Effect of Chemical Modifications on the Stability, Texture and Microstructure of Cooked Meat Batters

Andre Gordon

Shai Barbut
EFFECT OF CHEMICAL MODIFICATIONS ON THE STABILITY, TEXTURE AND MICROSTRUCTURE OF COOKED MEAT BATTERS

André Gordon¹* and Shai Barbut²

Dept. of Food Science¹ and Animal & Poultry Science²
University of Guelph, Guelph, Ontario, Canada N1G 2W1

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

Abstract

The effects of five chemical agents on the stability, texture and microstructure of cooked comminuted meat batters were studied. β-mercaptoethanol (β-ME), hydrogen peroxide (H₂O₂), ethylenediamine tetraacetic acid (EDTA), urea, and polyoxyethylene sorbitan monooleate (Tween 80) were used. The reduction of disulphide bonds by β-ME or the oxidation of free sulphhydrils by H₂O₂ prior to cooking did not affect liquid and fat losses during cooking as compared to the control (2.5% NaCl). However, texture profile analysis (TPA) showed that treatment with H₂O₂ resulted in a harder, more cohesive product than the control and β-ME treatments because a more fine-stranded, interconnected protein matrix was formed.

Treatment of batters with EDTA caused protein matrix aggregation which was accompanied by high liquid losses during cooking (p < 0.05) and resulted in a product with an unacceptable texture. However, EDTA did not cause fat destabilization and most of the fat globules remained surrounded by an intact interfacial protein film (IPF). Urea, which disrupts hydrogen and electrostatic bonds and solubilizes hydrophobic groups, produced extremely stable meat batters with a very cohesive texture. The micrographs of the urea treatment showed that the matrix was formed by a uniform, interconnected, fine-stranded protein network. Batters treated with Tween 80 showed large fat and moisture losses from the cooked product. This was responsible for the soft texture of the batter and corresponded to the very dense aggregates and the lack of an interfacial protein film around the fat globules observed in the micrographs.

Key Words: Chemical modification, microstructure, meat proteins, interfacial protein film, texture, disulphides, hydrophobic interactions, emulsifiers.

Introduction

In order to produce an acceptable meat batter, myofibrillar proteins should be exposed to conditions which favour the specific protein-protein and protein-lipid interactions that are essential for good fat binding and protein matrix formation. The commercial use of 2.5% NaCl in most finely comminuted products provides an environment which facilitates these desirable interactions (Barbut and Findlay, 1989). Hence, a meat batter made with 2.5% NaCl is the system which is usually studied when an understanding of meat batter formation and stabilization is desired. In order to clarify some of the mechanisms involved in structure/function relationships, several chemical agents have been used to alter protein structure by targeting specific residues. These include β-mercaptoethanol for the reduction of disulphide bonds (Stark, 1970), hydrogen peroxide (H₂O₂) for the oxidation of free sulphhydrils (Means and Feeny, 1971), chelating agents and urea which modify protein-protein binding (Means and Feeny, 1971; Nakai, 1983; Whiting, 1988) and polyoxyethylene sorbitan monooleate (Tween 80) which modifies protein-lipid interactions (Meyer et al., 1964; Whiting, 1987a; Goff and Jordan, 1989). Information on the effects of specific residues or interactions is gained by examining the functionality of the protein(s) before and after modification (Means and Feeny, 1971).

There is limited published research describing the chemical modification of meat proteins. Furthermore, most of the existing data was derived from work with pure isolated proteins or subfragments (Cheung, 1969; Ishioroshi et al., 1981; Samejima et al., 1981; Jiang et al., 1988). Properly understanding the mechanisms of batter stabilization requires the use of systems which relate more closely to those produced commercially. There are few studies which have attempted to do this (Whiting, 1987a) and fewer still which have examined the effects of chemical modification on the structure/function relationships of commercial-type meat batters (Gordon and Barbut, 1991). Consequently, a study which examines the role of specific protein-protein and
Table 1: pH and cooked batter stability and texture profile analysis of meat batters prepared with different chemical agents.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Batter Stability</th>
<th>Texture Profile Analysis</th>
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<tr>
<td></td>
<td></td>
<td>Cooked Losses</td>
<td>Hardness (N)</td>
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<td></td>
<td></td>
<td>Fat %</td>
<td>Water %</td>
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<tr>
<td>NaCl (2.5%)</td>
<td>5.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt; (0.3%)</td>
<td>5.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>β-Mercaptoethanol (0.25%)</td>
<td>5.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.94&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>EDTA (0.2%)</td>
<td>5.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea (4.5%)</td>
<td>5.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Tween 80 (0.66%)</td>
<td>5.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-d</sup>: Numbers in the same column with different superscripts are significantly different (p < 0.05).

<sup>*</sup>: No significant fracturability (brittleness) observed at the deformation applied (60% compression).

Materials and Methods

Batter Preparation

Six meat batters (750 g each) were prepared by comminuting chicken breast meat (65%) with pork back fat (25%), 10% added water and NaCl (2.5% of the meat block + water). One of the batters (containing only NaCl) served as a control and the other five were each treated with one of the following chemical agents: 0.3% H<sub>2</sub>O<sub>2</sub>, 0.25% β-mercaptopethanol (β-ME), 0.2% ethylenediaminetetraacetic acid (EDTA), 4.5% (0.75 M) urea and 0.66% Tween 80. All of these percentages were based on the total weight of the meat block (+ water). β-mercaptopethanol was obtained from Sigma Chemicals, St. Louis, MO; all other chemicals were obtained from Fisher Chemicals (Toronto, ON). The level of use of each chemical agent was designed to produce a detectable effect without affecting the pH of the batter and was determined as previously described (Gordon and Barbut, 1991). EDTA was an exception and was used to destabilize the batters by a combination of ionic and pH effects.

Lean breast meat was obtained from at least 30 birds, trimmed, preground and stored at -18 °C for up to 1 month prior to use. Semi-frozen pork back fat was also pre-ground, refrozen and stored at -18 °C. Proximate analysis (AOAC, 1980) was performed in duplicate on the meat and the pork back fat. The meat had a moisture content of 72.20%, fat of 1.41%, protein of 25.43%, and ash of 0.93% while the back fat had a composition as follows: moisture - 28.43%, fat - 67.4%, protein - 4.05%, ash - 0.12%.

The comminution protocol was designed to maximize the effects of the chemical modification on the proteins of the meat batter system (Gordon and Barbut, 1991). The preground, frozen meat was tempered for 18 hours at 4 °C to an internal temperature of 2 °C,
chopped with 2.5% NaCl for 1 minute at the high speed setting in a bowl chopper (Schneidemeister SMK 40, W. Germany) and stored at 2 °C for 18 hours to allow sufficient time for pre-extraction of some of the proteins. The meat was then chopped at the high speed setting for 30 seconds, water was added, the mixture chopped for a further 30 seconds, then the chemical agent was added and each batch was chopped for another 30 seconds. After this, the fat was added and the batter chopped for 3.5 minutes to give a total chopping time after pre-incubation of 5 minutes. The post-chopping temperature did not exceed 12 °C in any of the batters. A table top vacuum tumbler (Lyco, Madison, WI) was used to remove small air bubbles which were trapped during chopping (Gordon and Barbut, 1989).

**Batter Stability and Texture Profile Analysis**

The stability of the cooked batters was determined by cook out losses as previously described (Gordon and Barbut, 1989). Briefly, each meat batter (34 g) was weighed into a 50 ml test tube, centrifuged at low speed (600 x g) to remove trapped air and cooked to an internal temperature of 69 °C in 1.5 hours in a water bath. The amounts of fat and liquid released from each batter immediately after cooking was taken as an indication of batter stability and are reported in Table 1. Texture profile analysis (Bourne, 1978) was performed using an Instron Universal Testing Machine (UTM, Model 1122, Instron Corp., Canton, MA) as described previously (Gordon and Barbut, 1989). Because of the very brittle nature of some of the samples, 60% compression (instead of 75%) of the original height (10 mm) was used. Hardness (force at maximum deformation, N), cohesiveness (ratio of the area of the second curve to the area of the first curve mm²/mm²), brittleness (force to initial fracture, N) and springiness (distance from gauge length to slice surface on the second compression, mm) were determined. A total of seven samples per treatment were tested.

**Microscopy**

Cold stage scanning electron microscopy (cryo SEM), as described by Gordon and Barbut (1990a), was used to examine the meat batters 24 hours after cooking (stored at 4 °C overnight). Specimens were rapidly frozen in a liquid nitrogen slush, fractured, etched at -80 °C, sputter coated, transferred to the microscope stage and examined at 10 kV by SEM (Hitachi S-570, Tokyo, Japan). The Emscope SP2000A System was used for specimen preparation (Emscope, Kent, England). Both the preparation chamber and the microscope stage were kept at -165 °C. Samples for transmission electron microscopy (TEM) were prepared as described by Gordon and Barbut (1990a). TEM specimens were fixed in 2% glutaraldehyde/1% paraformaldehyde in 0.1 M PIPES buffer (pH 6.0), rinsed with buffer, post-fixed with 1% OsO₄, rinsed and dehydrated through a graded series of ethanols. Dehydrated specimens were infiltrated with Epon 812 in capsules and cured by heating (60 °C for 36 hours). A Reichert OMU3 ultramicrotome (Reichert, Vienna, Austria), was used to cut 70 nm sections, which were contrasted with uranyl acetate (10 minutes) and lead citrate (5 minutes) and viewed by TEM (JEOL JEM 100S) at 80 kV. For light microscopy (LM), the procedure of Gordon and Barbut (1990b) was used. Briefly, 1 μm sections were cut from the tissue blocks of specimen prepared for TEM (Reichert OMU3 microtome), stained with a 1% solution of toluidine blue and fixed (by heating) on to a slide. The sections were viewed and photographed (x100 magnification) with a Zeiss microscope (Zeiss, Bonn, Germany).

**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 each magnification) 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, NC). Tukey's test was used to detect significant differences between treatment means.

**Results and Discussion**

**Effect on Batter Stability**

Two previous studies have shown that the chemical agents used in this study were effective in influencing protein functionality without altering the pH of the system (Whiting, 1987a; Gordon and Barbut, 1991). Hence, except for the EDTA, the effect of pH on the batter stability and texture differences observed between the treatments can be regarded as negligible. The fat and water losses from cooked batters for the different treatments are shown in Table 1. The batters treated with H₂O₂ and β-mercaptoethanol were stable during cooking and the urea-treated batter had excellent fat and water binding when compared to the control. A previous study has shown that uncooked batters prepared with these chemical agents were also stable (Gordon and Barbut, 1991). In addition, Whiting (1987a) also found that H₂O₂ and β-mercaptoethanol had no effect on cooked batter stability and that urea significantly increased water binding in cooked batters when used at levels comparable to those used here.

The number of disulphide bonds present within the meat batters appeared to have no effect on cooked batter stability (Table 1) as was also reported for raw meat batters (Gordon and Barbut, 1991). Disulphide bond
formation in the raw batter or during cooking apparently does not impact significantly on the stability of meat batters and therefore plays no direct role in fat and water binding. This appears to contradict their proposed role in the initiation of heat-induced gelation (Shioroshi et al., 1981).

Tween 80 resulted in significant fat and water losses from the cooked batters; this was expected since raw batters treated with Tween 80 were previously shown to be unstable (Gordon and Barbut, 1991). These results also support the findings of Whiting (1987a). Non-protein emulsifiers such as Tween 80 are known to destabilize protein-stabilized emulsions by interfering with the adsorption of the proteins during interfacial film formation (Keeny, 1982; Goff and Jordan, 1989). They also cause fat separation in meat batters (Meyer et al., 1964; Whiting, 1987a, b). The high level of total liquid losses from cooked batters treated with Tween 80 (16.5%) indicated that the batters were grossly unstable (Table 1). In particular, the fat losses (4.80%) were very significant, especially when compared to the control and EDTA treatments. These results suggest that Tween 80 may act mainly by directly destabilizing the fat (by the above mentioned mechanism) which, in turn, causes enhanced water loss. Both fat and water losses from meat batters are closely related (Gordon and Barbut, 1989) and often show a high degree of cooperativity (Schmidt et al., 1986).

EDTA caused large water losses from cooked batters but no significant fat losses were found with either cooked (Table 1) or raw batters (Gordon and Barbut, 1991). It is interesting to note that the behaviour of EDTA was similar to that reported for CaCl2 which has also been shown to produce stable raw batters but unstable cooked batters (Gordon and Barbut, 1989; 1990b). However, unlike CaCl2, high water losses from the cooked batters were not accompanied by high fat losses (Table 1). Gordon and Barbut (1991) indicated that EDTA caused extensive protein matrix aggregation in raw meat batters but did not result in widespread interfacial film breakdown as was observed for CaCl2 cooked batters (Gordon and Barbut, 1989). This suggested that EDTA acts mainly on the proteins forming the matrix to cause aggregation. It may be possible that very little water was lost from the raw batters because the centrifugal method used for batter stability determination did not provide sufficient force to expel the water from the channels within the matrix (Gordon and Barbut, 1991). However, the thermal energy supplied to the water during cooking would have been sufficient to force it out of the matrix, hence its recovery only after cooking (Table 1). An alternative scenario may be that EDTA also acts to destabilize batters by increasing protein-protein aggregation during cooking thereby facilitating easier water loss. EDTA could possibly act by cross linking proteins through sites where divalent cations (e.g., Ca2+, Mg2+) are bound to the proteins. Regardless of the exact mechanism, because EDTA results in an increase in the number of electrostatic and H-bonds in the system, it appears that these types of interactions are important in determining batter stability, especially in terms of the quantity (and possibly the location) of these bonds within the myofibrillar protein network.

Urea acts to disrupt hydrogen and electrostatic bonds and increase the solubility (and hence the availability) of hydrophobic groups which can become involved in protein-protein binding. Hence, the high stability of the urea-treated batters, especially with respect to water losses during cooking (Table 1), indicates that hydrophobic interactions, in addition to H-bonds and electrostatic interactions, are important in batter stabilization in both the raw (Gordon and Barbut, 1991) and cooked state.

**Effect on Texture**

The textural characteristics determined in this study (i.e., hardness, brittleness, springiness, cohesiveness) have been shown, in the past, to correlate quite well to sensory texture (Montejano et al., 1985; Lee et al., 1987). The texture of the control (2.5% NaCl) treatment (Table 1) was typical of such meat batters (Barbut, 1988; Gordon and Barbut, 1989). The batter treated with H2O2 was similar to the control in terms of brittleness and springiness but was significantly harder (p < 0.01). The use of β-mercaptoethanol during batter preparation resulted in a batter which had a similar level of brittleness and cohesiveness to the control batter and which was as cohesive, but was less springy and less hard compared to both the control and the H2O2-treated batters (Table 1). Whiting (1987a) has reported that either of these chemical agents had an effect on texture in pork/beef meat batters as measured by gel strength (i.e., penetration of a single stainless steel probe into the gel).

H2O2 increases the number of disulphide bonds formed prior to cooking while mercaptoethanol reduces them. Consequently, if disulphide bond formation were important to the development of a particular textural characteristic, both of these treatments would show opposite effects on that characteristic. The formation of disulphide bonds prior to cooking (i.e., the H2O2 treatment) greatly increased the hardness of the batter and also increased cohesiveness while the reduction of these bonds (the β-ME treatment) significantly (p < 0.01) reduced hardness (Table 1). However, neither treatment produced a level of brittleness which was significantly different from that of the control batter, suggesting that this textural characteristic is a function mainly of other types of interactions. An interesting observation was the
significant decrease in the springiness (elasticity) of the meat batter when disulphide bond reduction took place prior to cooking. Springiness is among the more important textural characteristics and influences the "bite" of a batter-type meat product. It therefore appears that disulphide bond formation in the raw state is important to the development of an acceptable texture in meat batters. It influences the springiness of meat batters such that an acceptable level of elasticity can be achieved and is also a determining factor in the final hardness and cohesiveness of the product. However, excessive formation of disulphide bonds may result in a product which is too firm and resistant to deformation.

Treatment of meat batters with EDTA resulted in an unacceptable texture (Table 1). This was expected since EDTA led to large water losses during cooking. This treatment generally resulted in a decrease in the intensity of all of the textural characteristics as compared to the control. This may be a consequence of the extensive cross-linking of proteins (through salt bridge formation) which EDTA may cause, perhaps in a manner similar to that suggested for other anions (Hamm, 1970), as proposed earlier. The Tween 80-treated batter showed a reduction in the hardness and springiness compared to the control (Table 1). This was probably due to the excessive fat and water losses from this treatment during cooking since hardness and springiness are related to fat loss (Patana-Anake and Foegeding, 1985; Gordon and Barbut, 1989). Whiting (1987a, b) also found a significant deterioration in the texture of cooked batters treated with Tween 80. The cohesiveness of the batter was not affected by the high fat and water losses before or after cooking. This indicates that this parameter is independent of fat and water binding and supports the assertion that cohesiveness is an intrinsic property of the system and develops prior to cooking (Montejano et al., 1984; Patana-Anake and Foegeding, 1985).

The urea treatment produced similar hardness and springiness to that of the 2.5% NaCl batter (Table 1). It did not show any significant fracture behaviour at the deformation level used (60%) and showed higher cohesiveness than all of the other treatments. The very good textural characteristics of the urea batter and the excellent water and fat retention it displayed (Table 1) were indicative of the formation of a very desirable type of structure within the batter on cooking. Urea increases the effective hydrophobicity of proteins by solubilizing hydrophobic residues (Nakai, 1983; Whiting, 1988). However, Wicker et al. (1986) have shown that increases in effective hydrophobicity in Tilapia (Soro­therodon aureus) preceded the onset of thermally-induced gelation. Nakai (1983) has also indicated that such a situation will result in entropy-driven protein-protein interactions and hence aggregation. Aggregation is the process whereby proteins interact and initially form isolated aggregates which may then further interact to form an ordered gel or a disordered coagulum (Foegeding et al., 1986). Hence, aggregation normally precedes gelation (Acton and Dick, 1984) and, consequently, in the environment existing in the urea-treated batter, it should be expected that a very cohesive, strong gel structure would be developed on cooking. This would explain the desirable textural properties of this treatment since its high cohesiveness and resistance to fracture reflect the flexibility, strength and uniformity of the structure that should be formed in such a system. The textural characteristics of this batter also suggests that the number of hydrophobic bonds formed affect hardness and especially the cohesiveness of meat batters.

**Effect on Cooked Batter Microstructure**

Cooking meat batters results in increased protein-protein interactions, the nature of which determines the final cooked stability and textural properties of the batters. The microstructure of all of the batters (including the control 2.5% NaCl batter) changed as a result of protein aggregation during cooking (Figs. 1-4). H2O2 resulted in a cooked batter with a microstructure fairly similar to that of the control. However, the protein matrix had a finer structure with more inter-connections between the strands and smaller pore sizes (Figs. 1b, 2b versus Figs. 1a, 2a). This structure is much more cohesive and less discontinuous than was present in the raw H2O2 batter described in our previous study (Gordon and Barbut, 1991). This more cohesive matrix may represent the effect of some new interactions which were not very active during raw batter preparation but took place mainly during cooking. Most of the fat globules remained stable after cooking (Figs. 3b, 4b). Nevertheless, some unstable fat pools which appeared to have arisen from fat exudation through pores in the IPF of fat globules during cooking were evident (Fig. 3b). These fat pools, however, were small, well contained within the matrix and not well interconnected (the interconnections would indicate gross fat instability). The microstructure of the H2O2-treated batter was in accordance with its observed stability (Table 1). The more dense, highly interconnected protein matrix structure explains the greater TPA hardness and cohesiveness which the batter displayed in comparison to the control (Table 1). The β-mercaptoethanol batter (Figs. 1c and 3c) showed a microstructure which was very different to that existing prior to cooking as described by Gordon and Barbut (1991). Cooking resulted in a very dense, highly aggregated matrix with many large spaces and little continuity (Figs. 3c and 4c). The matrix appeared to consist mainly of dense, tightly aggregated cores (clumps) bound to each other by thin strands. This structure was
Figure 1. SEM micrographs of representative fields from cooked meat batters treated with: a) 2.5% NaCl; b) H₂O₂; c) β-mercaptoethanol; d) EDTA; e) Urea; and f) Tween 80; Fc - fat cells, cell clumps or fat pool. Bar = 20 μm.
Figure 2. High magnification cryo SEM micrographs of matrices of cooked meat batters treated with: a) 2.5% NaCl; b) $\text{H}_2\text{O}_2$; c) $\beta$-mercaptoethanol; d) EDTA; e) Urea; and f) Tween 80. Bar = 2 $\mu$m.
Figure 3. TEM micrographs of representative fields from cooked meat batters treated with: a) 2.5% NaCl; b) H₂O₂; c) β-mercaptoethanol; d) EDTA; e) Urea; and f) Tween 80; F - fat, M - matrix, F_p - uncoated fat particle, P - pools of fat entrapped within the matrix, FC - fat channels. Bar = 4 μm.
Figure 4. Light micrographs of representative fields from cooked meat batters treated with: a) 2.5% NaCl; b) H$_2$O$_2$; c) β-mercaptoethanol; d) EDTA; e) Urea; and f) Tween 80; F - fat, M - matrix, T - Tunnel. Bar = 50 μm.
somewhat similar to that which was formed in raw batter treated with H$_2$O$_2$ but was much more highly aggregated. This discontinuous matrix structure explains the reduced TPA hardness, brittleness and springiness of the cooked mercaptoethanol batter (Table 1) and indicates that the formation of more disulphide bonds than is normal during cooking (compared to the control) can produce an unacceptable texture in meat batters. Although the protein matrix was so highly disrupted, the fat within the batters generally remained very stable, existing mainly as round, protein-coated globules (Figs. 1c, 3c and 4c). This was in accordance with the stability exhibited by this cooked batter (Table 1) and indicated that extensive matrix disruption does not necessarily result in batter instability, an observation which we have previously reported (Gordon and Barbut, 1990b; 1991). It should be noted that some unstable fat which was derived from fat globules with broken or absent interfacial films was evident (arrows, Fig. 3c) and may explain the slightly higher but non-significant (p > 0.05) levels of fat loss in this treatment compared to the control. However, the occurrence of fat instability was not widespread throughout the batter.

The cooked EDTA batter displayed a very aggregated matrix (Figs. 1d, 2d, 3d and 4d). Raw EDTA batters have already been shown to be aggregated prior to cooking (Gordon and Barbut, 1991). This suggests that heat processing did not greatly alter the microstructure of the raw batter which was already highly aggregated. The fat within the cooked batter remained relatively stable and many globules appeared to retain their IPF, which corresponded well with the good fat stability observed for the cooked EDTA batter (Table 1). However, in some cases, fat stabilization by entrapment within the aggregated portions of the matrix was evident (Fig. 3d). The high water losses and unacceptable texture of the cooked EDTA batter (Table 1) resulted from the highly aggregated, discontinuous structure of the protein matrix (Fig. 4d) which allowed the easy release of water during cooking. The relatively small changes in the structure of this batter on cooking (compared to the raw batter) indicates that the type of interactions promoted by EDTA (H-bonds and electrostatic interactions) probably would normally occur during cooking. This is suggested since it appears that any other interactions which occurred during the cooking of this batter had a negligible effect on the structure once EDTA-mediated interactions had occurred.

The protein matrix of the urea-treated batter was very cohesive, fine-stranded and uniform (Figs. 1e and 3e). A highly interconnected network was formed with numerous small pores (Fig. 2e). This type of structure was different from the control (Figs. 1a, 2a and 3a) and other batters and accounted for the excellent fat and water binding properties and the very high TPA cohesiveness of this batter (Table 1). The TEM micrograph (Fig. 3e) illustrates the ability of the very cohesive matrix of this batter to bind fat by entrapment even in cases where the fat was irregular in shape.

In contrast, the cooked batter treated with Tween 80 showed increased protein matrix aggregation and disruption as a result of cooking (Figs. 2f, 3f and 4f). Very dense aggregates were formed (Fig. 3f), similar to those in the mercaptoethanol and EDTA treatments (Figs. 2c, 2d and 3c, 3d). Very few of the fat globules examined retained their protein coat (Fig. 3f); virtually all of them had no visible IPF remaining after cooking and fat instability was universal (Fig. 4f). In some cases, round fat particles without a visible IPF were evidently dispersed within the protein matrix (Figs. 3f and 4f). These possibly represent the central solid cores of what were originally fat globules but had lost much of the liquid fat during cooking due to the action of the emulsifier. This microscopic evidence further confirms the assertions that non-protein emulsifiers, particularly Tween 80, destabilize meat batters by interfering with the adsorption of myofibrillar protein to form an IPF (Gordon and Barbut, 1991).

**Summary**

The effects of different types of protein-protein and protein-lipid interaction on the stability, texture and microstructure of cooked meat batters were investigated by chemical modification of the batter proteins. With respect to the effect of H$_2$O$_2$ and β-ME it appears that disulphide bond formation occurs during cooking and plays an important role in influencing the development of the structure such that a texture with a desirable level of cohesiveness, springiness and hardness is formed. These three textural parameters are the ones most important in determining overall product texture (Patana-Anake and Foegeding, 1985; Gordon and Barbut, 1989). However, disulphide bond formation during cooking did not appear to affect batter stability. These findings are in accordance with those reported for isolated meat protein systems as reviewed by Asghar et al. (1985).

Hydrophobic interactions were found to be important in influencing the TPA cohesiveness and hardness of batters. They were also involved in structure development during cooking and contributed to an increase in the density of the matrix. The role of electrostatic and H-bonds in meat batters appears to be mainly restricted to assisting structure formation during cooking. Nevertheless, the use of ingredients which greatly increase the number of these types of interactions in meat batters should be avoided as this can result in cooked batter instability as evidenced with EDTA. Non-protein emulsifiers such as Tween 80 appeared to cause meat batter
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failure either by displacing the myofibrillar proteins from the fat/water interface or by preventing their adsorption altogether. This results in batter failure since the emulsifier-stabilized interface is of lower mechanical strength and is therefore unable to adequately localize the fat. In addition, non-protein emulsifiers cannot participate in interfacial film-protein matrix binding and therefore the physical immobilization of the fat globules by this mechanism (Gordon and Barbut, 1990c) is reduced to a minimum.

References


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Discussion with Reviewers

K. Samejima: Did the authors use a stock solution of EDTA adjusted to the desired pH? If so, please explain why the EDTA-sample was lower in pH than other samples?

Authors: The EDTA used was added to the batters in its solid form. The level used was based on Whiting (1987a) and had been shown in our preliminary studies to be just about sufficient to produce a detectable change in the batter although the pH was altered. Lower levels of addition which may not have affected the pH of the batter did not produce as easily detectable a difference. We therefore accepted an EDTA level that we knew would have a measurable effect on the batter and with whose pH effect we were familiar.

F.W. Comer: If pH differences were obtained between treatments, then pH adjustments to the control would separate out this effect.

Authors: A pH difference was obtained with one treatment only (EDTA). The pH of this batter was not adjusted as this would have introduced changes to the proteins, the extent of which would be unknown and which would have made interpretation of the results even more difficult.

J.G. Montejano-Gateau: Looking at the cooked losses for fat, it appears that the method employed is not sensitive enough to detect minor changes and that it has a relatively high standard deviation. Although fat loss was only significant for the batter treated with Tween 80 as compared to control, the fat loss in the urea-treated sample was about six times lower than the control and ten times lower that the batter treated with β-mercapto-ethanol, yet there was no statistical difference among those samples. Is this true? The textural data seem to be contrary to this finding.

Authors: The method used for batter stability was designed more to detect gross changes or differences in water and fat binding, than to detect subtle differences. Nevertheless, contrary to your suggestions, the standard deviations produced were relatively small, typically 10-15% of the value reported (Table 1). The differences between the treatments (except Tween 80) in amount of fat lost were small and this is perhaps why no significance was found. With respect to your observation regarding the relationship between the batter stability results and those for texture, it is not clear to us what this is based on.

C. M. Lee: Heating was applied to fix the specimen, how was it done? Was there any good reasons to apply the heat? Would the heating cause an alteration in the structure?

Authors: The slide was gently heated on a hot plate at a low heat setting. The heat was used in accordance with recommended practice (Dawes, 1988) to improve the adherence of the section to the slide and remove wrinkles (the section expands on heating). When done as described, the heat would have negligible effect on the specimen. Please remember also that the proteins within the specimen are already cooked and undergo further fixation with chemical fixatives.

R.C. Whiting: The SEM of the urea treated gels shows globular bodies (Fig 2e). Do you have any ideas on what they are?

Authors: The globular white bodies in Fig. 2e have been identified as ice crystals. We experienced great difficulty in removing the surface water from urea-treated meat batter specimens by sublimation. We feel that this was related to the extremely good water binding properties which the urea-treated batters displayed (Table 1). Several time/temperature combinations were tested but those which produced ice-free specimen were often unacceptable because they created other structural artifacts which were even more difficult to control. Consequently, the lesser of the evils was chosen and the standard protocol was used (as for all other treatments) which produced the artifacts which we could, at least, interpret. Further work needs to be done to address this problem.

F.W. Comer: No information is given about the kinetics and/or extent of chemical modification. The results are "single point" experiments. Have the authors collected data which show the effects of degree of modification upon microstructure and functionality, i.e., data from several experiments, rather than a single experiment?

Authors: The intention of this study was not to characterize and document the chemical modification of meat batters but to seek insights into the types of molecular interactions involved in meat batter production. As such, no attempt was made to investigate the kinetics of the action of each chemical agent on the meat protein or the effects of degree of modification on microstructure or functionality. However, we have investigated the extent of the modifications caused by the treatments as
compared to the control using spectrophotometric techniques (Gordon, 1990).

J. G. Montejano-Gateau: Despite the lack of statistical differences, when comparing fat losses for the batters treated with \( \text{H}_2\text{O}_2 \) and \( \beta \)-mercaptoethanol (almost doubled in the latter), the pattern observed is the same as in all the TPA parameters. Therefore, may we assume that the method for fat loss was not sensitive enough and that, in fact, disulfide bond formation plays a key role in fat and water binding that in turn gives stronger and more deformable structures?

Authors: The major factor in batter stability is the amount of water loss (Schmidt et al., 1986; Gordon and Barbut, 1989) and not fat loss. Table 1 shows that the difference between both the \( \beta \)-ME and \( \text{H}_2\text{O}_2 \) treatments in water loss was small. We do not agree that the pattern for fat loss between the two treatments is the same as for the TPA, nor do we think that fat loss is as important as water loss as index of batter stability. However, our results indicate that, as you suggest, disulfide bond formation contributes to firmer, more deformable cooked meat batters.

R.C. Whiting: The TEM and light microscopy images suggest to me that mercaptoethanol disrupted the matrix. This would be expected to cause a significant water loss, which was not observed. How do you interpret these micrographs?

Authors: Cooking of the mercaptoethanol (ME) treated batter resulted in a greater level of protein-protein interactions than in the control (2.5\% NaCl only) batter, and hence a more disrupted matrix (Fig. 3). However, especially the light micrographs indicate that the matrix was not totally disrupted and exhibited some continuity, although several spaces (discontinuities) were evident (Fig. 4). In addition, these discontinuities were not as large or as well interconnected as those in the EDTA and Tween 80 batters which both lost significant amounts of water. This may account for the relative stability of the ME batter. Furthermore, we previously showed that a highly disrupted EDTA raw batter matrix did not result in significant water loss (Gordon and Barbut, 1991). Consequently, the stability of the ME batters concurs with this earlier observation and further indicates that protein matrix disruption alone is not sufficient to cause batter failure. Other factors, such as the state of "openess" of proteins (i.e., the conformation) and protein-fat interactions (Schmidt et al., 1986; Gordon and Barbut, 1991) also determine whether or not water is lost from meat batters during cooking.

K. Samejima: Your results appears to be different from that of Ishioroshi et al. (1981) about the role of disulfide bonds in the initiation of heat-induced gelation of myosin. What is your explanation on this point?

Authors: Ishioroshi et al. (1981) have reported that disulfide bond formation (i.e., the oxidation of sulphydryl (SH) groups) was involved in the initiation of heat induced gelation. In your earlier work (Samejima et. al., 1981), you had alluded to the possibility that "cross-linking to consolidate the gel structure probably occurs at relatively low temperatures" (our emphasis) and is associated with the oxidation of SH groups and that "another type of aggregation ... superimposed on the SH dependent reaction". This, we feel, is close to the truth since it has been shown that gel formation does occur at low temperatures during raw batter preparation (Gordon and Barbut, 1990a, b) and that disulfide bond formation contributes to the integrity and cohesiveness (visual) of the structure formed (Gordon and Barbut, 1991). This present study has also shown that disulfide bond formation plays a role in heat-induced protein gelation and influences meat batter gel strength (firmness) and cohesiveness (Figs. 3 and 4; Table 1). However, raw batter gelation also readily takes place under conditions where disulfide bond formation is discouraged (\( \beta \)-ME treated batters). Consequently it appears that disulfide formation is not an absolute necessity for meat protein gelation to occur and is, therefore, not the initiating (critical) event in this process. The results of our studies suggest that gel formation is initiated through other types of interaction (hydrophobic, it appears) in the raw batter, with the SH-dependent aggregation either occurring simultaneously or superimposing itself on the preformed gel structure prior to and during cooking.

It is important to highlight some differences between our work and previous studies on the role of disulfide bonds. Our studies were not done with pure proteins but, instead, with a commercially relevant, complex meat batter system. Furthermore, the occurrence of protein gelation during raw batter preparation was recognized and considered in interpreting the results of the chemical treatments. In this system, several proteins other than myosin are involved in protein matrix gelation (Gordon and Barbut, 1990b, 1992) and their contribution to sulphydryl-dependent interactions and roles in protein matrix formation must also be considered. These factors, together, complicate the picture regarding the role of disulfide bonds in real meat batter systems and explain the differences in interpretation to which you have referred.

C. M. Lee: There are many statements regarding textural properties such as brittleness, springiness, cohesiveness, etc. Do authors know if these terms are appropriate ones to use, and how they should be
interpreted? Does the cooked meat batter have any brittle textural characteristics? Do these texture terms have any meaning to the stability and structure of the batters which have received various chemical treatment? The authors should have focused on one or two specific textural properties which can be readily identifiable with structural changes.

Authors: The texture of the treated meat batters was described using terms derived from TPA measurements. The characteristics chosen were the ones which were shown to correlate well with similar sensory characteristics (Montejano et al., 1985; Lee et al., 1987). Cooked meat batters do show some fracturability which affects the mouthfeel of the product and is therefore quite important. We have also shown previously that batter stability indices and microstructure are related to textural parameters as determined by TPA (Gordon and Barbut, 1989). Consequently, we have no reason to believe otherwise for chemically treated batters. Indeed, we feel that the results presented here provide further evidence of the relationship between microstructure, batter stability and texture. We concede, however, that some textural characteristics such as cohesiveness, are more easily identifiable with structural changes.

R.C. Whiting: Tween is said to "act mainly by directly destabilizing the fat which, in turn, causes increased water loss". Later effects on texture are also attributed to Tween. Could Tween affect protein gelation which then affects all the measured properties?

F.W. Comer: The authors have offered a plausible explanation of the effects of Tween 80 upon the IPF. Is there also an explanation of how Tween 80 affects the protein aggregation? The loss of stability and textural firmness is likely to be caused by the high degree of protein aggregation which is similar to that observed with the EDTA treatment.

Authors: It is possible that Tween 80 could affect protein gelation directly. However, work done in our laboratory has shown that Tween 80 does not greatly affect the proteins themselves directly (Gordon, 1990), especially if no fat is involved (Barbut and Mittal, unpublished). Furthermore, it has consistently been shown that Tween 80 interferes with lipid-protein interactions (Keeny, 1982; Goff et al., 1987; Goff and Jordan, 1989). We believe that the destabilizing effect of Tween is due to its selective adsorption to the water/fat interface in preference to myofibrillar proteins, a scenario we had earlier suggested (Gordon and Barbut, 1991). Tween 80, therefore, influences protein aggregation and, subsequently, gelation, by increasing the number of protein ligands available for interacting. This is a direct result of its blocking of the protein-lipid interaction necessary for IPF formation thereby freeing large numbers of protein ligands which then facilitate excessive protein-protein interactions, resulting in a highly aggregated protein matrix.

K. Samejima: You have mentioned that 2.5 % NaCl is commercially used for meat batters. However, the salt concentration has an inclination to decrease in recent years. I would expect that you will continue to investigate under the condition of lower salt concentration and the presence of phosphate.

Authors: We have done some work with reduced salt meat batters and pyrophosphate and intend to explore further in the future.

J.G. Montejano-Gaitau: Was there any possibility of increasing or decreasing moisture and fat losses during cooking of samples for texture evaluations as compared to samples for stability tests? The batter stability was evaluated on test tubes of certain dimensions and construction that may not be the same used for the samples for textural evaluation. Hence, this may help to explain some of the differences observed in samples with improved textural attributes that did not show significantly greater stability when compared to the control (example: fat loss in the urea treated batter).

Authors: The samples used for texture evaluations were derived directly from the cooked meat batters on which moisture and fat losses had been determined (see Materials and Methods). Hence the results of the textural analysis should directly reflect the amount of fat or water lost from the product or dislocated within the product.

Additional References


*CLA Gordon and A Gordon are the same person; CLA Gordon was the name required to be used by the University of Guelph for thesis submission.