Protein Lipid Interactions in Processed Meats

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Introduction

Sausage batters are a complex colloidal suspension of meat and fat articles, solubilized proteins, water, salt and other added ingredients. Figure 1 illustrates many of the components which might be observed in a typical finely comminuted cooked meat product. Numerous bonding energies and other physical forces are essential in maintaining the stable integrity of the colloidal suspension before, during and after thermal processing.

Figure 1.

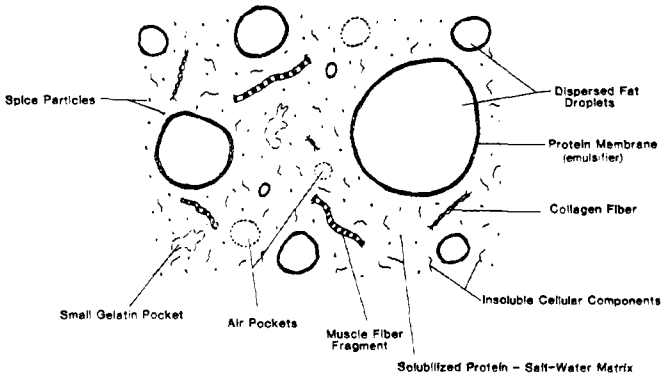

Figure 1. Structural components of a typical cooked sausage batter.

Comminuted meat systems, sometimes referred to as "meat emulsions," are not a true emulsion system and may be more appropriately termed a minced meat or meat batter system. It is now becoming more widely accepted that the gelation of muscle proteins is largely responsible for the physical and chemical stabilization of fat and water in comminuted meat products (Ziegler and Acton, 1984). Although it is true that comminuted meat systems do not possess the complete properties of classical oil-in-water emulsions, they nonetheless have characteristics which resemble an emulsion and thus the retention of the optional term, meat emulsion, Photomicrographs of raw and cooked batters have shown the presence of a proteinaceous membrane surrounding the surface of lipid particles or droplets (Hansen, 1960; Helmer and Saffle, 1963; Borchert et al., 1967; Theno and Schmidt, 1978; Jones and Mandigo, 1982). Some scientists have suggested that this proteinaceous membrane at the oil/water interface does in fact play a role in the stability of the comminuted meat system during thermal processing (Ivey et al., 1970; Jones and Mandigo, 1982; Li-Chan et al., 1984). However, the degree to which this interfacial protein membrane contributes to the fat binding (or holding) ability of the comminuted meat system and the subsequent inhibition of "fatting out" is unclear. Numerous other factors undoubtedly play a role in the comminuted meat system's ability to bind and hold fat during thermal processing. These factors include pH, ionic strength, melting point and cell membrane integrity of the lipid source, protein gelation properties and thermal processing conditions. Chopping temperature and time also play a role in the fat-binding ability of the system. However, these latter parameters are indirectly related to some of the factors previously mentioned (fat cell integrity, melting point, ionic strength and gelation properties).

The role of actual protein-lipid interactions in comminuted meat systems is related to the biophysical properties of the proteinaceous membrane which surrounds lipid particles. Other forces may be equally if not more important in maintaining a high degree of stability in the system. Certainly, the gelation properties of the proteins within the "continuous phase" or matrix of the system are a large determinant in the fat holding ability of the system. Physical hindrance of liquid fat migration during thermal processing effectively acts as an inhibitor of fat coalescence to some degree. However, the formation of an interfacial protein membrane must also be considered a mechanism responsible for the suppression of fat coalescence during thermal processing. Lastly, the physical characteristics of the fat also influence the stability of comminuted meat systems. Perhaps each of these phenomena are equally important in determining the fat binding ability of comminuted meat systems.

This paper will focus primarily on the properties of the interfacial protein membrane, since that is where the site of interaction between proteins and lipids occurs.

Interfacial Protein Film (IPF)

The mechanical strength of an interfacial protein film (IPF) and its resistance against external forces has long been known to determine the stability of classical two-phase emulsions (Tachibana and Inokuchi, 1953). In an effort to attain a more stable molecular arrangement at a lower free energy

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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 hydrophobicity of this membrane in part determine the stability of the emulsion (Tachibana and Inokuchi, 1953; Tsai et al., 1972; Schut, 1976).

The presence of an interfacial protein membrane (or film) surrounding lipid droplets in "emulsified" meat products and model systems has been well documented in the literature (Hansen, 1960; Borchert et al., 1967; Saffle, 1969; Theno and Schmidt, 1978; Jones and Mandigo, 1982). In recent years, considerable controversy has arisen regarding the functionality of the IPF in comminuted meat systems. In finely comminuted meat batters, simultaneous to the membrane formation around lipid droplets, a complex matrix of protein-protein and protein-water interactions occurs, forming a viscous gel which further entraps 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 are discussed in greater detail later in this text.

Physiochemical Properties of Myosin

In meat batter systems, myosin has been recognized as the most important protein in encapsulating fat particles (Saffle, 1968; Schut, 1976). This is attributed to the abundance of myosin as a meat protein and its ability to solubilize easily in 3% to 5% brine concentrations. The gelation properties of myosin, actomyosin and other muscle proteins have been widely investigated and are more thoroughly covered in the related papers in the proceedings of this conference.

The thermal transition temperatures (Tm) of myosin and actomyosin are very important when considering a potential functional role of these proteins as a membrane stability factor for lipid droplets. At least two transition temperatures ($Tm_1 = 43°C$ and $Tm_2 = 55°C$) are considered important in the heat denaturation of myosin molecules (Samejima et al., 1981). Transition temperatures represent points at which conformational changes (denaturation) occur in the native structure of a protein (e.g. as in a change from a helical to a random coil-type structure). Wright et al. (1977), Ishioroshi et al. (1979), Quinn et al. (1980) and Ziegler and Acton (1984) have reported that the pH and ionic environment of the meat system affect the Tm properties of myosin and actomyosin. Ziegler and Acton (1984) have shown a decrease in Tm_1 and Tm_2 of 5.5° and 1.1°C respectively when pH was lowered from 6.0 to 5.5 for natural actomyosin solutions (ionic strength = 1.0).

Since most meat batters are cooked to approximately 70°C, conformational changes must occur in the native structure of proteins surrounding fat globules. The heat-induced gelation of the IPF is thought to result in a semi-rigid structure which has definite viscoelastic properties and functions to prevent the escape of liquefied fat during thermal processing (Jones and Mandigo, 1982). Without such a membrane surrounding fat globules, liquefied fat would be free to migrate through void spaces (air pockets) to form pools of fat within the product or fat caps on the product surface. Of course, fat liquefication and subsequent migration can only occur during the thermal processing cycle and are also inhibited by the gelation properties of the batter matrix and the integrity of fat cell membranes.

IPF – Lipid Interactions

The importance of myosin and actomyosin transition temperatures becomes more readily apparent when one considers the melting profiles of various meat and poultry lipid sources (Table 1). Hermansson (1977) has stated that when part of the fat liquefies, as during the latter stage of comminution, then it can be emulsified with the myofibrillar proteins acting in the role of an emulsified-stabilizer. Ironically, in raw minced meat batters, virtually all the lipid particles are surrounded by a protein membrane (Borchert et al., 1967; Jones, 1982) even though the vast majority of the fat particles are still in a solid state. Without liquefied fat, it would seem improbable that such a protein membrane could be formed. This would suggest that a thin layer of liquid fat likely surrounds each fat particle as a result of tissue disruption and frictional heat during chopping.

Table 1. Melting Point Ranges Of Various Animal Fats[1].

Fat Source	*Melting Point Range (°C)*
Beef	40-48
Pork	33-46
Lamb	44-51
Poultry	31-33

[1]Source: Technical Bulletin No. 77. Archer, Daniels Midland Co.

The endpoint chopping temperatures in commercial comminuted meat systems seldom exceed 18°C which is well below the liquefaction point of animal and poultry fats (Table 1). This temperature is also considerably lower than the transition temperatures of myosin (43° and 55°C). This would indicate that relatively little heat denaturation of the myosin molecule occurs prior to the thermal processing cycle of sausage batters. During thermal processing, meat and poultry fat are liquefied in the temperature range of 31° to 51°C (Table 1). Although the thermal transition of myosin from sol to gel begins at approximately 30°C, maximum get strength is not reached until 60° to 70°C (Yasui et al., 1979; Ishioroshi et al., 1979). If one assumes that the batter matrix follows similar thermal transitions, fat liquefication occurs well before an optimal gel strength has been achieved in the matrix. These observations give credence to the role of the IPF as a stabilizing factor in comminuted meat systems.

Very little literature is available on the mechanisms of membrane formation around lipid particles in comminuted meat systems. The emulsifying proteins in classical two phase water-in-oil emulsions are known to unfold (denaturation) and orient hydrophobic and hydrophillic regions of the proteins at the oil/water interface to attain a more stable enthalpy. (Tachibana and Inokuchi, 1953).

Surface Hydrophobicity

Recent evidence might suggest that protein denaturation may not be necessary for solubilized myosin to act as an emulsifying protein during the early phases of the interfacial protein film (IPF) formation. The question of effective (surface) hydrophobicity of the contractile proteins has not received much attention in the past. However, the hydrophobic properties of such proteins largely determine their ability to form an interfacial membrane between two immiscible components (fat and water). Free myosin is a unique protein in that its surface hydrophobic properties are primarily confined to the head region or the HMM S-1 subfragment (Borejdo, 1983). Borejdo (1983) used the fluorescent marker, cis-parinaric acid, to identify hydrophobic sites on the myosin molecule. The presence of 1.34 hydrophobic sites per mol of myosin were observed, of which .65 hydrophobic sites were accounted for in each of the two HMM S-1 subfragments. Although many hydrophobic residues are buried in the interior of native proteins, some hydrophobic groups remain exposed at the molecular surface or in crevices. Few proteins exhibit the intensity in surface hydrophobicity that is observed in the confined region of the myosin head (Figure 2).

Figure 2.

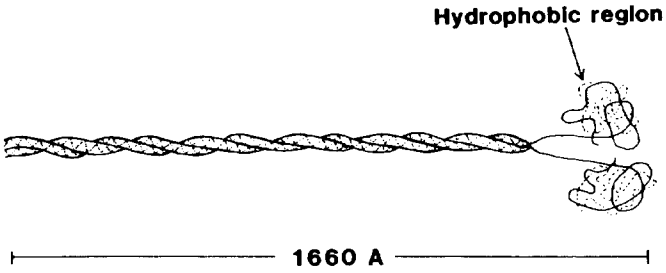

Figure 2. A three dimensional representation of a myosin molecule. Surface hydrophobic regions have been identified in each of the two globular appearing myosin heads.

In view of this information from Borejdo (1983) and the work of Kato and Nakai (1980) it would appear that myosin can, at least theoretically, form a protein membrane with only minimal denaturation or reorientation of the head region residues. Thus, free myosin may remain relatively intact in the early phases of membrane formation around lipid droplets in comminuted meat systems. The work of Kato and Nakai (1980) and Li-Chan et al. (1984) found significant high correlations between surface hydrophocity, protein solubility and 2 measures of emulsifying properties. The latter authors reported being able to account for 70% and 82% of the variability in emulsifying activity index and emulsion capacity, respectively, by surface hydrophobicity of the meat contractile proteins in their model systems. However, these high correlations were based on relatively few numbers. Protein solubility was also considered an important parameter of regression equations used for predicting the emulsifying properties of their model systems.

It is presently unknown to what extent free myosin (solubilized) occurs in minced meat batters as opposed to free actomyosin. It is further unknown how actomyosin may be involved in interfacial protein film formation. It is likely that actomyosin must undergo surface orientation and subsequent denaturation to a more stable state and lower enthalpy in order for it to act as an emulsifying protein.

Others who have extensively studied the gelation properties of myosin are in general agreement that the strongest cross linkages involved in gelation occur at the myosin head region (Samejima et al., 1981; Acton et al., 1983). Conformational changes that occur during the thermal denaturation of myosin are thought to be first initiated in the head region (Samejima et al., 1981; Ziegler and Acton, 1984). This latter observation would support the presence of surface hydrophobicity in the head region.

Figure 3 represents what may occur in the early phases of protein membrane formation at the lipid/water interface. Free myosin may exist relatively intact to form a surface monolayer as illustrated; or free myosin, actomyosin and possibly other proteins may undergo major conformational changes (denaturation) to form a membrane. It is likely that a monolayer of the most polar proteins occurs first (e.g. myosin); followed by slightly more random protein-protein interactions influenced by hydrophobic forces, covalent bonding energies, hydrogen bonding and weak attractive forces of the solubilized proteins.

Figure 3.

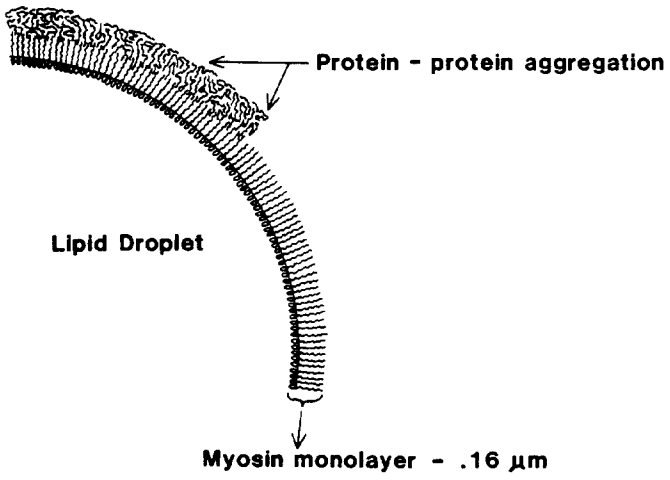

Figure 3. Diagramatic representation of events that may occur in the initial development of an interfacial protein film or membrane in a raw sausage batter. The initial formation of a myosin monolayer of relatively undenatured (intact) myosin molecules is a theoretical possibility, although has not been proven. Subsequent protein-protein interactions likely thicken and strengthen the protein membrane. The lipid particles in raw sausage batters are generally in a solid state but are probably covered with a thin layer of liquified fat.

Physical Characteristics

Scanning electron micrographs by Jones and Mandigo (1982) suggest that the nature of the interfacial protein membrane is highly dependent on the chopping temperature to which the meat batter was exposed. Micrographs of cooked frankfurter batters that were chopped to an endpoint temperature of 10°C and 28°C are illustrated in Figures 4 and 5, respectively. Batters were finely comminuted through an emulsion mill with identical raw materials, comminution time, and other factors held constant. Small lipid droplets appear to have exuded from numerous pores in the protein-encapsulated globule viewed in the batter chopped to 10°C (Figure 4).

Figure 4.

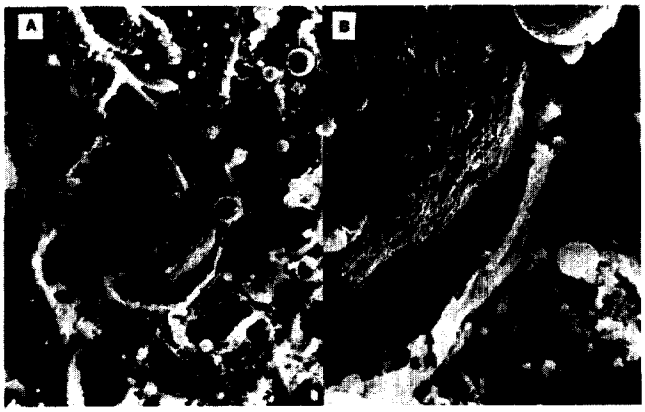

Figure 4. Scanning electron micrographs of cooked frankfurter batter that was chopped to an endpoint temperature of 10°C. 4A was taken at magnification of 625X and depicts a relatively large protein encapsulated fat globule lossely embedded in the surrounding batter matrix. 4B depicts the same globule in 4A, at a magnification of 2500X. Note the small pores on the surface of the interfacial protein film and the separation between the globule and the surrounding matrix. Legends: IPF = interfacial protein film; M = batter matrix; P = pores in IPF; S = separation between matrix and IPF. (modified from Jones and Mandigo, 1982).

The protein membrane surrounding the globule appears thin and flexible in nature. However, frankfurter batters that were comminuted at an endpoint temperature of 28°C revealed a markedly different gelled matrix and membrane properties. Large rupture holes were observed in approximately 20% of the larger fat droplets (those greater than 50 microns in diameter) as shown in Figure 5. Considerable loss in the integrity of the gelled matrix is also apparent in Figure 5. Frankfurter batters that were comminuted to endpoint temperatures of 16°C and 22°C (not shown) yielded micrographs with characteristics intermediate to those shown in Figures 4 and 5 (Jones and Mandigo, 1982). The "lumpy" appearing fat globule in Figure 4 suggests that the protein membrane became a rigid structure before the solid fat within reached its melting point. Otherwise, a smoother, more uniformly rounded protein encapsulated fat globule would have been observed. Also note the separation between the interfacial

Figure 5.

Figure 5. Scanning electron micrographs of cooked frankfurter batter that was chopped at an endpoint temperature of 28°C. 5A was taken at a magnification of 625X while 5B was taken at 1350X. Approximately 20% of the larger fat globules (those greater than 50 microns) exhibited large rupture holes in the interfacial protein film. The batter matrix displayed considerable loss of integrity when compared to the matrix in 4A. Legends: IPF = interfacial protein film; M = batter matrix; R = rupture hole. (Modified from Jones and Mandigo, 1982).

membrane and the surrounding batter matrix. Jones and Mandigo (1982) concluded that the interfacial protein films (IPF) observed in that study displayed definite variations in elastic properties, primarily due to IPF thickness. Sherman (1963), Ivey et al. (1970) and others have also reported that the stability of minced meat batters is dependent on the interfacial film thickness and subsequent viscoelastic properties.

Gel Strength and Lipid Characteristics

The most recent literature suggests that the strength of the gelled batter matrix is extremely important in the fat-binding properties of comminuted meat systems (Acton et al., 1983; Ziegler and Acton, 1984). In addition to gel strength, which has been very thoroughly covered in the two other papers of this session ("Protein - Protein Interactions" and "Protein - Water Interactions"), physical characteristics of the fat largely influence their stability and fat holding abilities. Coarse-cut sausage products seldom experience the "fatting out" problems which are sometimes observed in finely comminuted products. Since there is less fat cell disruption, cell membranes function to inhibit the coalescence of the fat in the heat set protein matrix. Also, larger lean and fat particles drastically reduce the particle surface area, thereby effectively increasing the ionic strength and subsequent stability of coarsely comminuted meat systems.

Conclusions

Three separate phenomena appear to affect the structural stability of the interaction between proteins and lipids in comminuted meat systems. These phenomena are as fol-

lows: 1) the presence of an interfacial protein membrane or film which surrounds lipid particles, 2) the gel strength of the batter matrix and 3) the physical characteristics of the fat (e.g. melting point and degree of fat tissue disruption). Each of these factors work together to produce a stable thermally processed meat product with little or no fat separation under the proper conditions. In coarse-cut sausage products, the interfacial protein film (IPF) is probably of little or no importance in determining the fat holding ability of the meat system. However, in finely comminuted sausage products the IPF appears to play a significant role in the stability of the sausage product. The gel strength of the batter matrix is probably the single most important factor affecting the overall stability and fat holding ability of most sausage products. The gel strength is primarily influenced by the ionic strength and pH of the comminuted meat system. The physical characteristics of the fat affect the stability of the comminuted meat system through variations in melting points and the apparent ability of fat cell membranes to inhibit coalescence of fat into large "pockets" during thermal processing.

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Discussion

Schmidt: I'd like to make two comments. First, the original work by Wilson, and more recently results from German workers, has shown that adding emulsifying agents to a meat batter has a negative effect on cook yield. The other comment I would make, (and neither one of these is a criticism), is that work which has been done with the scanning electronmicroscope, including our own work, is very subject to the creation of artifacts either by freezing, by gluderaldehyde fixation or by osmium textroxide fixation. Recently, when we had Dr. Hermansson visiting our lab, we tried a freezer fracture technique which uses no fixation. It involves freezing the meat batter, forming a carbon platinum template and then examining it under the electron microscope. With this system, we saw a more dense structure than you normally see with the scanning electronmicroscope and it was a much finer network than we've seen up to this time. There probably was a coating around the lipids. The lipids were probably recrystallized and in a few incidences we found tiny bubbles at the membrane interface surrounding the lipid. There is room for additional work and if we can develop techniques to prevent the formation of artifacts, we may add additional information. If you have any comments, I'm happy to hear them.

Jones: I'd like to ditto everything you said. There definitely is room for additional work. Artifacts are a problem, particularly in scanning electronmicroscopy. I would like to comment on one other thing, many of the cryofracturing techniques of fixed tissue, at least the ones that I've looked at, have problems with lipid stabilization in the matrix. I think it is not uncommon to get craters formed where the lipids were.

The first comment you made indicated that when emulsifiers were added to meat batters a decrease in stability was observed. Is that correct?

I think that is very likely. From the data and SEM pictures

that I presented, I would concur. The thicker the emulsifier becomes around a particular fat droplet, the lower the viscoelastic properties of the membrane. Therefore, it is less flexible during thermal processing.

Trout: I have two comments. First, about the work that you did in Nebraska on the effect of different chopping temperatures, on microstructure and cooking loss. I remember from reading your paper that you showed a lot of microstructural changes occurring with increased chopping temperature as well as increases in cooking loss. However, most of the cooking loss was water, not fat; a very small percentage was fat and the bulk of it (probably 80%) was water. So first of all, am I correct on that point?

Jones: You are correct in that the majority of the stability losses were moisture, which in fact is what one would expect when you look at a comminuted meat product. You are dealing with an immobilized water phase that is much larger than the lipid phase. Secondly, if you look at commercial products, moisture loss is a much larger problem than fat loss. We don't see many weiners fattening out any more.

Trout: Are these microstructural changes producing the changes in fat loss, as you implied? There are quite large microstructural changes but you don't see the same large changes in the amount of fat lost.

Jones: I think the majority of the changes, if we look at the pictures in total, are of the matrix. We see more dehydration of the matrix and loss of water as the time and temperature increase.

Trout: Another comment I have is that you mentioned the hydrophobic nature of the myosin molecule. You pointed out that most of the hydrophobic regions are in the head of the myosin molecule, but native myosin is in a helix and most of the hydrophobic regions are internal. So it may be that you have the major hydrophobic regions on the head region when it's undenatured but in the denatured or partially denatured state you may find as many hydrophobic groups in the tail region. Would you like to comment?

Jones: We're talking in this particular incidence about surface hydrophobicity. I guess there still remains a question as what is the exact state of myosin. In my mind, at least, myosin is at the protein oil or water oil interface. Myosin may be a unique protein – and let me say this for others' comment – and may not be denatured when it forms a film.

Acton: One question relates to your proposed model, or at least an idea for discussion: What are the roles of myosin and actomyosin? The majority of products in the country are probably made from postrigor meat, not prerigor meat, and actomyosin is the main form rather than myosin. Because both the gel structures of the two proteins are different as well as your model of the lipid-protein interface, they should not have the same.

Jones: I thought quite a bit about how actomyosin would interact in such a system. My initial thoughts were that if most of the meat proteins are in fact actomyosin, how could this be? But when one thinks about it further, I think we do see a fair degree of soluble myosin in any meat system and not actomyosin alone. That's not really answering your question, as actomyosin would not fit the model well; mainly because actin does not display the binding properties or the hydrophobic properties that myosin does and actin would be bound at the myosin head.