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COLLAGEN UTILIZATION IN COMMINUTED MEAT SYSTEMS

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COLLAGEN UTILIZATION IN COMMINUTED MEAT SYSTEMS

by

Kevin W. Jones

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COLLAGEN UTILIZATION IN

COMMINUTED MEAT SYSTEMS

BY

Kevin W. Jones

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TABLE OF CONTENTS

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Literature Review

General Introduction

The stability of finely comminuted meat products is dependent on a wide variety of factors ranging from physical production parameters to the type and quality of meat being utilized in the system. A short review of muscle structure and essential components involved in the emulsification process is necessary to understand the complexity of meat emulsions as compared to the classical emulsions such as mayonnaise.

Skeletal muscle tissue is composed of bundles of muscle fibers. The muscle fiber is the essential structural unit in all meat. The fibers are long cells (up to 30 cm in length) and vary in diameter from 10 to 100 um depending on their function and anatomical location (Price and Schweigert, 1971). The sarcolemma, a thin sheath of connective tissue, surrounds each individual fiber. Within each muscle fiber are longitudinally oriented myofibrils which are surrounded by the meat juices or sarcoplasm. The sarcoplasm contains approximately 25 to 30% of the total meat proteins. Myoglobin, which contributes the red meat color, and numerous enzymes are the principal sarcoplasmic proteins, also known as water soluble proteins (WSP). The myofibrils are thin threads of one to two um in diameter and are not soluble in water. Actin and myosin are the two primary proteins that make up the myofibrils. Numerous other proteins are also present in the myofibrils, but in such minute quantities that they have no significant impact in meat emulsion formation. In pre-rigor meat, actin and myosin are soluble in weak salt solutions and thus these myofibrillar proteins are also known as salt soluble proteins (SSP). In chilled post-rigor meat, the actin and myosin

form a more stable complex known as actomyosin. Actomyosin is less soluble than actin or myosin in salt solutions. The connective tissue within the sarcolemma that surrounds the muscle fiber is composed primarily of the protein collagen. Collagen, classified as a stromal protein, is essentially insoluble in both water and salt solutions, although very small quantities in young animals are soluble. The solubility of collagen is closely related to the degree of crosslinking within and between collagen molecules. As an animal matures, an increase in crosslinking occurs and the solubility decreases. Upon heating, collagen turns to gelatin to varying degrees depending upon the amount of crosslinking.

It is generally agreed that the myofibrillar meat proteins are the most important proteins that lead to the functional properties of a stable emulsion. Myofibrillar proteins are important in contributing to the water binding capacity of the emulsion system as well as being the primary emulsifying agent that reduces the interfacial tension between the two immiscible phases (fat and protein-salt-water).

The functional interrelationships of myofibrillar, sarcoplasmic and stromal proteins with fat and water in meat emulsion systems will be evaluated in this dissertation. A particular emphasis has been placed on the connective tissue proteins, as there is a definite lack of literature in this area. The ultimate goal is to improve the technological utilization of the stromal proteins in highly comminuted meat systems to yield a stable end product exhibiting low shrinkage and no gel pockets, or fat or water separation.

Section I: MEAT EMULSION CHEMISTRY

Definitions. Meat emulsions, meat batters and sausage batters are synonymous terms that have been reported in the literature. Some controversy

exists within the scientific community on the proper terminology that should be used. Therefore, it is essential that definitions pertaining to this subject be reviewed.

The classical definition of an emulsion was first reported by Sutheim (1946): "An emulsion is an intimate mixture of two immiscible liquids, one of them being dispersed in the other in the form of fine droplets." This definition was later modified by Friberg (1972) to: "An emulsion is a mixture of two immiscible liquids, one of them being dispersed into the other in the form of liquid droplets and/or liquid crystals." It becomes evident that neither of these two definitions are applicable to the comminuted meat system that has been termed a "meat emulsion". A meat emulsion is a much more complex system than a "true emulsion" which fits the classical definitions. However, the term "meat emulsion" has been widely accepted and used, and must have a more specific definition. Schut (1976) defined a meat emulsion as: "A two-phase system, consisting of a dispersion of a solid in a liquid in which the solid (fat) is not miscible. The liquid (external or continuous phase) is an aqueous solution of salts and proteins, and at the same time is a medium in which insoluble proteins and particles of muscle fibers and connective tissue are dispersed." Even this definition is not totally appropriate in defining a meat emulsion. For example, is the fat a liquid, solid, or semisolid? Of course, that depends upon the product temperature and the melting point of various fats. Likewise, these questions can be raised: must the continuous phase always be a liquid? Does a meat emulsion refer only to the raw batter, as Schut's definition would suggest, or does "meat emulsion" refer to both the raw and cooked product? Much of the literature would suggest the latter case. It becomes clear that a somewhat general yet clearly defined definition is still needed. Perhaps a more accurate definition

would read: "A meat emulsion is a complex two-phase system of which the continuous phase consists of a colloidal suspension of soluble proteins, insoluble proteins and cellular particles forming a matrix network, while the discontinuous phase is composed of finely dispersed lipid droplets entrapped within this matrix network."

"Emulsion" formation. Meat emulsions are made by grinding or chopping lean meat and fat meat with water (or ice) and NaCl (or certain other salts). Other ingredients are added to improve the flavor, color, yield and shelf life of the product, but are not essential in the formation of the "emulsion" itself. Meat emulsions are generally unstable unless another component, known as an emulsifying agent, is present. When fat droplets are in contact with water, there is a high interfacial tension between the two phases, resulting in a coalescense of fat droplets. Emulsifying agents, such as solubilized proteins, effectively reduce this unstable condition because of their affinity for both fat and water within the same molecule. Solubilized proteins unfold and align themselves around fat droplets with their hydrophobic portions oriented toward the fat and their hydrophilic portions away from the fat droplet and toward the aqueous matrix of the emulsion (Forrest et al., 1975). In sausage emulsions, soluble proteins dissolved in the aqueous phase act as emulsifying agents by coating all surfaces of the dispersed fat particles. The soluble proteins may be myofibrillar, sarcoplasmic, and to a limited extent, certain stromal proteins. However, the myofibrillar proteins are reported to be the most important emulsifying agent in contributing to emulsion stability (Hegarty et al., 1963). Myosin and Actomyosin are the most important of the myofibrillar proteins in terms of quantity and functionality as an emulsifying agent (Tsai et al., 1972; Galluzzo and Regenstein,

1978). Both are insoluble in water and dilute salt solutions. For this reason, salt is an essential ingredient required to solubilize these myofibrillar proteins into the aqueous phase so that they can become available for coating fat particles. Water improves the efficiency of the salt by increasing the rate at which swelling and solubilization of the proteins occur (Hamm, 1960).

The formation of an interfacial protein film or membrane around fat droplets has been an area that has received considerable attention in recent years. Becker (1965) described the hydrated interfacial film thickness (IFT) of an emulsion as half the average distance between the oil droplets. For emulsions with a uniform diameter of fat droplets the IFT would be the volume of protein extracted divided by the total calculated surface area of the fat droplets. For emulsions measured at the first detectable indication of inversion, Becker (1965) reported that the total surface area and IFT were limiting factors due to these components collapsing or failing to support emulsification. Ivey et al. (1970) stated that emulsifying capacity (EC) increased as the degree of dilution of the continuous phase was increased in a model system. These researchers found that the stability of emulsions formed with dilute protein extracts was dependent on the interfacial film thickness. They postulated that the reason a thinner IFT produced a more stable emulsion was due to the denaturation principle. A decreased concentration of emulsifying agent is thought to allow a greater degree of unfolding of the protein helix which allows for a higher amount of molecular orientation of hydrophilic and hydrophobic groups on the protein molecule. The model system work done by Ivey et al. (1970) suggested that the volume of fat that can be emulsified to give a high stability depends on the quantity of water added to the system. This was later confirmed by Morrison et al. (1971) in actual sausage type emulsions. The addition of protein was found to

be deleterious to emulsion stability, but the addition of an increasing amount of water increased the stability of meat emulsions. These scientists found a sharp decrease in emulsion stability when less than 16% water was added for fresh beef in an emulsion with a 30% fat level.

The presence of a membrane surrounding fat globules was shown by Borchert et al. (1967) using transmission electron microscopy. These researchers demonstrated that the membrane is disrupted during the cooking process. Saffle (1969) positively confirmed that a membrane surrounding lipid droplets is formed by detailed photomicrographs. A droplet of oil was extruded from a syringe into a salt soluble protein solution. The membrane formation that occurred was due to molecular orientation of the solubilized proteins around the oil droplet. When a protein molecule arrives at the boundary of the two phase system (e.g., an oil/water interface), orientation of the side chains is a logical result, because it allows the protein molecule to take up a conformation with a lower free energy, in which polar groups interact with the polar phase (water) and nonpolar groups with the nonpolar phase (oil or fat). As a consequence of this mechanism, interfacial protein denaturation occurs and a protein film arises. Schut (1976) reports that this denaturation is reversible. The mechanical strength of a protein film and its resistance against external forces have long been known to determine the stability of classical two-phase emulsions (Tachibana and Inokuchi, 1953). Pearson and Alexander (1968) investigated the viscoelastic properties of protein films and concluded that the mechanical strength of a protein film is characterized by a complex relationship of both elasticity and viscosity and is quite dependent on the surface concentration of the film. This work, as well as the work of Das and Chattoray (1980), suggested that the behavior of a protein film is not only dependent on the surface concentration but also the associated

stress. The degree of deformation of the interfacial film is a time and temperature dependent factor.

In light of these observations, the results obtained from emulsifying experiments with model systems can be interpreted better. When increasing amounts of oil or melted fat are emulsified in a protein solution, the total interface surface area gradually increases. At a point where the critical surface area is exceeded for a particular protein(s), the emulsion will collapse.

The role of temperature in meat emulsion production, according to Schut (1976), is of extreme importance and can be explained to a large extent by its influence on the rheological behavior of protein films. Film viscosity decreases at increasing temperatures and film elasticity is also adversely affected. Tachibana and Inokuchi (1953) found that the instantaneous elasticity of a protein film disappears completely above a certain temperature, which was reported to be 20 C for ovalbumin.

The role of a protein film as a stabilizer between the two phases in meat emulsions is a very complex entity whose stabilizing effect is dependent on the elasticity, viscosity, surface potential and surface concentration of the film. These parameters (viscosity, elasticity, surface potential and surface concentration) are in turn dependent on a wide variety of physical parameters such as temperature, pH, isolectric point of the protein(s), mechanical stresses and other factors (Schut, 1976). The nature of the protein film surrounding fat droplets from a mechanistic point of view is the most important factor in the formation of a stable emulsion system.

An over-simplified review of the events that occur in emulsion formation can now be more clearly understood. During the chopping of meat (with salt and water), muscle fibers are disrupted, proteins are solubilized, and meat and fat

particles are reduced in size. In an effort to attain a more stable molecular arrangement at a lower free energy level, protein molecules orient themselves at the interface between the two immiscible phases (fat and water). This orientation occurs such that a multimolecular membrane is formed whereby hydrophobic side chains of protein molecules are attracted toward the lipid phase and hydrophilic side chains are attracted toward the aqueous phase. The viscosity, elasticity, surface concentration and surface potential of this membrane in part determine the stability of the emulsion (Tachibana and Inokuchi, 1953; Tsai et al., 1972; Schut, 1976). Simultaneous to the membrane formation around lipid droplets, a complex matrix of protein-protein and protein-water interactions occurs, forming a viscous mass which further entraps the protein encapsulated fat droplets in a semi-rigid conglomerate. The interaction that occurs between this matrical network of proteins, water and extraneous material and the encapsulated fat droplets further influences the stability of the system. These interactions will be discussed later in this text.

Protein-water-fat relationships. The functional properties of meat proteins within an emulsion are largely dependent on the physiochemical state that these proteins exist in and their interrelationship with other components of the system such as fat or water. Therefore, it is essential to review the mechanisms of changes of state of proteins and how these changes in state affect their relationship with other components in meat emulsion systems.

The terminology that exists in the literature in regard to the physiochemical state of proteins and mechanisms of alteration is often confusing and poorly defined. It is thus necessary that this terminology also be reviewed.

Schmidt (1981) reports that a complex interrelationship between association-dissociation, precipitation, coagulation and gelation reactions exists in

protein systems. Protein association reactions refer to changes that occur at the molecular or subunit level, while aggregation reactions generally involve the formation of higher molecular weight complexes from association reactions. The terms gelation and coagulation are not as clearly defined. Schmidt (1981) defined gelation as a protein aggregation phenomenon in which polymer-polymer and polymer-solvent interactions and in which attractive and repulsive forces are so balanced that a well ordered tertiary network or matrix is formed. This matrix is capable of immobilizing or entrapping extremely large amounts of water. Coagulation, on the other hand, can be defined as a more random aggregation in which polymer-polymer interactions are favored over polymer-solvent reactions. Empirically, these definitions lead to an unavoidable overlap in terminology, as it becomes extremely difficult to differentiate between highly solvated coagulums and true protein gels.

Hermansson (1979) categorizes this confusing terminology somewhat more clearly. Protein-protein (or polymer-polymer) mechanisms are classified as aggregation, association, precipitation, floculation, or coagulation and are defined as follows:

Aggregation: a general collective term for protein-protein interactions

Association: refers to changes on the molecular level such as monomer $\longrightarrow$ dimer reactions which are characterized by weak bonds at specific binding sites.

Precipitation: random aggregation phenomenon that occurs as a result of neutralization of repulsive forces within a solution.

Floculation: random aggregation that is entirely a colloidal phenomenon where the interaction between protein molecules is determined by the

balances between electrostatic repulsive forces and Van der Walls' attraction.

Coagulation: refers to random aggregation which includes denaturation of protein molecules.

Contrary to the "association", aggregation, precipitation, coagulation and floculation refer to unspecified protein-protein interactions and the formation of complexes with higher molecular weights.

Hermansson (1979) categorizes protein-solvent mechanisms of interaction into the following classifications: solubilization, dissociation, swelling and denaturation. Solubilization refers to a state of equilibrium between a solid and a liquid. Denaturation can be defined as conformational changes from the native structure without alteration of the amino acid sequence. Swelling refers to the uptake of solvent (water) by a protein to increase the size of the protein without a significant change in conformation. Swelling is often regarded as the first step in the denaturation process. Interestingly, Hermansson (1979) does not classify gelation as a protein-protein or protein-solvent mechanism of interaction. He states that: "Gelation is often an aggregation of denatured molecules. Contrary to coagulation where the aggregation is random, gelation involves the formation of a continuous network, which exhibits a certain degree of order. The kinetics of the mechanisms (e.g., dissociation, swelling, denaturation and aggregation) will determine the structure and properties of the gel."

Protein-protein interactions. The structural characteristics of meat emulsions and a variety of food systems are complexly related to the physiochemical protein phenomena of aggregation, coagulation and/or gelation (Schmidt, 1981). These phenomena are reported to be physical manifestations of

protein denaturation processes which are highly dependent upon the type and amount of protein, processing conditions, pH and ionic environment. Hydrogen bonding, disulfide bridging and hydrophobic attraction are reported to play major roles in crosslinking and stabilizing the structures of a protein gel or coagulum (Schmidt, 1981). Meat emulsions, which have also been described as heat-induced gels (Hermansson and Akesson, 1975) are reported to be also quite dependent on ionic crosslinking as a partial mechanism of solvent (water) immobilization. The heat treatment required for gel formation is extremely critical when assessing the applicability of a given protein to a specific food formulation. For example, replacement of myosin (which has a relatively low gel temperature requirement) in a frankfurter formulation with a protein preparation requiring extremely high temperatures for comparable gelation would not be desirable. Peng et al. (1979) reported that significant protein-protein interaction of soy protein (IIS fraction) and myosin did not occur until temperatures reached 80 to 90 C. Siegel et al. (1979) investigated various other non-meat protein sources and concluded that the structure of heat-induced gels formed by these proteins and mixtures of them with crude myosin showed that a three dimensional network of protein fibers is not indicative of good binding ability. Rather, the type of molecular interactions stabilizing these gel structures was thought to be more important. Ishioroshi et al. (1979) reported a more in-depth study of the heat-induced gelation properties of myosin as affected by pH and salt concentration. These scientists found that optimal gelation temperatures of myosin occurred between 60 and 70 C at a pH of 6.0 as measured by a shear modulus technique. The optimum pH reported here is in agreement with findings reported by Trautman (1966) who studied the effect of pH on the gelling properties of salt soluble proteins from ham muscle. The existence of two transition temperatures in the heat-induced gelation of myosin

is thought to be important in the functional properties of this binding protein (Ishioroshi et al., 1979). The transition temperatures were thought to be related to conformational changes which occurred at 43 C and 55 C. These transition temperatures correspond to those for the helix-coil transition of the myosin rod reported by Burke et al. (1973), Goodno and Swenson (1975), and Samejima et al. (1976). Ishioroshi et al. (1979) hypothesized that the coincidence of the transition temperatures for myosin gelation with those for the thermal unfolding of the helical structures of the myosin rod suggest that the unfolding of the helical tail portion of the myosin molecule may play a role in the heat-induced gelation of myosin.

To summarize the impact of protein-protein interactions in comminuted meat systems, it can be said that the denaturation, aggregation and gelation properties of myosin and actomyosin play an important role in the formation of protein membranes and the lattice structure of the emulsion matrix. Temperature, ionic environment, protein concentration and pH are all physical phenomena that influence these denaturation, aggregation and gelation properties.

Protein-water interactions. The interactions that occur between meat proteins and water have been widely investigated and determine the water-binding ability (water-holding capacity) of meat and meat products (Hamm, 1960; Lumry, 1973; Schut, 1976; Hermansson, 1979; Schen, 1981). The water holding capacity (WHC) in turn has profound effects on product yields, texture, juiciness and overall eating qualities of the final product.

A review of the basic properties of water is essential before understanding the more complex interrelationships of water with the various meat proteins.

Water is the major component present in most food systems, being bound or entrapped by different bond types or physical phenomena (Fennema, 1976).

Water has often been described as either "free water" or "bound water" by various authors, creating some confusion in definitions in the literature. Fennema (1976) states that all water in biological material is bound to some extent and should thus be classified accordingly. This classification scheme lists four types of water based on water activity. Type I water is very tightly bound and is what some authors refer to as "true bound water". This water type is found as the mono- and possibly the bimolecular layers of water surrounding proteins and other substances which have an affinity for water due to electrostatic charges. Type I water has very little translational mobility, is unfreezable, and is present in small quantities (3%). Type II water refers to the multilayers of water surrounding the type I water. This water type still has a strong bonding affinity for the structures that it surrounds. Unlike type I water, type II water can be easily removed by conventional dehydration. Type III water is that water that is relatively easily removed and represents the majority of water in animal and food tissues. The water activity of this water type is only slightly less than pure water. When all of the type III water has been removed from a sample, the moisture content ranges from 12 to 25% and the water activity is about 0.8. Type IV water does not naturally occur in biological matter since it is water in the pure state. For the purposes of this review, Fennema's nomenclature on water types will be used.

Type I and type II water are tightly bound by hydrophilic groups of the side chains of proteins, such as carboxyl, amino, sulfhydryl and hydroxyl groups as well as the nonionized carboxyl and imido groups of the peptide chains (Schut, 1976). Most of the type III water is thought to be immobilized in a network of membranes and in the filaments of structural proteins in the case of muscle