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THE EFFECT OF CHLORIDE SALTS ON THE TEXTURE, MICROSTRUCTURE AND STABILITY OF MEAT BATTERS

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Abstract

The stability, texture and microstructure of six mechanically deboned chicken meat batters prepared with NaCl (1.25 and 2.5%) and replacement of the 2.5% NaCl with MgCl2, CaCl2, KCl and LiCl based on isoionic strength were examined. The uncooked MgCl₂ batter showed the poorest fat binding. The monovalent chloride salts produced stable cooked batters, whereas both divalent salts did not. CaCl₂ produced a more unstable batter than MgCl2. High correlation was found between water and fat loss and total cookout losses from cooked batters. Texture was significantly affected by the type of chloride salt used. The divalent chloride salt batters had low brittleness and were similar in texture. They had a different texture profile from monovalent chloride salt batters. Hardness and springiness were found to be related to batter stability.

Microstructural differences between treatments reflected differences in batter stability and appeared to explain some of the textural differences. The protein matrices of the monovalent chloride salt batters were all similar. However, LiCl produced a more tightly interwoven matrix than the others. Extensive coalescence was evident in the batters made with MgCl₂ and CaCl₂ which resulted in the formation of fat channels. In addition, their protein matrices were highly aggregated. Batter stability and texture appear to depend on the structure and integrity of the matrix as well as the formation of a stable protein film around fat globules.

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<u>KEY WORDS</u>: Salt reduction, emulsion stability, texture, microstructure, divalent cations, protein matrix, interfacial protein film, fat channels.

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Introduction

The production of finely comminuted sausages such as frankfurters requires the availability of adequate amounts of extracted myofibrillar proteins in the meat batter. Myofibrillar proteins are principally responsible for binding added moisture and fat and thus for the desirable texture associated with these products. The solubilization of myosin and actomyosin is of great importance in the formation of stable meat batters (Gillett et al., 1977). Sodium chloride (NaCl) is the salt most widely used to enhance the extraction of myosin and actomyosin during comminution (Agghar et al., 1985; Barbut and Findlay, 1989).

Two main models have been proposed to explain fat stabilization in such batters. According to the emulsion theory, the proteins form a film or membrane around the fat globule which stabilizes them (Jones, 1984), whereas the non-emulsion theory proposes that fat globules are physically entrapped within the protein matrix (Lee, 1985). During cooking, protein coagulation helps to immobilize the fat, water and other constituents.

Unstable batters are infrequent, but bothersome problems in routine meat processing operations. Research has therefore been directed at the mechanisms by which batters are stabilized. Early research demonstrated the existence of an interfacial protein film (IPF) around fat globules and suggested that it was responsible for the stability of meat batters during cooking (Hansen, 1960; Swift et al., 1961, Borchert et al., 1967). Several workers have since confirmed the presence of a protein film around fat globules (Theno and Schmidt, 1978; Lee et al., 1981; Carroll and Lee, 1981; Swasdee et al., 1982). In addition. Jones and Mandigo (1982) have investigated the 'pores' found in the protein film in the cooked batters and suggested that they serve as a pressure release mechanism during cooking. The matrix as well as the chemical and physical nature of the fat have also been shown to play an important role in emulsion stability (Meyer et al., 1964; Townsend et al., 1968).

Crowing concern has been expressed over the link between hypertension and dietary sodium intake (Kent, 1981). This has fueled a drive to reduce the levels of NaCl in meat products (Anon., 1980). However, reducing NaCl levels in meat products will reduce protein extraction which will result in an unacceptable product (Thiel et al., 1986; Whiting, 1987; Hand et al., 1987; Barbut and Findlay, 1989). In order to successfully reduce the sodium levels, an effective NaCl substitute must be found.

It has been suggested that the chloride ion is responsible for the functional efficacy of NaCl in meat products (Hamm, 1970; Offer and Trinick, 1983; Asghar et al., 1985). Consequently, the potential use of other chloride salts (KCl, MgCl₂ and LiCl) have been studied (Seman et al., 1980; Hand et al., 1982a,b; Barbut et al., 1988). This approach has met with some success, however its application has been limited because of some off flavour problems and insufficient knowledge of the mechanisms by which NaCl stabilizes meat batters.

There is currently an increasing body of information on the functional aspects of NaCl and other chloride salts in meat products. However, a fundamental understanding of the mechanisms involved can greatly speed up the process of developing NaCl alternatives. Very little work on the effect of chloride salts on the microstructure and/or texture of meat emulsions can be found in the literature (Knipe et al., 1985; Whiting, 1987; Barbut and Mittal, 1988; Barbut, 1989). Thus, the objectives of this work were to use a model system to obtain basic information on the effect of different chloride salts on emulsion stability and investigate their relations to product microstructure and texture.

Materials and Methods

Treatments

Five different chloride salts were used to produce six different poultry meat batters in three separate trials. NaCl was used at levels of 1.5% and 2.5% of the total weight of the batter. Four other chloride salts (MgCl2, CaCl2, KCl, LiCl) were used at levels which gave an ionic strength equivalent to that of 2.5% NaCl (Table 1.). The 2.5% NaCl represented the most widely used level for comminuted products today. The 1.5% NaCl represented a 40% reduction that would still provide a borderline stability (Whiting, 1984). Ingredients and Product Manufacture

Batters (0.5kg batches) were made from mechanically deboned chicken meat (MDCM) obtained from a commercial processing plant. The meat was kept frozen (-18°C) for up to one month prior to use. Proximate analysis of the raw meat as determined in duplicate (AOAC, 1980) was: 66.7% moisture, 16.1% fat, 14.3% protein and 1.1% ash. The composition of the cooked batters was not determined. All treatments were formulated with 6.0% added water (based on a finished product weight). The MDCM was the sole source of fat. The level of chloride salts (Fisher Co., Ontario) varied among treatments.

Batters were chopped for 4 min in a nonvacuum bowl cutter (Hobart, model 84/42, Troy, OH) using the high speed setting (33 rpm). Final chopping temperatures did not exceed 8°C in any of the treatments. To remove small air bubbles, batters were later vacuum tumbled in a pre-cooled 40 litre table top vacuum tumbler (Lyco, Columbus, WI) for 30 sec at a pressure of 0.15 atm. For cooking, 34g of batter was accurately weighed into 50ml plastic test tubes (to prevent evaporation) which were centrifuged (Fisher Centrific, Fisher, Ont.) at a low speed (600 g) for 5 min in order to evacuate any air trapped during stuffing (Whiting, 1984). Six tubes per treatment were cooked to an internal temperature of 69° C in a water bath gradually heated from 50^{\circ}C to 80° c within 1.5 hr. Three tubes were used to determine emulsion stability (ES) and the rest were stored in a cooler (2°C) overnight.

Sampling and Testing

The pH of raw batters was determined in triplicate (Chemcadet J-598, Cole Palmer, Chicago IL). Emulsion stability was assessed in triplicate on both the raw and cooked batters. For the raw batters, 34g of each batter was weighed into 50ml plastic centrifuge tubes and centrifuged at 18,000 g. The amount of oil separated was determined and used as a measure of the stability of the raw batter. Stability of the cooked batter was measured by the amount of 11quid and fat released from a 34g sample during cooking (Townsend et al., 1968).

Texture profiles were determined on seven cooked samples per treatment (Bourne, 1978). A central core (10mm high x 10mm in diameter) was removed from each specimen and compressed twice to 25% of its original height (Keeton et al., 1984; Zeigler et al., 1987) using the Instron Universal Testing Machine (Model 1122, Instron Corp., Canton, MA). Cross-head speed was 20mm/min and chart speed was 200mm/min. Hardness (force at maximum deformation, N), brittleness (force to initial fracture, N), cohesiveness (ratio of the area of the second curve to the area of the first curve, mm²/mm²), springiness or elasticity (distance from gauge length to slice surface of second curve, mm), gumminess (hardness x cohesiveness, N) and chewiness (gumminess x springiness, Nmm) were determined.

Electron Microscopy

Samples for scanning electron microscopy (SEM) were prepared by a modification of the procedure of Jones and Mandigo (1982). Two cooked batters per treatment were randomly selected from each treatment. A block (2 x 1 x 1cm) was cut from the centre of each and sliced into 3 mm cubes. They were broken using forceps such that the broken side would be used for SEM. The specimens from each treatment were fixed in 2% glutaraldehyde + 1% paraformaldehyde in HEPES buffer (pH 6.0) for 2 hrs, rinsed five times for 10 min with the buffer, and post-fixed with 1% OsO, for 4 hrs. This was followed by five more rinsings with buffer after which specimens were dehydrated through a graded series of ethanol of 50, 70, 80, 90, 95% and three changes of 100% for 10 min each. Samples were then critical point dried using CO2, mounted on stubs and sputtercoated with palladium/gold (Hummer VII, Anatech Ltd., Va.). The samples were examined by SEM (Hitachi S-570, Tokyo) at 10 kV.

For transmission electron microscopy (TEM), a standard procedure was used. The fixed and dehydrated samples were placed in acetone-Spurr's resin at ratios of 3:1, 1:1 and 1:3 for 30, 30 and 45 min, respectively. Samples were then infiltrated with 100% Spurr's overnight in a

	TREATMENT								
Parameter	NaCl (1.5%)	NaCl (2.5%)	MgCl ₂ (1.35%)	CaCl ₂ (1.58%)	KC1 (3.19%)	LiC1 (1.18%)			
Ionic									
Strength	0.26	0.43	0.43	0.43	0.43	0.43			
pH (raw)	6.74 ^{bc}	6.76 ^b	6.66 ^d	6.08 ^e	6.83 ^a	6.69 ^{cd}			
(3.0.)-	0.05	0.05	0.05	0.05	0.02	0.02			
Uncooked Fat Loss ^g (S.D.)	6.09 ^b 0.59	5.35 ^b 0.53	7.91 ^a 0.61	6.00 ^b 0.18	6.03 ^b 0.62	5.71 ^b 0.62			
Cooked									
Gel water									
Loss	4.62 ^c	3.09cd	21.06 ^b	23.94 ^a	4.09 ^c	1.94 ^d			
(S.D.)	0.69	0.33	0.26	0.37	0.55	0.30			
Fat Loss	0.15 ^{bc}	0.08bc	0.49 ^a	0.55 ^a	0.21 ^b	0.06 ^c			
(S.D.)	0.08	0.08	0.06	0.15	0.16	0.05			
Totalg									
Liquid Loss	5.06 ^c	3.32 ^{cd}	22.47b	25.56 ^a	4.71 ^c	2.11 ^d			
(S.D.)	0.74	0.33	0.30	0.50	0.69	0.34			

<u>Table 1</u> <u>Emulsion stability and pH of cooked and uncooked meat batters prepared with different chloride salts.</u>

a-e - means followed by different superscripts in the same row are significantly different (P<0.05).

f - standard deviation.

g - m1/100g of batter.

E mil/100E of buccer.

desiccator before curing for 16 hrs in capsules at $60^{\circ}C.$ Sections were cut, picked up on grids and stained for 10 min with uranyl acetate and 5 min with lead citrate. Sections were viewed by TEM (JEOL JEM 100CX) at 60 kV.

Statistical Analysis

The experiment was repeated three times. For the microscopical evaluation, samples from two of the trials were examined. The experiment was based on a complete randomized block design. Data were analyzed by analysis of variance using the General Linear Models (GIM) procedure (SAS Institute Inc., Cary, N.C.). Tukey's test was used to detect significant differences between treatment means. Correlation coefficients were determined on textural and emulsion stability parameters.

Results and Discussion

Emulsion Stability and pH

Fat was the only component released from the uncooked batters during centrifugation; no water was separated. Only the MgCl₂ treatment showed significantly higher fat release (p<0.05) than other treatments (Table 1). Whiting (1984) and Patama-Anake and Foegeding (1985) also reported no significant water loss prior to cooking in reduced NaCl batters and batters to which different binders were added. The difference in fat binding in the uncooked state suggests that the mechanisms by which MgCl₂ and CaCl₂ act to destabilize batters may be different, possibly because of differences in the type and quantity of extracted protein and protein-cation interaction. Divalent cations are thought to reduce batter stability by increasing protein aggregation through the formation of salt bridges (Hamm, 1970; Asghar et al., 1985).

In the cooked batters, both MgCl₂ and CaCl₂ resulted in high fat and water losses; however, the latter produced a less stable product (Table 1). There was no significant difference in fat loss between the two divalent salt treatments but CaCl₂ caused significantly more water loss than did MgCl₂ (p<0.05). This suggests that unstable batters are more often accompanied by water loss than fat loss. This conclusion was supported by high correlations (p<0.01) between water loss and total cook-out losses (Table 2). Other researchers have suggested that water loss is the main effect of batter instability (Patana-Anake and Foegeding, 1985; Schmidt, 1984).

Magnesium ions have been shown to destabilize meat batters prepared from pork and beef as well as poultry (Seman et al., 1980; Knipe et al., 1985; Barbut et al., 1988). MgCl₂ and CaCl₂ have different effects on pH and water holding capacity (WHC) in ground beef (Wierbicki et al., 1957). Whiting (1987) found no significant differences in fat exudation between beef/pork frankfurters made with MgCl₂ and CaCl₂. However, CaCl₂ caused more water loss from cooked batters. These findings agree with the results in Table 1. On the contrary, he found that MgCl₂ formed a stable

	PARAMETER Emulsion Stability								
Textural Parameter	pН	Gel Water Released ^b	Fat Released ^b	Total Liquid (cooked)	Fat Released (uncooked)				
Hardness 1	N.S. ^c	0.324*	0.302*	0.324*	N.S.				
Brittleness	N.S.	N.S.	N.S.	N.S.	N.S.				
Springiness	0.350*	-0.518**	-0.497**	-0.520**	N.S.				
Cohesiveness	N.S.	N.S.	N.S.	N.S.	-0.384*				
Gumminess	N.S.	N.S.	N.S.	N.S.	N.S.				
Chewiness	N.S.	N.S.	N.S.	N.S.	N.S.				
pH	-	-0.759**	-0.644**	-0.756**	N.S.				
Gel Water Released			0.913**	0.999**	0.285*				
Fat Released (cooked)				0.924**	N.S.				
Total Liquid (cooked)					0.284*				
Fat Released (uncooked)									

Table 2 Correlation coefficients for emulsion stability, pH and textural characteristics^a.

a - total no. of observations = 54.

b - gel water, fat released from cooked frankfurters (m1/100g).

c - not significant.

* - significant (P<0.05); ** - significant (P<0.01).

batter, a conclusion which supports the findings of Hand et al. (1982a) for both pork/beef and mechanically deboned turkey meat frankfurters. A tenuous relationship (p<0.05) was found between fat lost from the uncooked product and the ES of the cooked product (Table 2). This suggests that fat binding prior to cooking may influence the stability of the cooked product.

The other salts resulted in the formation of much more stable batters (Table 1). LiG1 formed the most stable batter when compared to the KG1 and 1.5% NaCl treatments but was not more stable than the 2.5% NaCl batter. In contrast, Hand et al. (1982a) and Whiting (1987) found no difference in stability between batters made with these salts. KCl has been found to be as effective as NaCl in promoting ES (Hand et al., 1987; Barbut et al., 1988). Whiting (1984) found significantly greater water loss from batters made with 1.5% than 2.5% NaCl but significant fat loss only occurred at less than 1.0% NaCl. LiG1 has been shown to have a positive effect on water retention (Hand et al., 1982b) but is not approved for use in food.

The lower pH of the MgCl₂ and especially the CaCl₂ treatments could possibly have caused lower protein extraction and greater protein-protein interaction resulting in the formation of a less open matrix. This could reduce water and fat binding, resulting in lower stability of the cooked batters (Table 1). Moderate correlation was found between pH, fat and water lost from the cooked products (Table 2). Clarke et al. (1987) have observed that a pH in the region of 6.0 resulted in poorer batters than a pH of about 6.3. However, Knipe et al. (1985) found that while MgCl₂ reduced the meat batter pH and ES, it was still able to extract enough protein to facilitate the formation of a batter.

If the pH effect is significant, then the higher pH resulting from KCl addition in this study should have facilitated greater protein extraction and better ES when compared to the standard 2.5% NGCl treatment. However, this was not the case. Further, LiCl produces a pH similar to that of MgCl₂ and the 1.5% NGCl treatments but formed a much more stable batter (Table 1). One must therefore be cautious about the pH effect when different ions are compared. Texture

The texture of the meat batter was significantly affected by the type of chloride salt (Table 3). The texture profile of the 2.5% NaCl treatment was different from that of all

	TREATMENT ^e								
Textural Parameter	NaCl (1.5%)	NaCl (2.5%)	MgCl ₂ (1.35%)	CaCl ₂ (1.58%)	KC1 (3.19%)	LiC1 (1.18%)			
Wardness 1	10 35cd	13.83ª	11.80 ^b	13.95 ^a	10.82 ^{bc}	9.27d			
(S.D.) ^f	1.80	2.82	1.63	2.97	2.07	1.86			
Brittleness	11.11 ^{ab}	11.86 ^a	6.77 ^c	7.60 ^c	11.95 ^a	10.45 ^b			
(S.D.)	1.72	2.59	0.95	1.52	2.43	1.80			
Springiness	5 88 ^a	5.91a	5.11 ^b	5.06 ^b	5.08 ^b	5.23 ^b			
(S.D.)	0.34	0.44	0.23	0.21	0.28	0.17			
Cohesiveness	0.22b	0.24ª	0.24 ^a	0.22 ^b	0.21. ^b	0.22ab			
(S.D.)	0.02	0.05	0.02	0.04	0.05	0.05			
Cumminess	2.22°	3.43 ^a	2.88 ^b	3.14 ^{ab}	2.37 ^c	2.05 ^c			
(S.D.)	0.36	1.25	0.5	0.98	0.88	0.71			
Chauiness	12 97cd	20.68ª	14.87bc	16.12 ^b	12.02 ^{cd}	10.91 ^d			
(S.D.)	1.95	8.25	2.70	5.42	4.51	3.76			

able 3	Textural parameters	of	frankfurters	prepared	with	different	chloride	salts.
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a-d - means followed by different superscripts in the same row are significantly different (P<0.05).</p>

e - IS of all treatments (except 1.5% NaCl) equivalent to 0.43.

f - standard deviation.

other treatments but the texture of the 1.58 NaCl and KCl treatments was very similar. The elasticity of both NaCl treatments was higher than the others. The 2.58 NaCl and CaCl₂ treatments were harder (p<0.01) than all the other treatments; lithium chloride produced the least hard frankfurter (Table 3). The 1.58 NaCl, 2.58 NaCl and KCl treatments were the most brittle (p<0.01) while the divalent salts produced the lowest brittleness. MgCl₂ and 2.58 NaCl produced the divalent chloride salts were rain texture except for differences in hardness and

The hardness of frankfurters is influenced by the protein concentration in the aqueous phase prior to cooking (Hamann, 1988). Therefore, the relative differences reported here may be due to different types and amounts of extracted protein as well as cation-protein interaction during cooking. The differences in hardness and cohesiveness between the ${\rm Mg}^{2+}$ and ${\rm Ca}^{2+}$ batters support the idea that they act by different mechanisms in destabilizing the batter. The MgCl2 batter was more cohesive, a textural characteristic believed to be formed prior to cooking. However, the CaCl, batter was harder and this characteristic is thought to be formed during cooking (Montejano et al., 1984; Patana-Anake and Foegeding, 1985). The differences in elasticity between the NaCl treatments and the others probably resulted from microstructural differences in the matrix structure and fat dispersion, as discussed below. Correlations between texture and ES are reported in Table 2. Hardness and springiness were the only textural characteristics related to batter stability. Patana-Anake and

Foegeding (1985) have also found that ES characteristics correlated with springiness and hardness but not with cohesiveness and Hardness, brittleness. springiness and brittleness appear to be the main characteristics which determine the final composite texture of the cooked batters. Szczesniak (1963) suggested that hardness and springiness were related to the attraction forces acting between particles and opposing disintegration. Patana-Anake and Foegeding (1985) suggested that springiness and brittleness have a similar microstructural basis and it is possible that they may be affected by the type and amount of protein extracted.

A small, inverse correlation was found between fat loss before cooking and cohesiveness (Table 2). Such a relationship would suggest that this textural parameter develops prior to cooking. Other workers have reported results which suggest that cohesiveness is among the intrinsic properties of the batter developed prior to cooking (Montejano et al., 1984; Patana-Anake and Foegeding, 1985). In addition, Hamann (1988) noted that cohesiveness depends on the functionality of the proteins extracted during manufacturing. Hence, it appears that proteinfat interaction in an uncooked batter may influence protein-protein binding, thereby affecting the cohesiveness of the final product. Microstructure

The 2.5% NaCl treatment showed an even distribution of fat globules throughout the matrix (Fig. 1A). Many of the fat globules seen under the TEM (Fig. 2A) were surrounded by a defined dark line which is believed to be the interfacial protein film encapsulating fat globules (Hansen, 1960). Borchert et al. (1967).



Figure 1.	Scanning electron micrographs of representative fields from the six chloride salt treatments.
	A = 2.5% NaCl; B = 1.35% MgCl ₂ (arrows show coalescence, a-aggregated matrix); C = 1.5% NaCl;
	D = 3.19% KC1; E = 1.58% CaCl ₂ ; F = 1.81% LiC1. (Bar = 100µm).

Lin and Zayas (1987) and Comer and Allan-Wojtas (1988) have also shown this protein film in TEM micrographs. The low NaCl treatment appeared to have a less even fat particle distribution with larger fat globules (Figs. 1C and 2C). The morphology of the low NaCl batter is probably a result of diminished protein extraction during comminution. This would make less protein available for interfacial film and matrix formation, which can lead to lower fat retention during cooking. Clarke et al. (1987) reported that an aggregated type matrix was formed as a result of high cook loss in a 1.3% NaCl low fat

The batter made with MgCl₂ had a fairly even distribution of fat throughout the matrix with the fat globules generally assuming irregular shapes (Figs. 1B and 2B). Extensive coalescence was evident in both SEM (noted by arrows) and TEM micrographs. However, a few very small, round globules could also be seen. CaCl₂ produced a matrix in which the fat appeared to be relatively evenly distributed (Fig. 1E). There appeared to be a great variation in the size of the fat globules in this treatment. The gross coalescence of fat is strikingly evident in the TEM micrograph (Fig. 2E) and was responsible for the virtual continuity of fat throughout the protein matrix. Residues of protein film were visible within the large, irregular shaped fat mass which seemed to be in the intermediate stages of coalescence. Some small, round globules were also seen.

The formation of fat channels in both divalent chloride salt treatments appear to be closely linked to the instability of the batters formed (Figs. 2C, 2E; Table 1). Fat channel formation in meat batters as well as changes in the protein matrix have been found to accompany decreased ES (Townsond et al., 1968) Carroll and Lee, 1981; Lee et al., 1981). The formation of fat channels is thought to create discontinuity in the protein matrix which causes a general destabilization of the batter and results in extensive fat and water loss (Lee, 1985). Fat



Figure 2. Transmission electron micrographs of representative fields from the six chloride salt treatments. A = 2.5% NaCl (m-matrix, p-protein film); B = MgCl₂ (f-fat, c-coalescence, a-aggregated area, g-round globule); C = 1.5% NaCl (a-aggregated area); D = 1.39% KCl; E = 1.58% CaCl₂ (r-protein film residue, t-tunnels forming fat channels); F = 1.81% LiCl (i-interwoven matrix, f-fat globule). (Bar = 10µm).



- Figure 3. Scanning electron micrograph of the matrix of the 1.39% KCl treatment (representative of the matrices in stable emulsions); p-pore. (Bar = 2µm).
- Scanning electron micrograph of the matrix of the 1.35% MgCl₂ treatment; ttunnel, f-bound fat, p-pore. (Bar = 2µm).
- Figure 5. Scanning electron micrograph of the matrix of the 1.58% CaCl₂ treatment; ttunnel. (Bar = 2μm).

particle size and distribution have also been found to affect ES; the smaller the fat particles and the more even their distribution, the more stable the resultant batter was (Lee et al., 1981).

The batters made with KCl and LiCl show fairly even fat particle distribution (Figs. 1D and 1F, respectively). The size distribution of particles within both treatments seemed to be fairly even. TEM micrographs showing their crosssections (Figs. 2D and 2F) indicated that some of the globules were irregular in shape probably resulting from the structural constraint imposed by the gelled protein matrix. The LiCl batter seemed to have a less uniform fat particle distribution than the 2.5% NaCl treatment but was otherwise similar (Fig. 2A vs 2F). The microstructures of the 1.5% NaCl and KCl treatments were very similar (Figs. 1C and 1D) and relate well to their ES. The Matrix Structure

The aggregation and denaturation patterns of the proteins in a frankfurter batter are of vital importance to the formation of the protein matrix as well as the protein envelope surrounding fat globules (Schmidt et al., 1981). While fat dispersion and fat particle size play a role in stabilizing the batter, some have suggested that the structure of the protein matrix may be the single most important factor determining batter stability (Jones, 1984). SEM micrographs of the matrices of the different treatments showed differences which might have influenced ES. The matrices of the stable batters had basically a similar appearance; a high magnification example is shown (Fig. 3). The protein matrix of the 2.5% NaCl treatment had a fine, thread-like structure which was highly interwoven and could have been the reason for its good water binding (Fig. 2A). The KCl and LiCl treatments had a similar appearance, but the LiCl appeared to produce a more highly interwoven, dense matrix (Fig. 2F). These microstructural differences seemed to be related to the observed differences in ES.

In contrast to the matrices formed by the more stable chloride salt treatments, those formed by $MgCl_2$ and $CaCl_2$ showed varying degrees of aggregation (Figs. 4, 2B and 2E, respectively). The $MgCl_2$ and $CaCl_2$ frankfurters had protein matrices which were highly aggregated with large tunnels evident throughout (Figs. 4 and 5). The matrix of the MgCl₂ batter appeared to have greater continuity and smaller tunnels than the CaCl₂ batter (Figs. 2B vs 2E and 4 vs 5). The matrix produced by 1.5% NaCl showed some aggregated areas interspersed with more open areas (Fig. 2C), suggesting lower protein extraction than the 2.5% NaCl. A constant feature observed in all treatments was the existence of small openings in the matrix (Figs. 3 and 4). These were noticeably fewer in the unstable treatments; the CaCl₂ batter had the least. These tiny openings were formed as a result of a finely interwoven, lacy matrix and are believed to hold water by capillary forces (Schmidt et al., 1984).

Jones and Mandigo (1982) and Schmidt et al. (1984) observed an apparent cooperativity between fat and water loss from products during cooking. It may be that water is lost at a faster rate and

in greater amounts than fat because it is continuous throughout the matrix and more mobile. It is likely that interprotein interaction in unstable batters causes the matrix to contract thereby creating tunnels through which water loss occurs more readily. Water loss increases the availability of protein ligands formerly involved in water binding, thus increasing protein-protein interaction. This can cause further matrix contraction, more water loss and can in turn create even wider tunnels through which the slower moving fat is able to flow. These tunnels may become filled with molten fat, forming fat channels (Figs. 2B and 2E). This could be further facilitated by the weak, thin and often incomplete protein film surrounding the fat globules in unstable batters (Fig. 2E).

Deng et al. (1981) found a large amount of broken protein film and fat separation from large fat globules in which the interfacial film was forced to assume irregular shapes. This inability of the protein film to stabilize large globules can contribute to the cooperativity between fat and water loss from unstable batters. The fact that no water was separated from the raw batters suggests good water binding in this state. Therefore, it appears that the main reasons for fat and water loss in the unstable batters were the formation of tunnels in the matrix of the cooked product and the inability of the insufficient amount of protein to form a coherent film to stabilize the fat during cooking. Microstructure Related to Texture

The microstructure of the chloride salt treatments in this study appeared to be related to their texture. Both NaCl treatments had similar springiness and brittleness which could be due to similarities in the structure of their matrices. Textural differences were possibly caused by what appears to be differences in fat particle size and dispersion as well as water loss during cooking. Angel et al., (1974) and Cassens and Schmidt (1979) have related firmness to fat globule size and distribution. The 2.5% NaCl and KCl treatments seemed to have similar fat particle distribution which may explain their similar brittleness values. However, their different texture overall may be the result of different degrees of interlinking within their matrices (Fig. 2).

The textural difference between LiCl and 2.5% NaCl treatments could possibly have resulted from the highly interwoven matrix of the LiCl treatment combined with its relatively uneven fat particle dispersion (Fig. 2A vs 2F). This would enhance its water holding properties and affect those textural characteristics (such as gumminess) which are affected by water content and brittleness which may be dependent on fat dispersion (Szczesniak, 1963; Zeigler et al., 1987).

The low brittleness of the divalent chloride salt treatments may have been due to the continuity of the fat throughout their matrices. The percentage fat content of these treatments would have increased as a result of high water losses. Greater contribution from the plastic nature of the fat to batter texture could therefore influence brittleness (Zeigler et al., 1987). The low springiness was likely a result of the low degree of integrity of their matrices. Montejano et al. (1984) found that beef muscle gels with a sponge-like texture and large pores had low elasticity. They also indicated that aggregated-type surimit gels had lower shear stress at failure but higher shear strain than nonaggregated gels. Shear stress and shear strain have been correlated respectively with hardness and cohesiveness (Hamann, 1988). The gels formed by the divalent chloride salts in this study appeared to be very similar in texture to both the beef and surimi aggregated gels described above.

The results of this study supported the findings of some previous studies on beef or pork systems. However, there were clear differences with other studies especially with respect to the effect of different chloride salts on batter stability and texture. The texture of cooked batters was found to vary depending on the chloride salt and was significantly affected by batter stability. The monovalent chloride salts formed more stable batters than $MgCl_2$ and $CaCl_2$. KCl (3.19%) produced a batter similar in texture to 1.5% NaCl. The 3.19% KCl was more similar to 2.5% NaCl than was 1.18% LiCl. Although commercial conditions were not used, it appears that proper formulation may allow the successful use of KCl in combination with other salts in the production of high quality reduced-sodium frankfurters. Product texture was also related to microstructure. The results suggest that batter stability and texture depend on the structure and integrity of the protein matrix as well as the formation of a coherent interfacial protein film around the fat globule and fat particle dispersion.

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Discussion with Reviewers

<u>Reviewer #4</u>: Test tube experiments have their limitations. Have you prepared frankfurters in commercial type formulations, eg. 11% total protein, 25% fat, to verify conclusions reached from this study?

<u>R.G. Cassens</u>: On what grounds can the results of your model system be related to a commercial frankfurter despite the differences in formulation and preparation?

Authors: It is true that test-tube experiments have limitations. However, while the fat content of our batters (ca 16%) was low compared to many commercial formulations, the protein content (ca 14%) compares favourably to levels found in some commercial products (Ockerman, 1989). The fat in our formulation was derived solely from the MDCM and was not supplemented in order to simplify the interpretation of results. Although this experiment was based on a model system, products with similar formulations have been prepared under commercial conditions and have shown comparable results (Barbut et al., 1988). Further, the results obtained for the 2.5% and 1.5% NaCl treatments are consistent with those of frankfurters prepared with standard formulations under commercial conditions (Whiting, 1984; Hand et al., 1987). In addition, Hand et al. (1982b) prepared poultry (mechanically deboned turkey meat) and pork/beef frankfurters with four of the salts used in this study and found great similarities in the behaviour of these salts in both meat systems. Their results showed pH differences between treatments similar to ours and their conclusions with respect to the effect of KCl and MgCl2 on product texture agree with ours.

<u>R.G. Cassens</u>: Was nitrite used? Was data for all three trials combined for statistical analysis? How many samples were actually viewed for microscopy in order to obtain a conclusion?

<u>Authors</u>: Nitrite was not used since we did not want to introduce additional ions into the system which could complicate the interactions that might affect product texture and microstructure. This is especially true in the case of the Mg^{2}_{+} , Ca^{2}_{+} and Li⁺ ions where their nitrite salts are not commercially available. The data from all three trials were pooled for statistical analysis after being checked for significant block effects. Any block effect which existed was adjusted for in the model used for the analyses.

For microscopy, five (5) fields from each of three (3) specimens per treatment from the first two trials were viewed. This gave a total of 30 fields per treatment.

<u>C.M. Lee</u>: Would you explain why you used such a short chopping time (4 min) and low temperature (6°C) even at a high speed? How can you assume that this preparation regimen facilitated adequate solubilization and fat dispersion? Have you considered looking at the structure under a light microscope?

Authors: The deboning process involves separation of the meat from the bone particles and connective tissue by passing the ground mix through small diameter holes in a metal screen. This results in a comminuted product with a paste-like texture in which the original myofibrillar structure has been severely disrupted. Therefore, four (4) min of chopping at high speed can provide an acceptable batter in terms of adequate protein solubilization and fat dispersion. The chopping time used was found to be sufficient to give adequate fat dispersion when mechanically deboned poultry meat is used to produce meat batters (Barbut, 1988), The final low temperature of 6-8°C was used in order to avoid the melting of the poultry fat which is more unsaturated than pork or beef fat and therefore melts at lower temperatures (Townsend et al., 1968).

In this study, light microscopy (LM) was not used. However, we are planning to use LM together with an image analysis system to further investigate the fat dispersion patterns.

Reviewer #4: Have you carried out any sensory tests to support the statements on textural differences derived from the instrument values? Authors: No sensory tests were carried out in this experiment for several reasons. The formulations used included KCl and MgCl2, both of which have been consistently shown to be objectionable in terms of taste when used by themselves in frankfurter formulation as was done here (Hand et al., 1982b; Terrell, 1983; Barbut et al., 1988). In addition, LiCl is not Generally Recognized as Safe (GRAS). Studies relating sensory texture to instrumental measurements show high correlation between TPA fracturability and sensory springiness and firmness (Keeton et al., 1984) and between cyclic compressive forces at 70% deformation and sensory elasticity, firmness and chewiness (Lee et al., 1987). TPA cohesiveness relates well to sensory cohesiveness and TPA hardness has been related to sensory hardness (Montejano et al., 1985).

<u>G.R. Schmidt</u>: In Table 1, it is shown that fat loss only varied from 0.06 to 0.55% whereas water loss varied from 2 to 24%. Isn't this convincing evidence for the theory of water binding by the protein matrix being the factor of predominant importance in batter stability?

Authors: The results in Table 1 do provide overwhelming evidence that water binding by the

protein matrix is the predominant factor in determining batter stability. Nevertheless, at least two other factors must be considered. First, the proteins which are responsible for matrix formation and therefore water binding, are the same proteins which form the interfacial film (Galluzzo and Regenstein, 1978 a,b). Consequently, poor protein extraction or protein aggregation can both result in extensive water loss and cause a weak, unstable interfacial film to be formed. In addition, even if large amounts of broken interfacial film exist, fat will be lost at a much slower rate than the less viscous water. Another factor worthy of consideration is the effect of fat spread throughout the matrix on texture. The fat may not be lost from unstable batters because of reasons already discussed, but it will be redistributed throughout the matrix (Figs. 2B and 2E). This will have undesirable effects of the product texture and acceptability as has been shown by Barbut et al. (1988).

<u>G.R. Schmidt</u>: The pH was probably of little importance since all values were above the critical level of 6.0. <u>Authors</u>: We agree with your conclusion on the

Authors: we agree with your conclusion on the effect of pH.

Authors: Fat coalescence does not necessarily result in excessive fat losses in all cases. Moreover, it is difficult to discern coalescence in SEM because fat generally appears ovoid in the plane of fracture and many of the interconnecting channels are buried within the matrix. Hence, transverse sections as in TEM or light microscopy (LM) will more easily reveal the nature and extent of coalescence. We would like to point out that the arrows in Fig. 1B are pointing to fat channels connecting two ovoid "globules". Studies are currently under way in our laboratory to examine the fat dispersion of these chloride salt treatments using LM. It may also be of interest to note that during sample preparation for TEM, semi-thin sections are viewed by LM prior to the final trimming of the embedded sample for sectioning. The fields viewed during this process showed similar fat coalescence patterns to those shown by TEM in Figure 2.

<u>G.R. Schmidt</u>: There is a general question of cause and effect for channelling and coalescence of fat and water. The large deposits of fat and water shown in Figures 2B and 2E could be the result of free water and melted fat flowing in large pores of an aggregated protein matrix. Are these deposits of fat and water seen in the raw batter?

Authors: No water was expelled after centrifugation from any of the raw batters examined in this study. This alludes to the fact that there was no 'free' water in the raw batters of any of the treatments; all of the water was bound by the protein matrix. However, it seems that cooking caused matrix shrinkage especially in the Mg²⁺ and Ca²⁺ batters which resulted in the water losses observed (Table 1). Conversely, fat was released from the MgCl₂ treatment prior to cooking and this may indicate that unstable fat does exist in the raw batter of this treatment. The microstructure of raw batters was not examined in this study. However, work is currently under way in our lab to examine the relationship between raw and cooked batter microstructure.

J.C. Acton: Brown and Toledo (J. Food Sci. 40:1061, 1975) demonstrated that fat stabilization and water stabilization were closely linked in meat batters cooked after chopping to different temperatures. If water and fat flow create the "tunnels" that you described in the matrix, what textural parameter(s) of the final product would likely be related to the degree of tunneling or channeling found? Would the described "small openings in the matrix" of the batters lead to more extensive appearance of tunnels in the case of treatments involving the chloride salts of Mg⁺⁺ and Ca⁺⁺?

Authors: The results obtained in this study indicate that the fracturability (brittleness) and the elasticity (springiness) were the textural parameters most likely affected by the degree of tunnelling in unstable batters. Fracturability has been related to sensory springiness (Keeton et al., 1984). Further, the high moisture losses in the unstable batters should lead to a relative increase in the percentage of fat in these batters and, because of its plastic nature, the fat should cause a reduction in springiness (Lee et al., 1987; Zeigler et al., 1987). The "openings in the matrix" result from the interlaced nature of the protein network and are probably sites of water binding by capillary forces. We think that in Ca²+ and Mg²+ destabilized batters, cooking leads to extensive protein-protein aggregation and matrix shrinkage. This could result in an enlargement of some of these "openings" which may therefore be the source of the fat-filled tunnels observed

<u>Reviewer #4:</u> The problem of small field size makes the interpretation of differences in the "protein matrices" difficult. Could you visibly detect, with the naked eye, major differences in the continuous phase of cooked homogenates prepared with divalent cations versus those prepared with monovalent cations? How would you describe these visual differences?

Authors: Visual differences between cooked batters made with the divalent cations and those made with monovalent cations were easily detectable. The monovalent chloride salt batters had continuous phases which were smooth and uniform in appearance. On the contrary, the divalent cations produced batters with a very coarse, uneven appearance.

<u>G.R. Schmidt</u>: The higher magnification shown in Figures 3 - 5 are more convincing than the lower magnifications shown in Figure 1. The fineness of the protein matrices shown in Figure 2 are the most convincing. At higher magnification, is the fineness of the protein aggregates shown in these treatments clearly associated with cook yield? Figures 2B and E appear to have very large aggregates and the others very fine aggregates, expecially 2F.

<u>Authors</u>: It appears that there is a relationship between the fineness of the protein matrices (Fig. 2) and water and fat binding (Table 1) and therefore cook yield. The LiCl treatment had the most highly interwoven (fine) matrix and also had the least amount of liquid lost during cooking. On the contrary, MgCl₂ and CaCl₂ produced highly aggregated matrices (Figs. 2B and 2E) and showed extensive fat and water losses.

<u>J.C. Acton</u>: What do you think was the cause or source of the interfacial film "pores" found at the surface of the dispersed fat globues by Jones and Mandigo (1982)?

Authors: Jones and Mandigo (1982) suggested that holes ("pores") were formed at weak points in the interfacial film as a result of the internal pressure built up by the expanding fat during cooking. They further suggested that increasing end-point chopping temperatures created thicker, less flexible protein films which were ruptured by thermally expanding fat. The results of our study indicate that the protein film around fat globules ruptured when it was not strong or elastic enough to restrain the expanding fat during cooking. Furthermore, a companion study on the mechanism of pore formation conducted in our lab (unpublished work) supports the theory of Jones and Mandigo (1982) and indicates that the occurrence of pores may be dependent on the nature of the protein film itself. Huber and Regenstein (1988) observed that not enough protein is extracted in meat batters to completely surround the fat globules. This may suggest that some pores are in existence prior to cooking and that fat exudation occurs through these pre-formed holes; however, additional information is needed to clarify this situation.

J.C. Acton: Do you think that the protein forming the interfacial film is interlinked with protein remaining within the aqueous phase, a) in the raw batter below 10°C of chopping, and b) in the final cooked product? The SEM micrograph of Theno and Schmidt (1978 - Figure 8) indicated a linkage between the film and matrix in a cooked frankfurter, but this is not readily evident in your SEM micrograph for cooked poultry meat batters.

It is our belief that the proteins of Authors: the interfacial film and those of the protein matrix are interlinked in both raw and cooked Hermansson (1986) hinted that all of batters. the proteins within a meat batter may be part of a continuous system. We have recently seen this binding using cryo SEM in both raw and cooked meat batters (Gordon and Barbut, submitted). It therefore appears that fat globules are bound to the protein matrix in the raw batter as well as in cooked batters. We have also seen this physical binding in conventional SEM preparations of cooked batters (unpublished data) but the micrographs in Figure 1 do not show it.

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