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Andre Gordon

Shai Barbut

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EFFECT OF CHEMICAL MODIFICATIONS ON THE MICROSTRUCTURE OF RAW MEAT BATTERS.

André Gordon* and Shai Barbut

Department of Food Science and Animal and Poultry Science,
University of Guelph, Guelph, Ontario, Canada N1G 2W1.

*Current Address: Product Development Division, Grace, Kennedy & Co., Ltd.
7-1/2 Retirement Road, Kingston 5, Jamaica, West Indies

Abstract

The microstructure and stability of raw meat batters treated with five chemical agents were investigated by electron and light microscopy. Six batters were made with NaCl (2.5%), five of which were treated with either hydrogen peroxide (H_2O_2), β-mercaptoethanol (β-ME), ethylenediamine- tetraacetic acid (EDTA), urea or Tween 80. The Tween 80 treatment produced a highly unstable raw batter with significant ($P > 0.05$) fat and water losses. None of the other treatments produced an unstable raw batter.

Microstructural examination revealed that, except for Tween 80, the chemically-treated raw batters were stable and showed some similarity to the control in their microstructure although they had distinctive differences between themselves. The H_2O_2 and β-ME batters differed in their microstructure. This appeared to reflect the differing levels of disulphide bonds present in these batters. The EDTA-treated batter had a very discontinuous matrix but contained stable fat globules surrounded by an interfacial protein film (IPF). The urea-treated batter showed a good fat globule distribution in a cohesive matrix, while Tween 80 resulted in a highly aggregated matrix and widespread fat globule destabilization. Very little of the fat present in the raw batters of this treatment was localized within an IPF.

The results suggest that hydrophobic interactions are important in raw batter gelation. The findings further indicate that non-protein emulsifiers may act mainly by blocking the adsorption of meat proteins to the fat surface to form an IPF and that IPF formation is the major mechanism by which fat is stabilized within meat batters.

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Direct inquiries to S. Barbut
Telephone number: 519 824 4120 x3669
Fax number: 519 836 9873

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Introduction

Finely comminuted meat products are made by chopping meat, fat, salt (usually 2.5% NaCl) and water together to form what is known as a meat batter (Whiting, 1988), which is subsequently cooked. These meat batters appear to be stabilized by a combination of the physical entrapment and binding of fat within a protein gel matrix and the formation of an interfacial protein film around the fat globules (Jones, 1984; Lee, 1985; Gordon and Barbut, 1990a,b). The successful manufacture of an acceptable meat batter is therefore influenced by the amount of the myofibrillar proteins (myosin, actomyosin and others) extracted into the aqueous phase during comminution (Schut, 1978; Regenstein, 1988; Gordon, 1990). However, the functionality of the proteins is, perhaps, even more important than the total amount of these proteins extracted.

The functionality of proteins is directly dependent on their conformation within a given environment. Even small changes in environmental conditions can lead to large differences in protein conformation as has been shown for myosin in the presence of different chloride salts (Cheung and Cooke, 1971; Szilagyi et al., 1975). Conformational changes are influenced by the native structure of the protein which determines the types of amino acid residues exposed to, and reacting with, the environment (Tanford, 1968). Hence, a combination of intrinsic and environmental factors predetermine the types of molecular interactions which are possible for each protein under a given set of conditions. Therefore, investigating the effects of controlled modification of these factors can help to clarify their roles in meat batter formation and stabilization.

The controlled modification of meat protein functionality can be achieved by using selected chemical agents (Whiting, 1988). These agents can target sulphydryl and disulphide bonds or modify protein-protein or protein-lipid interactions. They include chemicals such as hydrogen peroxide (H_2O_2), mercaptoethanol and urea (Means and Feeny, 1971; Whiting, 1987a). Some of our earlier studies have shown

Table 1. pH and Raw Batter Stability of Meat Batters Prepared with Different Chemical Agents.

TREATMENT	pH	Batter Stability[d] Raw Losses	
		Fat	Water
Control*	5.89[a]	0.00[b]	0.00[b]
(SD)	0.10	0.00	0.00
H_2O_2 (0.3%)	5.90[a]	0.00[b]	0.00[b]
(SD)	0.12	0.00	0.00
ß-Mercapto-ethanol (0.25%)	5.91[a]	0.06[b]	0.12[b]
(SD)	0.12	0.04	0.00
EDTA (0.2%)	5.29[b]	0.00[b]	0.12[b]
(SD)	0.01	0.00	0.01
Urea (4.5%)	5.92[a]	0.09[b]	0.00[b]
(SD)	0.09	0.07	0.00
Tween 80 (0.66%)	5.90[a]	0.82[a]	2.32[a]
(SD)	0.10	0.18	0.23

* - contains only 2.5% NaCl, meat,water and fat; all other also contain the chemical agents

[a-c] - numbers in the same column with different superscripts are significantly different ($p < 0.01$).

[d] - total losses/100g i.e., % losses.

that several interactions occur prior to cooking which affect the microstructure and texture of the cooked product (Gordon and Barbut, 1990b,c). It follows, therefore, that if specific interactions which occur in raw meat batters can be influenced by using selected chemical agents, then useful information as to the importance of these interactions to batter formation can be obtained. Consequently, the objective of this study was to investigate the role of specific protein-protein and protein-lipid interactions on raw batter stability and microstructure, especially as these relate to the properties of the final cooked product.

Materials and Methods

Batter Preparation

Six meat batters (750 g each) were prepared from chicken breast meat (65%),and pork back fat (25%) and water (10%). NaCl was added to the meat block at a level of 2.5%. One of the NaCl batters served as a control and the other five were treated with the following chemical agents: 0.3% H_2O_2, 0.25% β-mercaptoethanol (ME), 0.2% ethylenediamine tetraacetic acid (EDTA), 4.5% (0.75M) urea and 0.66% Tween 80. All of the percentages were based on the total weight of the meat block (meat + fat + water). All chemicals were obtained from Fisher Chemicals (Toronto, ON) except β-mercaptoethanol (Sigma Chemicals, St. Louis, MO). Usage level of each of these chemicals was pre-determined in preliminary experiments and was designed to produce the desired effect without affecting the pH of the batter. An exception to this was the EDTA which was used to destabilize the batters mainly by ionic effects (Whiting, 1987a) but also affected the pH (Table 1).

The lean breast meat source was prepared from chicken breasts from a composite of 30 birds (commercial broilers, 7 wks old). The meat was processed 24 hrs post slaughter, trimmed to remove excess connective tissue and all visible fat, preground through 9 mm and 3 mm plates, respectively, and stored at -18°C for up to 1 month prior to use. The chicken used had an average weight of 5 lb and were 7 weeks of age. The pork back fat (from Yorkshire breed, live weight 90 kg) was ground (in a semi-frozen state) through a 9 mm plate, refrozen and stored at -18°C. Proximate analysis (AOAC, 1980) was performed in duplicate on the meat (moisture 73.25%, fat 0.41%, protein 25.43%, ash 0.93%) and the pork back fat (moisture 28.43%, fat 67.4%, protein 4.05%, ash 0.12%).

The comminution protocol was designed to optimize protein extraction from the meat so as to maximize the effects of chemical modifications on the functionality of the proteins within the system. Optimal comminution times were determined in preliminary experiments. The preground, frozen meat was tempered at 4°C for approximately 10 hours and chopped with 2.5% NaCl at the high speed setting in a bowl chopper (Schneidemeister SMK 40, W. Germany) for 1 min. The pre-salted meat was stored (2°C for 18 hr) to allow sufficient time for pre-extraction of the salt soluble proteins. The meat was then chopped (30 s) at the high speed setting after which water was added and the mixture chopped for an additional 30 s. Each chemical agent was then added to its respective batter which was chopped for 30 s, after which the fat was added and the batter chopped for a further 3.5 min; the total chopping time after pre-incubation was 5 min. The final chopping temperature did not exceed 12°C in any of the batters. A table top vacuum tumbler (Model 10G, Lyco, Columbus, WI) was used to remove small air bubbles which were trapped during chopping (Gordon and Barbut, 1989).

Batter Stability

The stability of the raw batters was determined by centrifugation as previously described (Gordon and Barbut, 1989). Briefly, 34 g of each raw meat batter was placed in a polycarbonate centrifuge tube and subjected to 18,000 x G for 10 min in a Sorvall RC2B centrifuge (Sorvall Inc., Norwalk, CN). The liquid separated was taken as an index of raw batter stability. In this study,

water as well as liquid fat (oil), was recovered from some of the raw batters on centrifugation and the values are reported separately (Table 1). The pH of the raw batters was measured as described by Gordon and Barbut (1989).

Microscopy

The meat batters were examined (2 hrs after preparation) by cold stage scanning electron microscopy (cryo SEM) as described by Gordon and Barbut (1990c). Samples from the batters were processed for microscopy within 2 h after batter preparation. Specimens for cryo SEM were prepared using the Emscope SP2000A System (Emscope, Kent, England). Rapidly frozen, fractured specimens were etched at -80°C, sputter coated, transferred to the microscope stage (kept at -165°C) and examined by SEM (Hitachi S-570, Tokyo, Japan) at 10 kV. Samples for transmission electron microscopy (TEM) were prepared as described by Gordon and Barbut (1990b). Specimens for TEM were fixed in 2% glutaraldehyde/1% paraformaldehyde in 0.1 M PIPES buffer (pH 6.0) for 2 hr, rinsed, post-fixed with 1% OsO_4, rinsed and dehydrated through a graded series of ethanols. Dehydrated specimens were infiltrated with Epon 812 in capsules and cured by heating at 60°C for 36 hr. Sections (70nm) were cut using a Reichert OMU3 ultramicrotome (Reichert, Vienna, Austria), stained with uranyl acetate (10 min) and lead citrate (5 min) and viewed by TEM (JEOL JEM 100S) at 80 kV. For light microscopy (LM), the procedure of Gordon and Barbut (1990b) was used. Briefly, specimens prepared for TEM (sectioned on a Reichert 0MU3 microtome; Reichert, Vienna, Austria), were stained with a 1% solution of toluidine blue, rinsed and heat fixed onto a slide. The sections were viewed and photographed (x100 magnification) with a Zeiss microscope (Zeiss, Bonn, W. Germany) equipped with a yellow filter (wavelength of 567nm).

Experimental Design and Analysis

The experiment was repeated three times. For the microscopical evaluation, samples from two of the trials were examined. Ten fields per sample (at both the higher and lower magnifications) were examined. The experiment was based on a randomized complete block design. Data were analyzed by analysis of variance using the General Linear Models (GLM) procedure (SAS Institute Inc., Cary, N.C.). Tukey's test was used to detect significant differences between treatment means.

Results and Discussion

Effect on Batter Stability

The chemical agents used in this study were chosen because they affect the range of interactions which are thought to be important to protein functionality in meat batters (Whiting, 1988) and do so in ways which are relatively well characterized (Means and Feeny, 1971). In addition, none of the chemicals, except for the EDTA, significantly changed the pH of the batters ($p<0.01$) compared to the control at the levels used (Table 1). Whiting (1987a) has reported similar observations for these chemical agents at similar levels of usage. Hence, the effect of pH on any differences observed between the treatments (except for the EDTA) can be ignored. The fat and water losses from raw batters as affected by the different treatments are shown in Table 1. The batters treated with H_2O_2 and β-mercaptoethanol did not show any differences in raw stability when compared to the control, while the urea-treated batter showed good fat and water binding. EDTA did not significantly increase fat or water losses from the raw batters. Tween 80, however, resulted in extensive fat and water losses from raw batters.

Interpretation of the effects of the different chemical agents on meat batter properties requires an understanding of how they function within the system. Hydrogen peroxide (H_2O_2) is a powerful oxidizing agent and oxidizes mainly the free sulphydryl groups of exposed cysteine side chains (Stark, 1970; Tanford, 1970). β-Mercaptoethanol is known to effectively reduce exposed disulphide bonds in proteins and urea affects protein structure by disrupting non-covalent bonds [hydrogen bonds (H-bonds) and electrostatic interactions] and increasing the solubility of hydrophobic groups (Tanford, 1968; 1970; Stark, 1970; Shimada and Matsushida, 1981; Whiting, 1988). Polyoxyethylene sorbitan monooleate (Tween 80) is a very powerful non-ionic detergent and an effective emulsifier (Nawar, 1985; Goff and Jordan, 1989). EDTA reduces the pH of meat batters and participates in electrostatic and H-bonds, both of which increase protein cross-linking (Whiting, 1987a; 1988). Many of these agents will exert their effect in the raw batter during comminution and in the earlier stages of heat processing since once sufficient thermal energy is supplied to the system, the kinetics of the system changes to favour those types of interactions with a significant activation energy barrier, i.e., more endothermic in nature (Nakai, 1983; Cheftel et al., 1985).

Increasing or decreasing the number of disulphide bonds formed within the batter had no effect on raw batter stability. This suggests that disulphide bond formation within the gel structure set up in the raw batter (Gordon and Barbut, 1990b) is not important to the stability of the uncooked system and therefore plays no direct role in fat and water binding. Disulphide bonds are believed to be involved in the initiation of heat-induced gelation (Ishioroshi et al., 1981); however, there is strong evidence which disputes this (Hamm and Deatherage, 1960; Hoffman and Hamm, 1978; Asghar et al., 1985). Tween 80 and other non-protein emulsifiers have consistently been shown to cause meat batter instability, albeit in cooked meat batters (Meyer et al., 1964; Whiting, 1987a,b). The high fat and water losses which accompanied raw batter instability were excessive (Table 1) and indicate that Tween 80 may act mainly by directly interfering with fat-protein interactions. Fat and

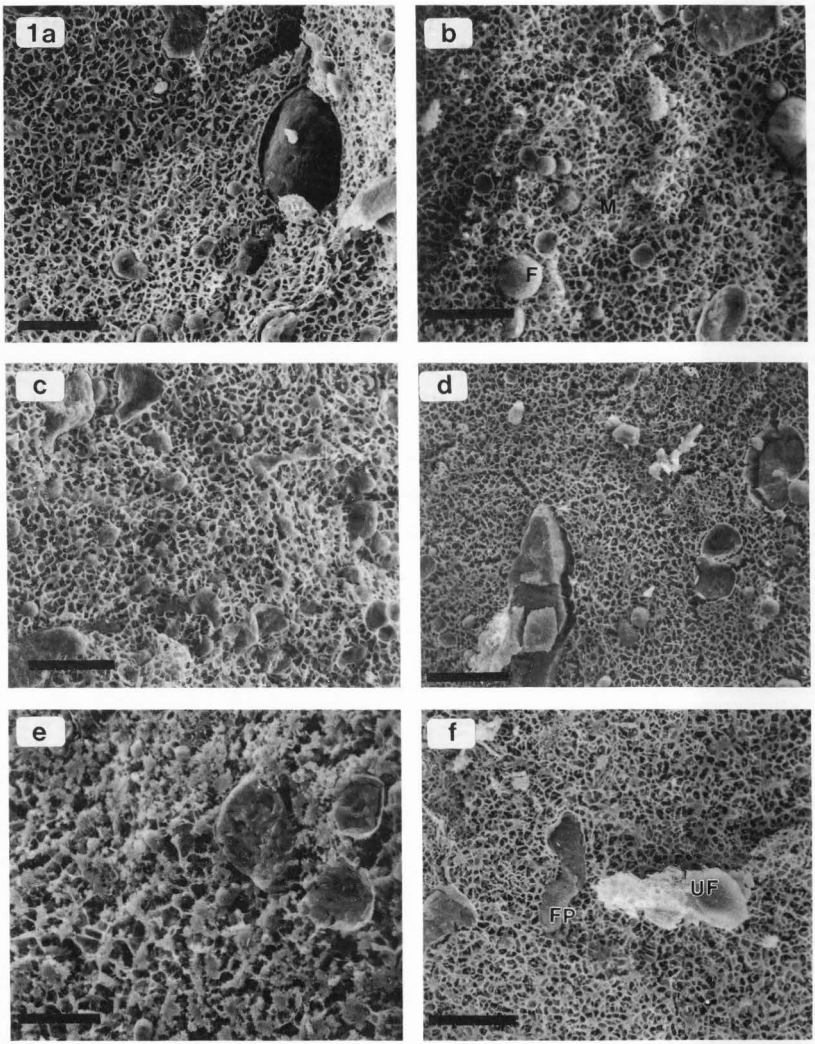

Figure 1. Scanning electron micrograph (SEM) of representative fields from raw meat batters treated with chemical agents **a)** 2.5% NaCl, **b)** H_2O_2, **c)** β-mercaptoethanol, **d)** EDTA, **e)** Urea, **f)** Tween 80; FP - fat pool; UF - unstable fat. Bar = 20 μm.

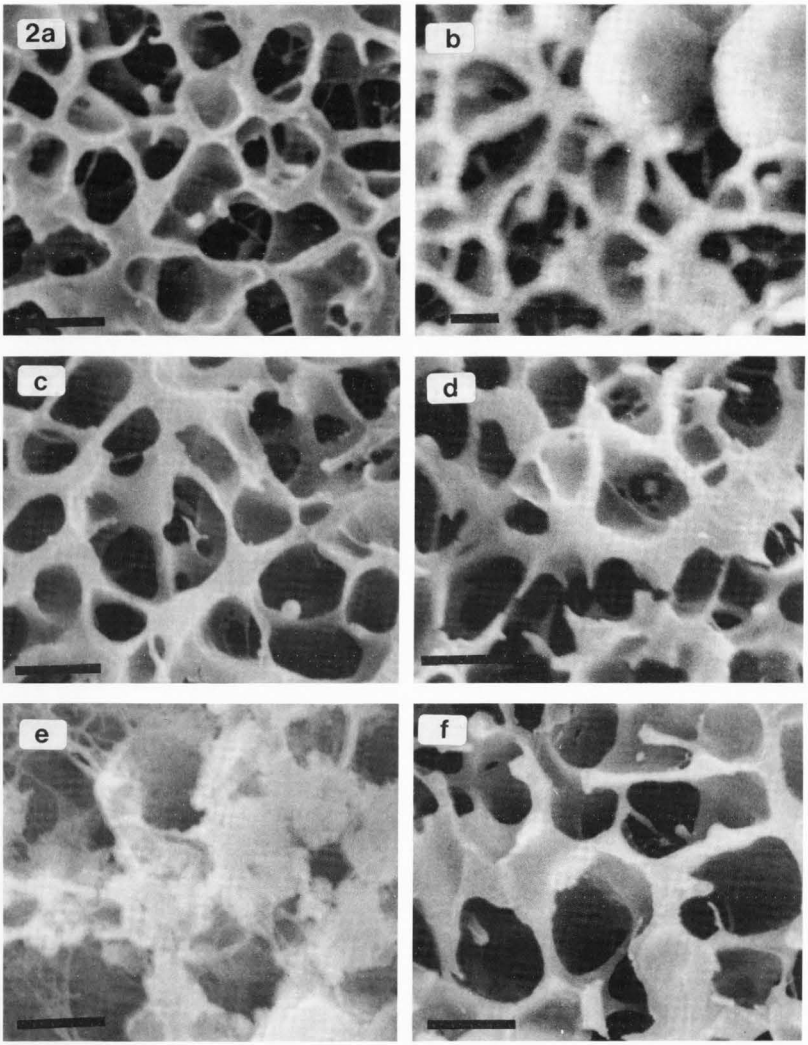

Figure 2. High magnification cryo SEM of matrices of raw meat batters which have been treated with chemical agents **a)** 2.5% NaCl, **b)** H_2O_2, **c)** β-mercaptoethanol, **d)** EDTA, **e)** Urea, **f)** Tween 80. Bar = 2 μm.

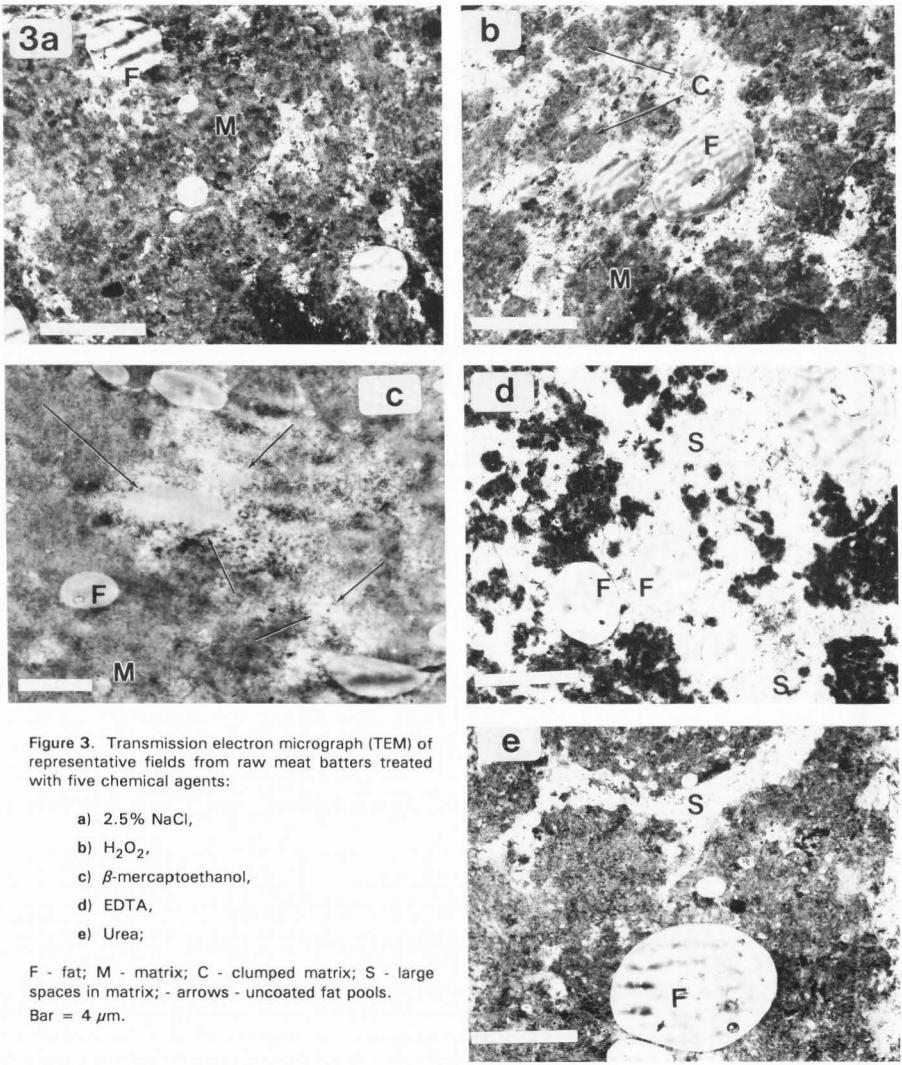

Figure 3. Transmission electron micrograph (TEM) of representative fields from raw meat batters treated with five chemical agents:

- **a)** 2.5% NaCl,
- **b)** H_2O_2,
- **c)** β-mercaptoethanol,
- **d)** EDTA,
- **e)** Urea;

F - fat; M - matrix; C - clumped matrix; S - large spaces in matrix; - arrows - uncoated fat pools.
Bar = 4 μm.

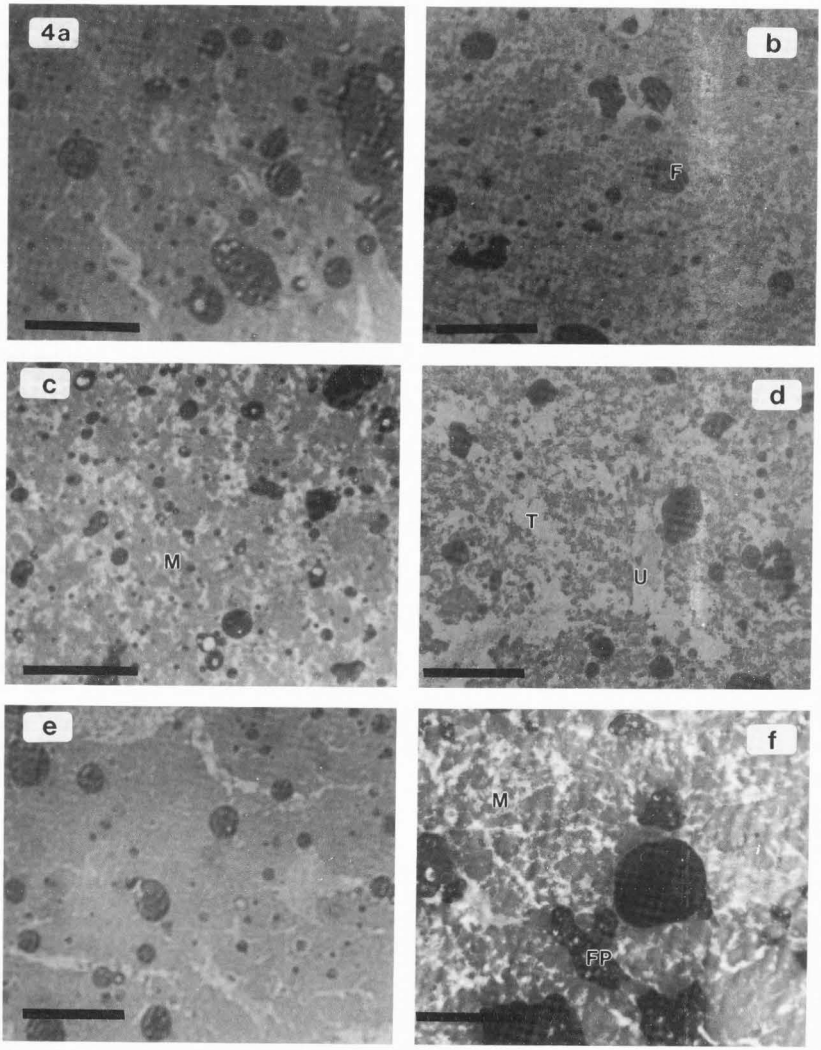

Figure 4. Light micrographs of representative fields from raw meat batters treated with five chemical agents **a)** 2.5% NaCl, **b)** H_2O_2, **c)** β-mercaptoethanol, **d)** EDTA, **e)** Urea, **f)** Tween 80; F - fat; FP - fat pools; T - tunnel; U - unstable fat. Bar = 50 μm.

water losses from unstable batters have been shown to be closely related (Schmidt, 1984; Gordon and Barbut, 1989).

The EDTA appeared to behave in a manner similar to that reported for $CaCl_2$ which has also been shown to produce stable raw batters (Gordon and Barbut, 1989; 1990b; Gordon, 1990). This may indicate that EDTA also facilitated extensive protein-protein aggregation in the raw batter which, by physical entrapment, prevented water and fat separation when the batters were subjected to a centrifugal force. The protein-protein interactions in this treatment would mainly be the result of increased electrostatic and H-bond formation (Whiting, 1988). Urea disrupts hydrogen and electrostatic bonds while increasing the availability of hydrophobic groups for binding. Therefore, the stability of the urea-treated batter (Table 1) may indicate that hydrophobic interactions are important in stabilizing the raw batter.

Effect on Raw Batter Microstructure

The microstructure of the raw batters of each treatment (Figs. 1, 2 and 3) showed some distinctive differences which tended to correspond to the differences in raw batter stability noted earlier (Table 1). The 2.5% NaCl (control) treatment showed a cohesive, well structured matrix with a highly interconnected network of strands in which well stabilized fat globules were evenly distributed when examined by cryo SEM (Figs. 1a and 2a). Transmission electron microscopy (TEM) and light microscopy (LM) also showed the fine, lacy structure of the protein matrix and indicated that the great majority of the fat globules were round and coated with protein (Figs. 3a and 4a). This structure is typical of 2.5% NaCl raw meat batters (Swasdee et al., 1982; Gordon and Barbut, 1990a).

The H_2O_2-treated batter, when examined by SEM, displayed a similar microstructure to that of the control and had a protein matrix with similar pore sizes but slightly smaller strands (Figs. 1b and 2b). However, TEM revealed that although this batter had a good dispersion of stable fat particles, the matrix itself had a different structure from that of the control (Fig. 3b). The matrix was not extensively disrupted (Fig. 4b) but tended to be composed of clumps joined to each other (Fig. 3b) rather than the even, well linked structure which was observed in the control (Fig. 3a). In some cases, the clumps were approximately spherical in shape (see "C") and were only bound to the rest of the matrix at a few points. This resulted in a slightly higher incidence of open spaces than is normal in raw batters (Fig. 4a vs. 4b). The structure of this raw batter matrix may have been due to the formation of inter- (and perhaps intra-) molecular bonds between the nearest available free sulphydryl (SH) groups due to the oxidizing action of H_2O_2 on the cysteine side chain (Means and Feeny, 1971). This would result in a less well interlinked matrix, since proteins in close proximity to each other would be cross-linked by disulphide bridges but would not be bound to those further away. In the more extreme cases, this group of proteins may almost exclusively bind between themselves, exhausting most of the free SH groups and thereby reducing the linkage of these "clumps" to the rest of the matrix (Fig. 3b). However, the general structure of the batter was sufficiently cohesive to provide good water and fat stabilization (see Table 1).

β-Mercaptoethanol resulted in the formation of a diffuse, even matrix which appeared to have slightly thicker strands and was therefore less dense than the control batter (Figs. 1c-4c). The fat particles were relatively evenly distributed throughout the matrix. Some fat instability was evident (Fig. 3c), but the unstable fat formed small pools which were not very well interconnected and were suitably localized by the diffuse protein matrix (Fig. 3c). It was noted that these pools of unstable fat appeared to be lacking a protein coat (see arrows, Fig. 3c). However, most of the fat within this treatment was stable and appeared to be surrounded by an IPF. The formation of a diffuse, stable raw batter under conditions which discourage disulphide bond formation indicate that the formation of disulphide bonds is not a prerequisite for raw batter gelation and suggests that other types of interactions (possibly hydrophobic interactions) are the main driving forces behind structure formation in raw batters. It should be noted, however, that the higher incidence of air spaces as shown by LM (Fig. 4) indicate that disulphide formation in the raw state does aid in forming a continuous, cohesive raw batter gel matrix.

The EDTA batter displayed a very dense protein matrix when examined at low magnification by cryo SEM (Fig. 1d). Higher magnification (Fig. 2d) showed that the matrix was discontinuous with poor linkage between its protein strands. This was the result of a very highly aggregated structure which led to large open spaces within the matrix (Figs. 3d and 4d) and should have resulted in high water losses from the raw batter. Although some of the fat particles within this treatment were irregular in shape (Figs. 1d and 4d), most of them were round and almost all of those examined were stabilized by an easily discernable IPF (Fig. 3d), even when they were located in some of the large spaces within the matrix. These observations tend to contradict the physical entrapment theory which says that fat is stabilized in meat batters mainly by physical entrapment within the protein matrix (Lee, 1985). In this EDTA batter, the protein matrix was highly discontinuous and yet no significant fat or even water loss was observed (Table 1). This suggests that the stabilization of fat within the EDTA raw batter was due mainly (if not solely) to the existence of a stable IPF around the globules (Fig. 3d). The fact that little water loss from the raw batter occurred (Table 1) may possibly be related to the cooperativity often observed between fat and water loss (Schmidt, 1984; Gordon and Barbut, 1989). Alternatively, any water expressed during centrifugation may have been reabsorbed by the protein matrix after centrifugation (especially if the quantity was small) and therefore was

not measurable by the method used. The fat, on the other hand, would not be easily reabsorbed by the matrix because of its greater viscosity and would therefore be more easily recovered from the raw batters if it were released during centrifugation.

Urea produced a raw batter with a microstructure unlike that of any of the other treatments, including the control (Figs. 1e, 2e and 3e). The protein matrix appeared to be fairly rough and tended to have large, fairly well interconnected tunnels running throughout it (Figs. 1e and 2e). This was also observed in transverse sections examined by TEM and LM (Figs. 3e and 4e), but these also showed that the majority of the protein matrix had a very fine, lacy structure. This may have been due to the fine strands which were evident at higher magnifications (Fig. 2e). Generally, the raw batter matrix tended to consist of aggregated areas with tunnels connected by fine, lacy protein strands. Although the tunnels appeared to be large when viewed by TEM, LM showed that they were actually relatively small and did not adversely affect the continuity of the matrix as compared to the control (Fig. 4).

Treatment with urea disrupts most of the non-covalent bonds within proteins systems (Stark, 1970). Furthermore, besides the peptide bonds which form the backbone of polypeptides, disulphide bonds are the only other major covalent bonds in proteins (Tanford, 1968; Means and Feeny, 1971). Consequently, the structure of the urea-treated raw batter probably reflected the effects structure formation as a result of "normal" levels of disulphide bond formation in the absence of a significant contribution to the structure from hydrophobic interactions. This structure was totally different from that formed in the oxidized batter (Figs. 1b-4b) where not only was excessive disulphide bond formation promoted, but contribution to the structure from hydrophobic interactions would also have occurred. However, the contribution of some (relatively few) hydrophobic "bonds" to the structure of the urea treatment cannot entirely be ruled out. This is because the high availability of hydrophobic groups in this batter could possibly have resulted in some amount of spontaneous, free energy-driven hydrophobic interactions (Nakai, 1983; Wicker et al., 1986). The more even cross-sectional appearance of the urea batter as compared to the H_2O_2 batter (Figs. 3b vs. 3e), may be a result of this scenario. It should be noted however, that the much greater similarity in structure to the control shown by LM for the urea batter as compared to the H_2O_2-treated batter (Fig. 4.) does not correspond well with the results of SEM and TEM (Figs. 1-3). This underscores the need for caution when using LM alone to study the microstructure of meat batter systems and justifies the use of several different types of microscopy in such studies. Nevertheless, the overall findings for the urea-treated raw batter tend to indicate that disulphide bond formation may play a role in structure formation in raw batters.

The preparation of raw batters using Tween 80 led to a microstructure which corresponded to the high levels of water and fat losses observed from this batter (Table 1). The protein matrix was very dense and diffuse in some areas and exhibited large discontinuities in other areas (Figs. 1f, 2f and 4f). The discontinuities were due to the formation of well connected channels throughout the matrix (Fig. 4f) which provided an exit route for water and fat. The fat within this treatment was mainly unstable and generally existed as fat pools (FP, Fig. 1f and 4f) or uncoated fat (UF, Fig. 1f). Some globules were present but few of these had an intact IPF when examined by TEM (not shown). Those which did have some protein coat on their circumference were only partially coated; the protein coat was very unevenly distributed around the globule and formed thick clumps in some areas but was absent in others.

Studies involving the use of non-protein emulsifiers, particularly Tween 80, are consistent in their findings that these emulsifiers cause gross batter instability (Meyer et al., 1964; Whiting, 1987a,b). This has been widely used as proof that fat emulsification by meat proteins is not a major contributor to batter stability and that the gelation aspects of the proteins are much more important (Lee, 1985; Whiting, 1987a; Regenstein, 1988). However, investigations into the role of emulsifiers in dairy processing have shown that those with high hydrophilic-lipophilic balance (HLB) values, such as Tween 80, destabilize protein-stabilized emulsions (Lin and Leeder, 1974; Goff and Jordan, 1989). The emulsifiers were shown to be preferentially adsorbed by the fat globules due to their higher HLB values than the milk proteins and therefore reduced protein-lipid interactions by interfering with the adsorption of protein molecules to the fat globule surfaces. This reduced the amount of protein adsorbed per unit area (Oortwijin and Walstra, 1982; Goff et al., 1987) and resulted in fat globule breakdown.

Tween 80 is the emulsifier most commonly used in the dairy industry to partially destabilize emulsions (Keeny, 1982) because it is the most potent. It is therefore very likely that, in raw meat batters, Tween 80 acts in a manner similar to its mode of action in dairy products and reduces the adsorption of the myofibrillar proteins by the fat globules. Furthermore, a myofibrillar protein-based IPF is required for binding the fat globules to the protein matrix (Theno and Schmidt, 1978; Gordon and Barbut, 1990a,b,c) which further immobilizes the fat. If the emulsifier at the water/fat interface is non-protein in nature, this binding will not take place. It can therefore be concluded that "classical" emulsifiers such as Tween 80 act by a different mechanism than meat proteins within protein-stabilized emulsions. Their main mode of action seems to be by interfering with protein-lipid binding and consequently destabilizing the fat. In addition, this will increase the number of protein ligands available for protein-protein interactions, thereby causing protein matrix aggregation which can result in excessive water loss as was observed for the raw batters treated

with Tween 80 (Table 1). Hence, while the gel forming ability of the meat proteins is central to the development of desirable texture (Montejano et al., 1984; Patana-Anake and Foegeding, 1985), the interfacial film plays an important role in fat stabilization (Jones, 1984; Gordon and Barbut, 1989; Koolmees et al., 1989) and its contribution to the system as a whole cannot be discounted on the basis of the inability of non-protein emulsifiers to stabilize meat batters.

Summary

The effects of different types of protein-protein and protein-lipid interaction on the stability and microstructure of raw meat batters were examined by chemical modification of the meat proteins. The use of 0.3% H_2O_2 and 0.25% β-mercaptoethanol indicated that the formation of disulphide bonds occurred during raw batter preparation. The formation of a high number of disulphide bonds in the control batter during comminution was not required for raw batter gelation. However, they appeared to play an important role in influencing the development of the structure formed in raw batters.

Hydrophobic interactions appeared to be the main driving force behind the phenomenon of raw batter gelation (structure formation). The role of electrostatic and H-bonds in stabilizing raw meat batters appears to be somewhat restricted. However, the results in this respect were unequivocal and, therefore, a strong inference can not be made. Non-protein emulsifiers appeared to destabilize meat batters by displacing the myofibrillar proteins from the fat/water interface as a result of their preferential absorption because of their higher HLB values. This causes instability because the lower mechanical strength of the emulsifier-stabilized interface is unable to successfully localize the fat. This is exacerbated by a reduction in fat immobilization by the mechanism of IPF-protein matrix binding since the non-protein emulsifiers block the adsorption of the meat proteins to the interface to form an IPF between the fat and the matrix. The myofibrillar proteins are required for IPF-protein matrix binding, a function which the non-protein emulsifiers are unable to fulfill.

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* NOTE: Gordon CLA and Gordon A are the same person; CLA had to be used as a requirement of the University of Guelph for thesis preparation.

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Discussion with Reviewers

R. G. Cassens: The authors present conclusions about disulphide bond formation, but direct chemical measurements were not made. Is this justifiable?

R. C. Whiting: Do you have any estimate of the stoichiometry of the hydrogen peroxide and mercaptoethanol and the sulfhydrl groups? Is the added mercaptoethanol sufficient to reduce all, most or some of the disulphide bonds?

Authors: No direct quantitative measurements of the number of disulphide or sulphydryl bonds present within these meat batter systems were made. However, in a companion study (Gordon, 1990), the effect of these chemical agents on the presence and level of exposure of sulphydryl and disulphide groups (as compared to a control meat batter) was evaluated with the use of both ultraviolet spectrophotometry and spectrofluorimetry. This study showed conclusively that the incorporation of hydrogen peroxide into the meat batter reduced the availability of free sulphydryls while increasing the level of disulphides. Mercaptoethanol addition had the opposite effect and was shown to reduce enough of the disulphides present to result in changes in the conformation of the protein in this batter. This was sufficient to affect their functionality in the meat batter to an extent where differences from the control were discernible (Figs. 1-4). This was entirely in keeping with the original objectives and therefore the conclusions presented are justifiable.

F.W. Comer: Mono- and di-glyceride emulsifiers have been found to have a negative effect upon batter stability. Although the results reported in this paper do not show evidence of "gross batter instability", it is clear that some agglomeration of fat globules has occurred. What causes this to occur? Is it because there is insufficient water and/or inadequate shear to produce an emulsion? The fat agglomerates are not so large that the batter is unstable, i.e. they are largely contained within the protein matrix. What evidence do you have that an interfacial protein film must be bound to the protein matrix to stabilize the fat globules? Have you tested this hypothesis by evaluating emulsifiers with low hydrophilic-lipophilic balance values?

G.R. Schmidt: Tween 80 gave the only significant raw losses. This, the author's note, disagrees with the role assigned to emulsifying capacity in batter stability, although usually discussed for cooked batters. This work does not appear to enlighten either side of this argument.

Authors: In this study, it was shown that non-protein emulsifiers, such as Tween 80 act by a different mechanism from protein-based emulsifiers in meat batters. The Tween 80 caused fat globules breakdown, coalescence, protein matrix aggregation and, as a consequence of these, batter instability. This is in sharp contrast to caseinate and other protein-based emulsifiers

which aid in meat batter stabilization (Schut, 1978; Asghar et al., 1985). It has been shown that the interfacial film functions as an intermediary for the binding of the fat globule to the protein matrix (Theno and Schmidt, 1978; Gordon and Barbut, 1990a). This acts to physically restrict the movement of most protein-coated fat globules, thereby preventing coalescence (Gordon and Barbut, 1990b,c; 1991). Emulsifiers with high HLB values such as Tween 80 are preferentially adsorbed at the interface and reduce protein-lipid interaction by displacing much of the protein already present at the interface or interfering with further adsorption of protein molecules (Goff and Jordan, 1989; Courthaudon et al., 1991; Heertje, personal communication). Consequently, an interfacial film with lower viscoelastic strength and reduced ability to participate in IPF-protein matrix binding is formed, resulting in batter failure.

We do not believe that shear rate or water content influence the effect of Tween 80 in meat batters. In response to your final question, we have not tested the hypothesis as suggested but intend to do so on future studies. However, it is interesting to note that a follow-up study (Gordon and Barbut, submitted) provides evidence of the effect of Tween 80 in disrupting the proteinaceous interfacial protein film.

F.W. Comer: My overall impression of the microscopy results, taking into account some inconsistencies noted by the authors, is that the treatments had relatively minor effects upon the protein matrices. Did you obtain any measurements of the extent of chemical modification produced by the treatments. For example, how many disulphide bonds were created, or broken - how many are there to begin with? What percentage of the hydrogen bonds are disrupted - how many are there? What are the electrostatic interactions - and how are they altered? The authors are on the right track in identifying molecular structure as a determinant of microstructure, and there is a need to obtain molecular structural information to begin to understand the effects upon food structure.

Authors: Some treatment did have a significant effect on the protein matrix. For example, both Tween 80 and EDTA, when added to the batters at relatively low levels (0.66% and 0.2%, respectively) resulted in protein matrix aggregation (Figs. 3d and 4d,f). However, only in the Tween 80 treatment was significant batter instability observed (Table 1, Fig.4). These physical and microstructural pieces of evidence were used to postulate that the protein matrix aggregation was not the major prerequisite for meat batter failure.

This study sought to evaluate the importance of specific protein-protein interactions as well as protein-lipid interactions on raw meat batter stability, this was done by evaluating differences between treated meat batters and a control (2.5% NaCl only) batter. Previous studies (Whiting, 1987a; Gordon, 1990) have established that, at the levels used, the chemical agents affect protein functionality sufficiently to create a measurable difference in batter characteristics. These differences were the basis for the conclusions drawn from this study. No direct measurements were made (see answer to the first question regarding measurements) as this would have been beyond the scope of the present study. Perhaps future work can address the need for this kind of quantitative information.

RC. Whiting: What temperature is the batter when centrifuged in the stability test? If ambient temperature, the fat would be a solid and the centrifugal force would be forcing the solid particles through the protein matrix. Would protein-lipid interactions and interfacial protein films play a major role in stabilizing the batter? The separated fat is described as not easily reabsorbed by the matrix because of its greater viscosity. If the fat was liquid I would attribute not being reabsorbed to hydrophobicity and surface tension instead of viscosity. If solid, the fat would not be expected to be reabsorbed.

Authors: The temperature of the batters during centrifugation was 20 $\pm$ 1.5°C and therefore much of the fat would be solid. However, some of the pork fat would also have become liquid at these temperatures (Townsend et el., 1968). In addition, the process of communition produces some liquification of fat and creates a thin layer of oil on the surface of solid fat particles. This is the layer in which the protein lipid interactions take place and results in IPF formation (Jones, 1984). Consequently, protein-lipid interactions and IPF formation would be important in localizing and stabilizing the fat (whether solid or completely liquid), as we have indicated in previous studies (Gordon and Barbut, 1990 a,b; 1991). The fat separated during centrifugation is mainly liquid (Gordon and Barbut, 1989). However, some crystalline (solid) fat is sometimes also recovered. Your suggestion that hydrophobicity and surface tension act to prevent reabsorption of this fat is possibly correct. However, we also feel that the greater viscosity of the oil (compared to water) also acts to prevent fat reabsorption when the protein matrix re-expands after centrifugation.

R. C. Whiting: EDTA is a chelator. Would you rationalized its effects on the batter by that property? EDTA is stated to facilitate extensive protein-protein aggregation and form a stable IPF. Is this the same mechanism or are these statements conflicting?

Authors: The statements are not conflicting. It is our belief that EDTA is functioning as a multivalent chelator in meat batters, participating in forming extensive cross-bridges between proteins (via bound metal ions), thus resulting in extensive protein-protein aggregation and matrix disruption (Figs. 2d and 3d). However, EDTA does not cause IPF breakdown and consequently, most of the fat globules in this treatment remain stable, (i.e.

enclosed within an interfacial film; Figs. 3d and 4d). This may be because EDTA acts mainly on the insoluble proteins within the matrix while not affecting the more soluble ones which mainly form the IPF.

R. G. Cassens: Most emulsion products are sold as heat-processed items. Do the present results on raw batters relate directly to this normal situation where the items are heat-processed.
G. R. Schmidt: All of this work discusses raw batters. What happens when these samples are heated? Is raw batter stability ever a real practical problem?
Authors: We focused mainly on the raw batter in this study because it is during batter preparation that problems most often occur (i.e., over chopping, high end-point chopping temperature, etc.). Poor processing technique at this stage can lead to batter failure, poor texture and economic losses due to rejection of the final cooked product. This is the stage at which the gel structure which determines the texture of the product is set up (Gordon and Barbut, 1990a,c). This is also the stage at which a stable interfacial film is formed (Borchert et al.,1967; Swasdee et al., 1982). In addition, previous work has shown that batters which are unstable in the raw state (eg. $MgCl_2$ batters) will fail upon cooking (Gordon and Barbut, 1989; 1990c). Hence, raw batter stability is an indicator of future problems with the finished product and, if the mechanisms involved in raw batter formation, can be better understood, our control over the whole process of making better-type meat products can be improved.

R.C. Whiting: Do you view the IPF as a true emulsifying agent or a continuation of the gel matrix around the fat particle?
Authors: This is a very important question because it addresses the fundamental role of the IPF in meat batter stabilization. Much of our recent work supports the importance of "fat emulsification" in batter stabilization (Gordon and Barbut, 1989, 1990 b,c,d). By this the localization of fat (within an interfacial film) serves to retard the tendency for fat to coalesce. However, a consistent feature observed is the continuity found between the IPF and the protein matrix (Gordon and Barbut, 1990 a,b,d). As a result, it is our opinion that the interfacial film not only serves to localize the fat, but is an integral part of the gel matrix. This idea has been previously suggested by Hermansson (1986) and was also supported by Katsaras and Peetz (1989).

F.W. Comer: Based on batter stability and microscopy results, all treatments produced stable protein matrices, i.e. there is no evidence of severe fat channels. However, several of the treatments, particularly urea, modified the matrix structure. Changes to the matrix often result in textural effects as well as stability effects. For example, the SEM of batters containing non-meat protein fillers is not unlike that of the micrographs 2e and 1e, and these fillers increase batter stability but the texture is somewhat drier and less firm than all-meat controls. Have you determined the textural properties of the batters? What were the differences?
Authors: The rheological properties of the raw meat batters in this study were not investigated. However, in a follow-up study, the textural properties of the chemically modified cooked batters were evaluated using texture profile analysis and related to microstructural differences (Gordon and Barbut, submitted). There were several textural differences between the treatments. The textural properties were related to the microstructure of the cooked batters and could also be related to the raw batter microstructure discussed here. Among the major differences were the findings that both EDTA and Tween 80 resulted in poor texture but both differed in the effects on the individual components of the texture profile. The level of free sulphydryls or disulfides present was also found to affect product hardness and cohesiveness.

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