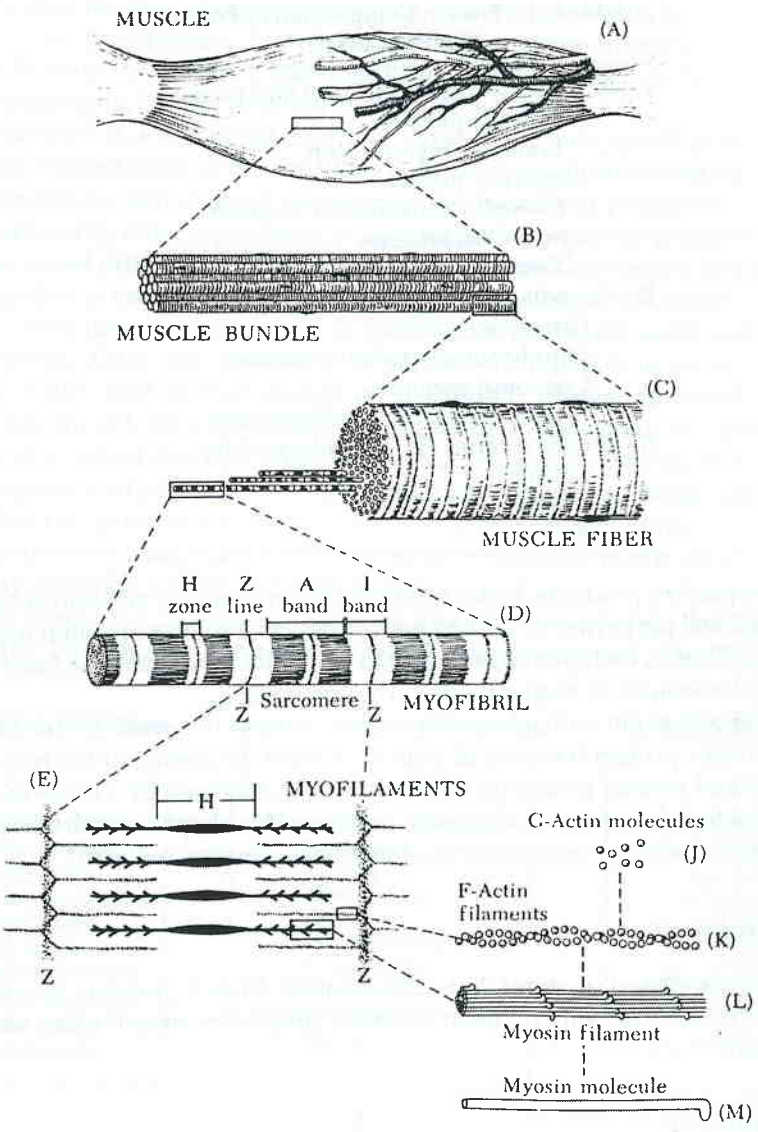

Figure 11.2 Organization of skeletal muscle structure. (From Hedrick, H. B., et al., *Principles of Meat Science*, Third Edition, Kendall/Hunt Publishing Company, Dubuque, IA, 1994. With permission.)

long thin molecule with dimensions of about 150 nm in length by 1.5 nm in width in the rod region and 8 nm in width in the globular head region. Poultry skeletal muscle myosin is a large molecule of about 520 kDa and is comprised of 6 polypeptide chains or subunits (Figure 11.3). The subunits include two heavy chains of about 222 kDa each and 2 pairs of light chains ranging from 17 to 23 kDa. Each heavy chain has a globular head region and a fibrous tail or rod region. The light chains are designated as alkali light chains or DTNB light chains and are associated with the globular head region. The globular head of myosin heavy chain also contains the actin binding site. The tail or rod region is comprised of a

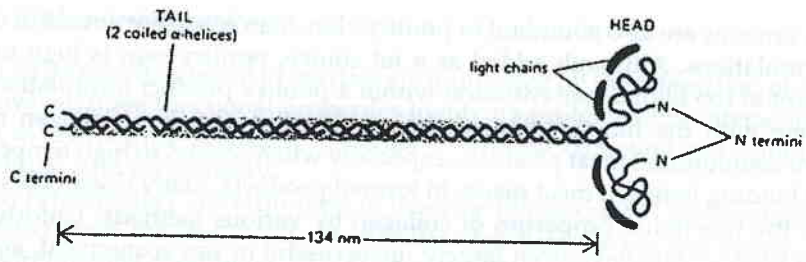

Figure 11.3 Schematic diagram of the myosin molecule.

coiled-coiled alpha helix. This is the region of myosin that is responsible for filament formation under physiological conditions. The head and fibrous tail regions of myosin exhibit distinct biochemical and functional properties. Chicken skeletal myosin contains 43 sulfhydryl groups and no disulfide bonds. The isoelectric point (pI) of myosin is about 5.3 and it is the pH at which the protein has no net charge in solution due to an equal number of positive and negative charges on the molecule.

Actin is the second most abundant myofibrillar protein and comprises about 20 to 25% of this fraction. G-actin is a globular protein with a molecular mass of about 42 kDa. The isoelectric point of actin is about 4.8. Actin, along with the regulatory proteins, troponin and tropomyosin, make up the thin filaments of the sarcomere. Myosin binds reversibly to actin in the thin filaments during muscle contraction. In post-rigor muscle, the globular head or subfragment-1 region of myosin binds irreversibly to actin to form a complex known as actomyosin. This cross-linking between actin and myosin in post-rigor muscle influences meat tenderness in intact muscle.

The contractile proteins, myosin and actin, have a large influence on muscle protein functionality. Myosin, in pre-rigor muscle, and actomyosin, in post-rigor muscle, are generally considered to contribute several functional properties to processed meat products and have been extensively studied.[3,6,7] Since actin is usually complexed with myosin in post-rigor muscle, actin modifies the functionality of myosin in both comminuted and formed poultry products. The ratio of actin to myosin, as well as the ratio of free myosin to actomyosin, influences the functional properties of a poultry product. Sarcoplasmic and stroma proteins modify the functional properties of the myofibrillar proteins.

Sarcoplasmic and stromal proteins

Sarcoplasmic proteins are located inside the muscle cell membrane in the sarcoplasm and comprise about 30–35% of the total muscle protein. These proteins are soluble in water or low ionic strength solutions ($<0.6\ \mu$). Proteins in this category include oxidative enzymes, myogloblin, and other heme pigments, the glycolytic enzymes responsible for glycolysis, and lysosomal enzymes. Myoglobin is the protein primarily responsible for meat color, but in general, these proteins play only a minor role in meat protein functionality.

The stroma proteins, often referred to as connective tissue proteins, hold together and support the muscle structure by surrounding the muscle fibers and entire muscle. Connective tissue surrounding the muscle is called the epimysium. Connective tissue surrounding bundles of muscle fibers is called perimysium, while that surrounding individual fibers is called endomysium. Stroma proteins usually comprise about 3 to 6% of the total protein of poultry skeletal muscle. The major stroma protein is collagen. Elastin and reticulin are minor constituents of the stroma fraction. All of these proteins are insoluble in water and salt solutions. Meat tenderness often decreases with age of the animal due to the increased cross-linking and other modifications that occur to collagen.[8]

Stroma proteins are also abundant in poultry skin. Skin is a major source of collagen in poultry formulations. Although added as a fat source, poultry skin is high in collagen. When present at too high a concentration within a poultry product formulation, collagen may interfere with the functionality of the myofibrillar proteins. Collagen may cause shrinkage of comminuted meat products, especially when cooked to high temperatures, or interfere in binding between meat pieces in formed products. Many researchers have tried to improve the functional properties of collagen by various methods. Unfortunately, all approaches tried to date have been largely unsuccessful or not economical, and thus the amount of skin that can be included in a processed poultry formulation must be kept below certain critical levels.

Role of proteins in comminuted products

To prepare a comminuted poultry product, meat, water, salt, phosphate and perhaps other ingredients are ground or chopped to form a paste-like batter. The meat batter is then stuffed into a casing of the desired shape and cooked. More details on the actual procedures used to prepare comminuted products are described in Chapter 12.

Meat batters are complex systems consisting of solubilized muscle proteins, muscle fibers, fragmented myofibrils, fat cells, fat droplets, water, salts, phosphates, and other ingredients. Comminuted products, such as frankfurters, bologna and sausages, typically contain about 17 to 20% protein, 0 to 20% fat, and 60 to 80% water. Thus, a relatively small amount of protein has to bind a relatively large amount of water and fat. In meat formulations about 1.5 to 2% salt is typically used to allow for the extraction and solubilization of the myofibrillar proteins.

Comminution, sometimes referred to as chopping, physically disrupts the muscle tissue by damaging the sarcolema (muscle cell membrane) and the supporting network of connective tissue. In the presence of salt, the muscle fibers swell, myofibrils are fragmented into shorter pieces, and myofibrillar proteins are extracted and solubilized. These events lead to the formation of a thick, paste-like batter which holds water and stabilizes fat. Upon cooking, the extracted and solubilized muscle proteins in the batter form a cross-linked gel matrix that binds the water and fat and forms the typical texture associated with cooked comminuted products.

Role of proteins in formed products

Formed poultry products are made from chunks or pieces of meat that are bonded or glued together. Turkey breast rolls and chicken cold cuts are common examples of these products (please refer to Chapter 12 for more details on processing). Similar events occur during the production of both comminuted products and formed products. The major exception is that during the production of formed products, most of the changes occur on the surface of the meat pieces. Tumbling, massaging, or mixing in the presence of salt are used to disrupt the muscle cells, disintegrate the muscle fibers, and extract the myofibrillar proteins from the surface of the meat pieces. A tacky myofibrillar protein exudate is formed on the surface of the meat pieces. This extracted protein exudate forms a gel on cooking that acts like a glue to hold the pieces of meat together. The myofibrillar protein, myosin, is thought to contribute most to the binding strength of the protein exudate. Collagen has been found to interrupt the binding of the meat pieces when present on the surface.

Protein-water interactions

In general all protein functional properties are influenced by the interaction of protein with water. However, three functional properties involving protein-water interactions are very important in raw poultry products. These are (1) protein extraction and solubilization, (2) water retention, and (3) viscosity.

Protein extractability is a term used to describe the amount of protein that is released or dissociated from the organized myofibrillar structure during processing. Under the proper environmental conditions, an extracted muscle protein is soluble. Solubility is primarily dependent on the distribution of hydrophobic and hydrophilic amino acids on the surface of a protein and on the thermodynamics of the protein-water interactions. Muscle protein extractability and solubility are affected by pH, salt concentration, type of salts, and temperature.

Water retention describes the ability of a protein matrix to retain water or absorb added water in response to an external force, such as during cooking, centrifugation, or pressing. The water may be chemically bound to the protein, held via capillary action, or physically entrapped within a protein structure. The proteins in the highly organized myofibrillar structure chemically bind water. Water is also physically held within the interfilamental spaces of the myofibril. The water-binding ability of a protein is also influenced by pH, salt concentration, the type of salts present, and temperature.

Viscosity, defined by rheologists as the resistance of a material to flow, has a large influence on the stability of the raw product prior to cooking. The viscosity of the meat batter increases during comminution when the muscle fibers swell and absorb water. Extracted proteins that are large, fibrous, and highly soluble, such as myosin, can increase solution viscosity, even at very low concentrations. Batter viscosity must be high enough to stabilize the raw product, but low enough to allow pumping and handling within the plant.

Effect of salt and pH on protein-water interactions

The effect of salt on the water-binding ability of a turkey muscle homogenate is illustrated in Figure 11.4. Water binding increases most rapidly as the salt concentration is increased from about 0.3 (1.8%) to 0.6 *M* (3.4%) NaCl in both breast and thigh meat.[9] The addition of salt reduces electrostatic interactions between protein molecules to increase protein extractability, solubility, and water binding. Chopping or tumbling of meat in the presence of salt disrupts the muscle tissue allowing the muscle fibers to absorb water and swell, which leads to an increase in viscosity of the batter. Also, the organized thick and thin filaments of the sarcomere are disrupted due to solubilization and extraction of the myofibrillar proteins. Individual myofibrils are released from the muscle fibers and are fragmented into shorter pieces. The extracted proteins, especially myosin, also bind water and increase the viscosity of a poultry meat batter which helps to stabilize dispersed fat. For these reasons, about 1.5 to 2.0% salt is added to most poultry product formulations. Although higher concentrations of salt may improve water binding, the salty flavor is undesirable.

The pH of the poultry meat batter also has a large influence on the extractability, solubility and water-binding ability of the muscle proteins.[9] The effect of pH on the water-binding ability of a turkey muscle batter is illustrated in Figure 11.5. Water binding is lowest at the isoelectric point of myosin and actin (near pH 5.0). The proteins have no net charge at the isoelectric point and tend to associate to form aggregates. The water-binding ability of the muscle homogenate is increased as the pH is adjusted away from this isoelectric point. As the pH is increased, the proteins become more negatively charged. A higher net negative charge leads to an increase in repulsive force between the proteins within the myofilament which subsequently allows the myofibril to swell and hold water.

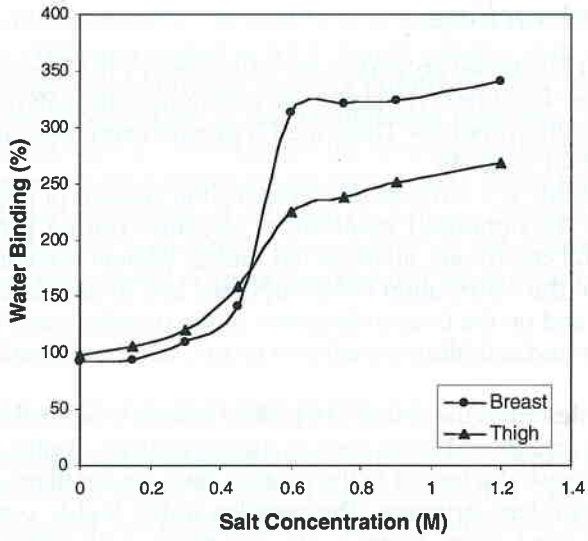

Figure 11.4 Salt concentration affects the water-binding ability of raw turkey meat batters at pH 6.0. (Adapted from Richardson, R. I. and Jones, J. M., *Int. J. Food Sci. Technol.*, 22, 683, 1987.)

The effect of pH on the concentration of extractable protein for a turkey meat homogenate is illustrated in Figure 11.6. Protein extractability and solubility are low near the isoelectric point of the myofibrillar proteins. As the pH is increased, the extractability and solubility of the myofibrillar proteins are increased as the proteins become more negatively charged. Alkaline phosphates are commonly used in poultry products. Alkaline phosphates increase the pH of the meat batter, usually by about 0.1 to 0.4 of a pH unit, to increase the water binding ability of the muscle proteins.

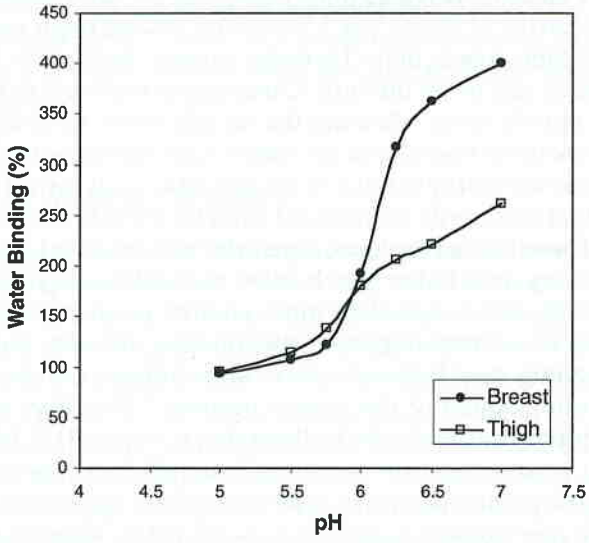

Figure 11.5 pH affects the water-binding ability of raw turkey meat batters containing 0.5 *M* NaCl. (Adapted from Richardson, R. I. and Jones, J. M., *Int. J. Food Sci. Technol.*, 22, 683, 1987.)

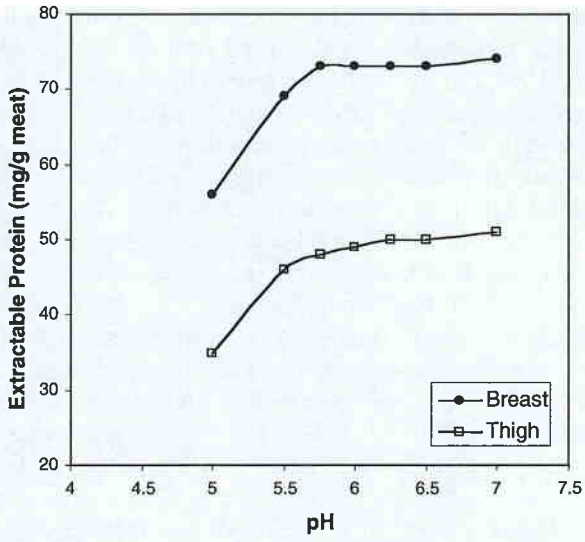

Figure 11.6 pH affects the extractable protein content of turkey meat batters containing 0.5 *M* NaCl. Water binding was defined in this study as the ability of raw meat batter to hold added water upon centrifugation. (Adapted from Richardson, R. I. and Jones, J. M., *Int. J. Food Sci. Technol.*, 22, 683, 1987.)

Processing factors affecting protein-water interactions

The time and temperature of chopping of comminuted products and tumbling of formed products must be carefully controlled during processing. Chopping and tumbling are required to disrupt the myofibril and to solubilize and extract the myofibrillar proteins as described above. However, excessive chopping or tumbling can lead to protein denaturation, usually due to increased temperature or excessive shearing. Thus, chopping and tumbling time must be optimized to maximize protein extraction while avoiding protein denaturation. Denaturation occurs when the native protein structure is destabilized and partially unfolded. Denatured muscle proteins usually form insoluble aggregates that have poor water-binding and film-forming abilities (see following section). Excessive chopping or tumbling may also lead to excessive disintegration of the muscle fibers and to a reduction in batter viscosity, which reduces the quality of the cooked gel network.

Protein-fat interactions

In coarsely chopped comminuted products, such as formed products and many sausages, fat is largely retained within intact fat cells. In these products, fat loss is not usually a problem during handling or cooking as fat is trapped within a cell membrane. The viscosity of the batter and the intact fat cell membrane prevent problems caused by fat instability.

In highly comminuted products, such as bologna and frankfurters, the fat cell is disrupted and fat droplets more typical of those found in emulsions may be formed. An emulsion is made of two immiscible phases, one of which is dispersed as fine droplets within the other continuous phase. In comminuted products, the fat droplets form the dispersed phase, while the continuous phase is comprised of water, protein, and salt. Energy is required to form an emulsion. This energy input occurs during comminution of the meat batter. In general, the greater the energy input, the smaller and more numerous are the fat droplets in the discontinuous phase of a meat batter.

At high temperatures and with sufficient energy input, the fat cell membranes are disrupted and the solid fat is melted and emulsified into liquid droplets. Most poultry fat begins to melt at about 13°C, but due to the variety of lipids present, poultry fat is not completely melted until a temperature of 33°C is reached. Fat droplets may be spherical when the fat is primarily liquid or irregular in shape when the fat is partially solid or crystalline. Liquid fat droplets are highly unstable and readily coalesce on standing. Coalescence is the process in which small fat droplets combine and form large, unstable fat droplets. Coalescence of fat is highly undesirable as it leads to several quality defects in comminuted products. If temperatures are kept low enough, the fat within the droplets may be partially crystallized and less likely to undergo coalescence.

In highly comminuted products, the liquid fat droplets must be stabilized to withstand the stresses of holding, pumping, and cooking. This is accomplished in two ways. First, the high viscosity of the meat batter helps to prevent coalescence of the fat. Second, the fat droplets are surrounded by a protein film that reduces the interfacial tension between the fat and water (the dispersed and continuous phases, respectively) and stabilizes the droplets.

The protein film is comprised of solubilized and extracted myofibrillar proteins. During emulsification, the solubilized and extracted proteins must diffuse to the surface of the oil droplet and then adsorb onto the surface of the droplet. Denatured proteins usually exist as large insoluble aggregates and do not diffuse as readily as smaller, soluble proteins. Once the protein is at the surface, its will unfold or rearrange such that polar regions of the molecule are oriented toward the water and non-polar or hydrophobic regions are oriented toward the oil droplet to minimize free energy. Also, the protein must be present in sufficient concentration so that the protein molecules can interact to form a continuous, stable film on the surface of the oil droplet. There must be a sufficient quantity of extracted protein so that all of the fat droplet surfaces are covered with a protein film. One reason highly comminuted batters are unstable is that very small droplets have a very large surface area and thus require more solubilized and extracted protein to form the stabilizing film. Myosin is the major component of the interfacial film surrounding the fat droplets and is thought to play a key role in stabilizing the fat droplets during holding and during the early stages of cooking.[10] An electron micrograph of the protein film at the surface of a fat droplet in a raw meat batter is shown in Figure 11.7.

Protein-protein interactions

Protein-protein interactions during cooking lead to the formation of a protein gel matrix. A protein gel is formed during heating when muscle proteins unfold and aggregate to form a continuous, defined solid cross-linked network or matrix. The formation of a continuous protein gel network has a large influence on the textural and sensory properties, as well as the cooking yields, of poultry products. The gelation of the myofibrillar proteins occurs during thermal processing of both comminuted and formed products and is probably the most important functional property in processed poultry products during cooking. However, connective tissue and sarcoplasmic proteins may interfere with the ability of the myofibrillar proteins to form a strong gel.

Myofibrillar proteins form thermally irreversible gels. This means that the cross-linkages or chemical bonds formed between proteins during heating are not appreciably altered by cooling or reheating. A schematic diagram illustrating the steps involved in the formation of a thermally irreversible myofibrillar protein gel is shown in Figure 11.8. When muscle proteins are heated they unfold or denature once a critical temperature is reached. In the second step, these unfolded molecules aggregate into small clumps to form an

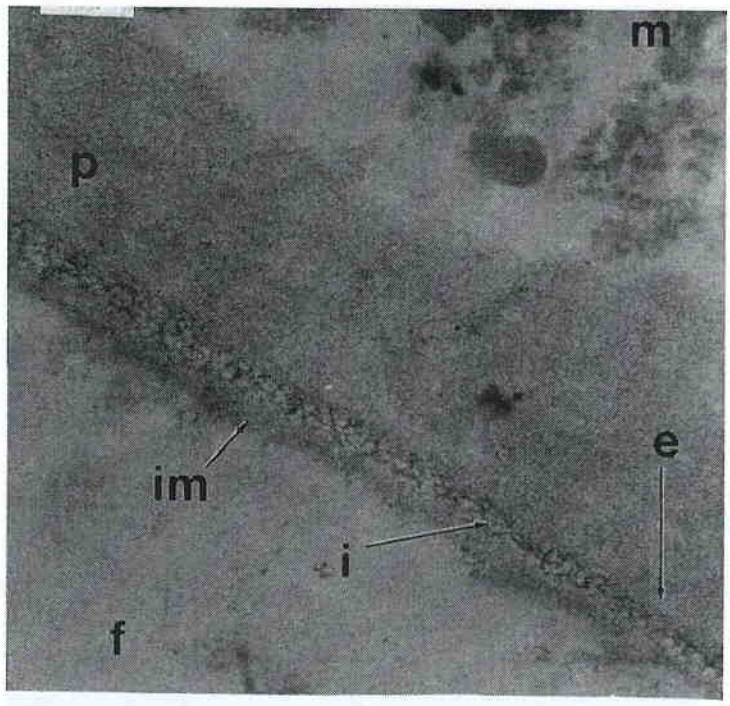

Figure 11.7 Electron micrograph showing the protein film formed on the surface of fat dropets in a highly comminuted poultry meat batter. f, fat droplet; p, proteinaceous material surrounding; i, interface between the fat droplet and the proteinaceous meat batter; m, matrix; e, outside of the protein film; im, inside of the protein film. (From Gordon, A. and Barbut, A., *Food Struct.*, 9, 77, 1990. With permission.)

increasingly viscous solution. The gel point is reached when the aggregates rapidly cross-link into a continuous gel matrix. Muscle protein gels are formed by a combination of hydrogen bonds, electrostatic interactions, hydrophobic interactions, and disulfide bonds. Upon cooling, slight changes occur in the relative importance of the chemical bonds forming the final gel matrix.

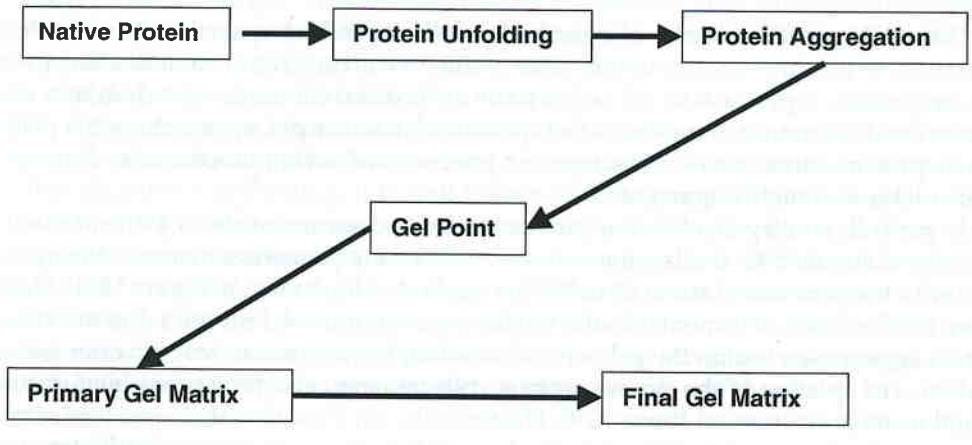

Figure 11.8 Diagram illustrating the steps necessary to form a heat-induced protein gel.

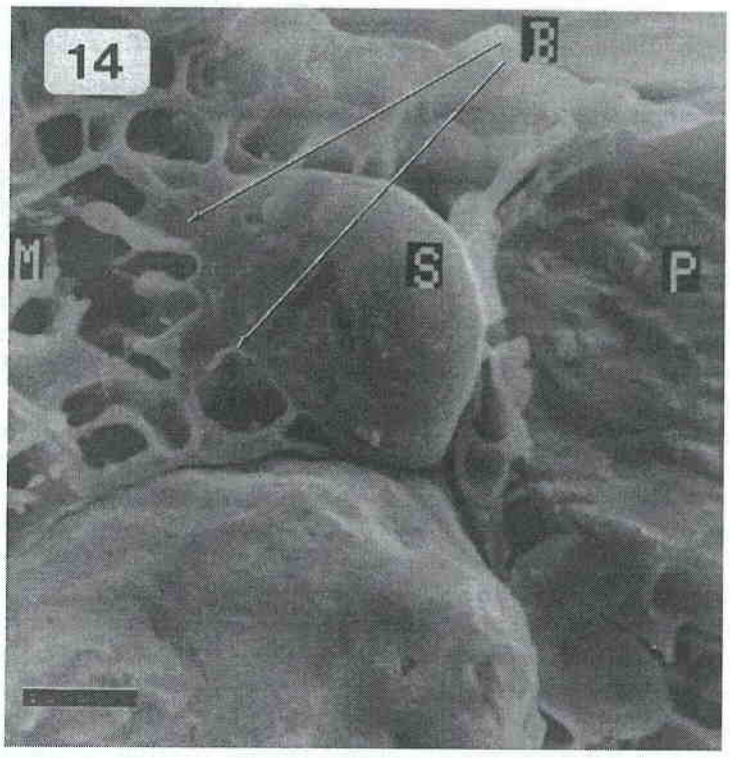

Figure 11.9 Electron micrograph of a cooked chicken meat batter made with 2.5% salt. M, protein gel matrix; S, fat droplet coated with protein; B, junction zone between protein film coating the fat droplet and the gel matrix. (From Gordon, A. and Barbut, A., *Food Struct.*, 9, 77, 1990. With permission.)

The microstructure of the gelled matrix of a poultry meat batter is shown in Figure 11.9. Protein gels hold large amounts of water within their network structure, bound by both chemical reactions and physical entrapment. The protein gel matrix physically restricts coalescence of fat within a cooked meat batter. Upon cooking, the interfacial protein film around the fat droplets also forms cross-links with the continuous protein gel matrix.

Different types of gel networks can be formed, depending upon the pH and salt concentration, to produce poultry batters with distinctive textural and water-binding properties. In general, a pH of 6 to 6.5 will maximize textural hardness and desirable elastic properties of comminuted products. Gels produced at lower pH, approaching the pI of the muscle proteins, often have soft texture and poor water-binding properties as the proteins are insoluble and highly aggregated.

In general, poultry myofibrillar proteins begin to denature at about 4°C and reach the gel point at about 55°C. Gel hardness and water-binding properties increase during cooking until a temperature of about 65 to 70°C is reached as illustrated in Figure 11.10. Heating above 70°C is often detrimental to the quality of a comminuted product due to extensive protein aggregation within the gel network, leading to syneresis or loss of water from the product. The gelation of the stroma protein, collagen, may also be responsible for syneresis and water loss observed above 70°C. Heating rate can also affect the type of gel network formed and subsequent quality of cooked poultry products. It is thought that a slower

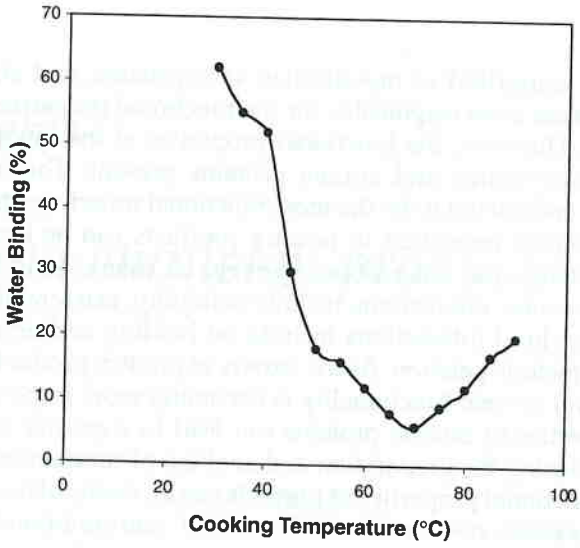

Figure 11.10 Effect of cooking temperature on the ability of ground meat to hold water added after cooking.

heating rate will result in the formation of more ordered gel structures with higher water-binding abilities. Thus, low fat frankfurters are cooked more slowly than their higher fat counterparts to form a protein gel network with higher water-binding ability.

Model systems in protein functionality research

Many different model systems have been used to study the functional properties of poultry meat proteins. Certainly, the easiest system to use is a whole muscle homogenate. However, it is often difficult to determine the true cause and effect in such a complex system, due to the myriad of ingredients and potential interactions. Simplified model systems have been used to limit the number of ingredients and decrease complexity. Researchers have used model systems comprised of fractionated proteins including: myofibrils, myofibrillar protein, salt-soluble protein, actomyosin, and even myosin, to try to understand how proteins function in a poultry product. Myosin has been studied extensively by biochemists, however, much of the work has been done under conditions of pH and salt concentration that are not typically found in poultry products.

In model systems, it is often difficult to compare work among researchers as the composition of the proteins in a particular fraction may change due to preparation procedures. For example, the salt-soluble protein fraction is comprised of a mixture of 15 or more proteins that all interact on heating. It is well known that the composition of the salt-soluble fraction can change depending on extraction conditions and starting material. Thus different amounts of total myosin or different ratios of actin to myosin in the salt-soluble fraction may affect the results obtained. Due to these limitations it is necessary to select a test system with care. For product development work, it may be best to work with the actual product or to select a system as similar to the product as possible. For more basic research, it may be best to start with a simplified system, such as pure myosin, and then move toward more complex systems to determine if the relationships discovered in a simple system are still true when other components are added.

Summary

Muscle proteins are comprised of myofibrillar, sarcoplasmic, and stroma fractions. The myofibrillar proteins are most responsible for the functional properties typically observed in poultry products. However, the functional properties of the myofibrillar proteins are modified by the sarcoplasmic and stroma proteins present. The myofibrillar protein, myosin, is generally considered to be the most functional muscle protein.

Functional properties important in poultry products can be broadly classified into those involving protein-water interactions, protein-fat interactions, and protein-protein interactions. Protein-water interactions include solubility, extractability, water retention, and viscosity. Protein-lipid interactions include fat holding and emulsification. Protein-protein interactions include gelation. As the variety of poultry products increases, the need to modify and control protein functionality is becoming more important. Understanding the functional properties of muscle proteins can lead to a greater understanding of the changes that occur during the preparation and cooking of comminuted and formed poultry products. The functional properties of proteins can be manipulated to allow for the utilization of less expensive meat sources, the use of non-traditional meat sources, the improvement of existing products, and the more efficient utilization of non-meat ingredients. The functionality of poultry proteins can also be manipulated to control processing and energy costs, as well as reduce production waste.

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