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CHANGES IN THE MICROSTRUCTURE OF A COMMINUTED MEAT SYSTEM DURING HEATING

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Abstract

As part of a series of experiments on heating of a comminuted meat system, image analyses were conducted to quantify microstructural changes that could be visualised by light microscopy. Three different batches of comminuted meat were investigated. Two batches (A and B) were prepared without polyphosphate; one of these (B) was deliberately overchopped. One batch containing 0.1% polyphosphate was prepared (C). All batches were heated at 11 different temperatures ranging from 40 to 115°C. The microstructure of the three types of meat batters at the end of the comminution process differed. These types of meat batters also revealed a different pattern of fat and jelly release after the different temperature treatments. This coincided with microstructural changes. The latter included changes in the area percentage, numerical density, shape factor, and size distribution of pores in the protein matrix measured in defatted histological sections. The highest mobility of fat and jelly was associated with the highest area percentage (A and C) and number of pores (A, B and C).

Key Words: Microstructure, protein matrix, image analysis, comminuted meat batter, fat channels, porosity, jelly release.

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Introduction

Raw meat batters represent a complex multiphase system consisting of fat, water, protein and other ingredients. Usually, they are formed by chopping. Extracted muscle proteins are necessary to form the continuous phase of comminuted meat batters which is required to stabilise added water, fat and other tissue components (Aguilera and Stanley, 1990; Asghar *et al.*, 1985). Therefore, the extraction and solubilisation of myosin and actomyosin during chopping is of great importance in the formation of the proteinaceous gel matrix, and thus, for the production of thermo-stable comminuted meats (Barbut, 1989; Gordon and Barbut, 1989). Although myosin is regarded as the major emulsifying protein in meat batters, collagen may also play an important role as a subsidiary emulsifier because of its hydrophobic nature (Bailey and Light, 1989).

Based on microscopical observations, two models have been introduced to explain fat stabilisation in meat batters. According to the emulsion theory, the myofibrillar proteins form a thin layer around the fat particles by which the latter are stabilised (Gordon and Barbut, 1990; Hansen, 1960; Jones and Mandigo, 1982). The non-emulsion theory suggests that fat particles are physically entrapped within the sponge-like protein matrix (Lee, 1985; Van den Oord and Visser, 1973). It is likely that both mechanisms play a certain role, depending on the batter composition and processing conditions (Smith, 1988).

During heating, fat, water and other components are immobilized by the protein network formed by protein aggregation, and the raw flowable batter is transformed into a solid product (Acton *et al.*, 1983). Instability, which is often induced by heating, involves fat coalescence or separation and exudation of water, pockets of gelatine or jelly from the porous protein matrix. As long as the integrity of the protein matrix is not disturbed, hardly any coalesced fat or jelly will be released. In meat processing, problems with instable batters still occur. Microscopical research on comminuted meat products has therefore been focused on the stabilisation mechanisms of fat and water (Aguilera and Stanley, 1990; Barbut, 1989).

The microstructure of stable meat batters is characterized by a uniform distribution of fat pools, fat cells, partly disintegrated adipose tissue, and many small fat particles, and by the presence of a continuous protein matrix, which retains its integrity during heating. During the heating process of meat batters, different reactions between the various proteins take place. By irreversible interactions, such as shrinkage, coagulation, and aggregation, discontinuity of the protein matrix may occur. It is likely that during the heating process the mobility of fat and jelly through larger pores and channels is higher in a coarse and highly aggregated matrix with dense zones than in a fine interwoven protein matrix (Hermansson, 1986; Pfeiffer *et al.*, 1985; Schmidt *et al.*, 1985).

During the last years, image analysis has played an increasingly important role in the examination of microstructural and thus functional properties of meat products. The additional information provided by image analysis can be useful in process control (Hermansson, 1988; Katsaras, 1991; Koolmees *et al.*, 1989; Olsson and Tornberg, 1991).

The objective of this study was to obtain basic information on the microstructural changes in a standardised meat batter during the different stages of the heating process. Three different batches of meat batters, prepared to create clear differences in the microstructure at the end of the comminution process, were used as model-systems for stable and instable meat products. Image analysis was used to relate the microstructural changes with morphological parameters and with the mechanism of fat and jelly release. Particular attention was paid to the porosity of the protein matrix, which included the area percentage and the number and size distribution of pores measured in defatted sections at a low microscopical magnification (x 25).

Materials and Methods

Three different batches of comminuted meat were investigated. Batches A and B were prepared without polyphosphate, while batch C contained 0.1% polyphosphate (Latuw, commercial mixture of tetrasodium diphosphate and potassium polyphosphate at a 1:2 ratio, containing 60% P_2O_5, Degens, Vlaardingen, The Netherlands). Batch A was chopped for 6.5 minutes, batch B was 'overchopped'. The batches were prepared according to the recipe given in Table 1. Beef neck meat (4°C), pork rind, ice and salt (containing 0.6% $NaNO_2$) were chopped in a bowl chopper (Laska, model KT 60-3MV, Linz, Austria) for about 7 minutes at a knife speed and a bowl speed of 2677 and 20 rpm respectively, until a temperature of 9°C was reached. Subsequently, the other ingredients (4°C) were added and further comminuted. The comminution process was continuously monitored by an WTW electrical conductivity sensor (Wissenschaftlich Technische Werkstätten GmbH, Weilheim, Germany) and a Hunter D25M-9 Tristimulus colorimeter (CTM). By means of these conductivity and colour measurements, especially lightness (L*) values, the different stages of the comminution process could be followed. The absolute values of L* and conductivity curves are of lesser significance, but their pattern of inclination is more important. The optimal moment to interrupt the comminution process, i.e., resulting in the least instability upon heating, was indicated during monitoring by the calculation of the inclination of light reflection (Δ CTM/Δ time). In this way, the monitoring procedure can be applied to predict the thermal stability of the batter (Oelker and Oelker, 1987; Van Wijk and Wijngaards, 1991).

In batches A and C, the comminution process was interrupted at the moment when the conductivity and L* values had reached a plateau (Figures 1A-1C). In batch B, the chopping procedure was stopped after these values had decreased. In this way, three batches with a different microstructure were obtained. The comminution process of batch A was stopped at a L* value of 41.0 and a conductivity of 0.58 mS/cm. At that point the batter was chopped for 6.5 minutes and had reached a temperature of 23°C. Batch B was overchopped. Here, the comminution was interrupted at a L* value of 41.5 (highest value 45.2) and a conductivity of 0.57 mS/cm (highest value 0.67). At that moment, the chopping time was 10 minutes and the batter temperature was 25°C. Batch C was chopped for 9 minutes. The temperature of this batch rose to 28°C. The chopping process was interrupted at a L* value of 48.1 and a conductivity of 0.73 mS/cm. In order to evaluate the relation between the monitoring system used and the heat stability of the three different batches of meat batter during the different stages of the comminution process, fat and water losses were determined. At different time intervals during the comminution process, 200 g cans were filled with batter. These were heated at 115°C (F_0-value 2.0) for 75 minutes. After cooling to 23°C, fat and water losses were determined (Figures 1A-1C). As described, the comminution process was monitored by conductivity and L* values, rather than by temperature. End-temperatures > 23°C are not commonly used in practice. However, the purpose of this experiment was only to create clear differences in the microstructure of the meat batters at the end of the comminution process.

The chemical composition of the unheated meat batters was determined by the International Organization for Standardization procedures [ISO/R937 (1969); ISO 1442 (1973); ISO 1444 (1973)] and is shown in Table 1.

Immediately after the comminution process, 200 g cans were manually filled with the meat batter. Unheated cans served as control samples. The remaining cans were heated for 75 minutes at 40, 45, 50, 55, 60, 65 and 70°C in waterbaths and for 75 minutes at 100, 105, 110 and 115°C in an autoclave. The latter temperature was chosen in order to simulate a retort process at a F_0-value of 2.0. The core temperatures were recorded with thermo-couples. After cooling to 23°C,

Table 1. Recipe and chemical composition of the meat batters in percentages.

Batches	Batch A without phosphate	Batch B without phosphate overchopped	Batch C with 0.1% phosphate
Beef neck meat	26.0	26.0	26.0
Pig head meat	31.0	31.0	31.0
Precooked pork rind	5.5	5.5	5.5
Pork fat	20.5	20.5	20.4
Added ice/water	15.0	15.0	15.0
Salt	2.0	2.0	2.0
Polyphosphate	0.1		
Fat	29.1	29.4	30.2
Moisture	56.7	56.8	55.9
Total protein	12.5	13.2	12.0

fat and water losses were determined as described by Tinbergen and Olsman (1979). In the same material, the consistency (penetration value in mm) of the batters after different temperature treatments was measured by means of a penetrometer (C type PNR8, SUR, Berlin, Germany) at 15°C. By means of this device, the distance in mm, penetrated in the batters by a conical steel probe (weight 31.5 g, angle 30°) during 5 seconds, is measured. For each sample, the penetration value was measured 10 times. The value thus measured, provides information about the consistency of finely comminuted meats. A high value corresponds with a lower consistency; in tough batters with a high consistency the conical steel probe hardly penetrates.

For microscopical examination, eight samples (each measuring 1.5 x 1.5 x 0.5 cm) of batters after the different temperature treatments (40, 50, 60, 70, 100 and 115°C, and unheated controls) were taken and fixed in a buffered neutral formalin solution for 24 hours. Simultaneously, samples each measuring 1.0 x 1.0 x 0.5 cm were collected and frozen in isopentane cooled with liquid nitrogen. The frozen samples were cryosectioned (Reichert-Jung 2800 Frigocut N), after which 8 μm thick sections were stained for fat with Oil red O (Cassens *et al.*, 1977) and for connective tissue with picro Sirius red F3BA (Flint and Pickering, 1984). The formalin-fixed samples were embedded in paraffin and sectioned at 5 μm thickness using an American Optical 820 microtome. Subsequently, the sections were stained for protein with toluidine blue (Swasdee *et al.*, 1982).

All sections were examined by light microscopy at different magnifications to observe structural differences between the three batches and between the different temperature treatments. During microscopical examination, particular attention was paid to swelling and shrinkage of intact collagen and muscle particles, the porosity of the protein matrix, and the fat distribution. Micrographs of control samples and of the major changes observed in the batters after the different temperature treatments were made (Axioskop Photomicroscope, Zeiss, Oberkochen, Germany).

To quantify the observed changes, the following morphological parameters (Russ, 1990) were determined in the defatted paraffin sections by image analysis: the density of pores, defined as the number of pores per 2.74 mm² (area of the digitized image); the area of all pores as a percentage of the total area measured; the size distribution of the individual pores, plotted in histograms of eight class ranges between 100 μm² and 1 mm²; and the shape factor of individual pores, indicating the degree of roundness (e.g., 1.0 describes a circle, while lower values correspond with irregular shapes). Image analysis was done with an IBAS system (Kontron Bildanalyse GmbH, Eching b. München, Germany) and a television camera (Panasonic WV-CD50) connected directly to the microscope. The measurements were conducted at a microscopical magnification of 25x. At this magnification, the number of pixels per pore is too small to allow accurate measurements of pores < than 100 μm². Hence, pores < 100 μm² were disregarded in the image analysis. The latter area corresponds with a diameter of 11.3 μm.

Data from the different morphological variables from the three batches were subjected to Student t-tests. Correlation coefficients were determined on stability and morphological variables.

Results and Discussion

Batter stability and consistency

The fat and water loss percentages and the L* and conductivity values of the three different batches during the comminution process are presented in Figures 1A-1C. These results reveal that changes in the inclination pattern of L* and conductivity, particularly when a plateau was reached, were associated with an increase of the thermal instability which was determined afterwards. It seems that this monitoring procedure as described by Van Wijk and Wijngaards (1991) can be applied to predict the thermal stability of finely comminuted meat batters.

The fat and water loss percentages and the results of the consistency measurements of the three batches after heating at different temperatures are shown in Figure 1D-1F. In batches A and C, the jelly release started at 60°C and showed a marked increase between 70 and 100°C. In batch A, both fat and jelly losses still rose gradually at temperatures above 100°C, whereas these losses remained at the same level in batch C. This means that the addition of polyphosphate prevented higher fat and jelly losses after heating at temperatures > 100°C. Fat losses only occurred at higher temperatures in both batches. As expected, batch B showed much more instability and a different pattern of fat and jelly release. Here, fat and jelly losses occurred from 40°C onwards at the same time. A sharp increase was observed between 50 and 60°C,

Table 2. Correlation coefficients for batter stability and microstructural characteristics[a].

	Batch A: without phosphate, overchopped	Batch B: without, phosphate	Batch C: with 0.1% phosphate
Area percentage of pores/jelly released	0.97^{**}	0.96^{**}	0.81^{*}
Area percentage of pores/fat released	0.94^{**}	0.97^{**}	0.23^{NS}
Jelly/fat released	0.87^{*}	0.97^{**}	0.70^{*}
Area percentage of pores/shape factor of pores	0.33^{NS}	0.82^{*}	0.92^{**}
Area percentage of pores/density of pores	0.90^{**}	0.34^{NS}	0.97^{**}
Shape factor of pores/density of pores	0.54^{NS}	0.26^{NS}	0.82^{*}

[a]number of observations = 6 (different temperature treatments).
**significant ($p < 0.01$); *significant ($p < 0.05$); ^{NS}not significant.

while only slight changes in loss percentages were seen at higher temperatures. There was a good correlation between jelly and fat losses in all batches (Table 2). The overall picture of fat and jelly release observed in the three different batches suggest that water loss is the main result of instability of meat batters. Gordon and Barbut (1989) and Schmidt (1984) came to the same conclusion.

The penetration measurements provided information about the consistency of the meat batters after heating at different temperatures. In batches A and C, the meat batters were rather solid after heating at 55°C. In batch B, this was the case after heating at 65°C. The consistency of this batch remained at the same level also after heating at higher temperatures. This is probably due to the large amount of fat and jelly which is already released after heating at 65°C. In batches A and C, the batters became somewhat softer with increasing fat and jelly release after heating from 100°C onwards.

Microscopical examination

For easier comparison of structures, micrographs (Figures 2A-2C, 3A-3C, and 4A-4C) are presented together.

The different patterns of fat and jelly release of the three types of meat batters after different temperature treatments coincided with microstructural changes as observed by light microscopy. In batch A, the microstructure of the unheated batter was characterized by a continuous protein matrix of disintegrated muscle and collagenous tissue in which intact adipose tissue, individual fat cells, and many smaller fat particles were evenly distributed (Figure 2A). The presence of intact fat cells was confirmed by the observation of intact fat cell membranes in the frozen sections stained with picro Sirius red. After heating at 40°C, a distinct swelling of the precooked rind was observed (Figure 5) which resulted in a more dense microstructure. Between the partly denatured pork rind particles (fading of nuclei and fibrous structure normally present in raw connective tissue) and the surrounding protein matrix, a transition area consisting of gelatine can be observed. No substantial changes were observed between 40 and 70°C. After heating at 70°C, some jelly originating from the pork rind was squeezed out of the structure (Figure 2B). A different microstructure was visible after heating at 100 to 115°C, indicating that the protein matrix could no longer resist the pressure differences caused by heating of the different tissues. The matrix showed some discontinuity while considerable amounts of fat and jelly were squeezed out of the structure through channels. Between 100 and 115°C, the fat and jelly release still increased resulting in a rigid matrix with a higher porosity. Only small amounts of gelatine remained in the matrix (Figure 2C).

Figure 1A-1C (on the facing page). Lightness (L*) and conductivity (mS/cm) values as a function of time and temperature, recorded during the comminution process. Fat and water loss percentages after heating at 115°C were determined at different time intervals during the comminution process.

In batch B, the microstructure of the unheated control was characterized by large channels which disturbed the integrity of the coarse, aggregated matrix (Figure 3A). After heating at 40°C, the pork rind particles started to gelatinize and disturbed the relatively soft and viscous meat batter by pressure. In this meat batter heated at 50°C, channels partially filled with gelatine were observed (Figure 3B). The porosity of the matrix increased between 40 and 60°C and a large amount of fat and jelly was released. After heating at 60°C, this fat and jelly release together accounted for 27 weight percentage of the starting material (Figure 1D-1F). From 60°C onwards, a more dense microstructure with less large channels was observed. This was probably caused by shrinkage of the protein matrix and the fact that relatively more myofibrillar muscle protein remained to form a more dense matrix with a lower porosity. At temperatures between 45 and 80°C, the ability of myosin to form a three dimensional network of fibers and to bind meat particles is increased

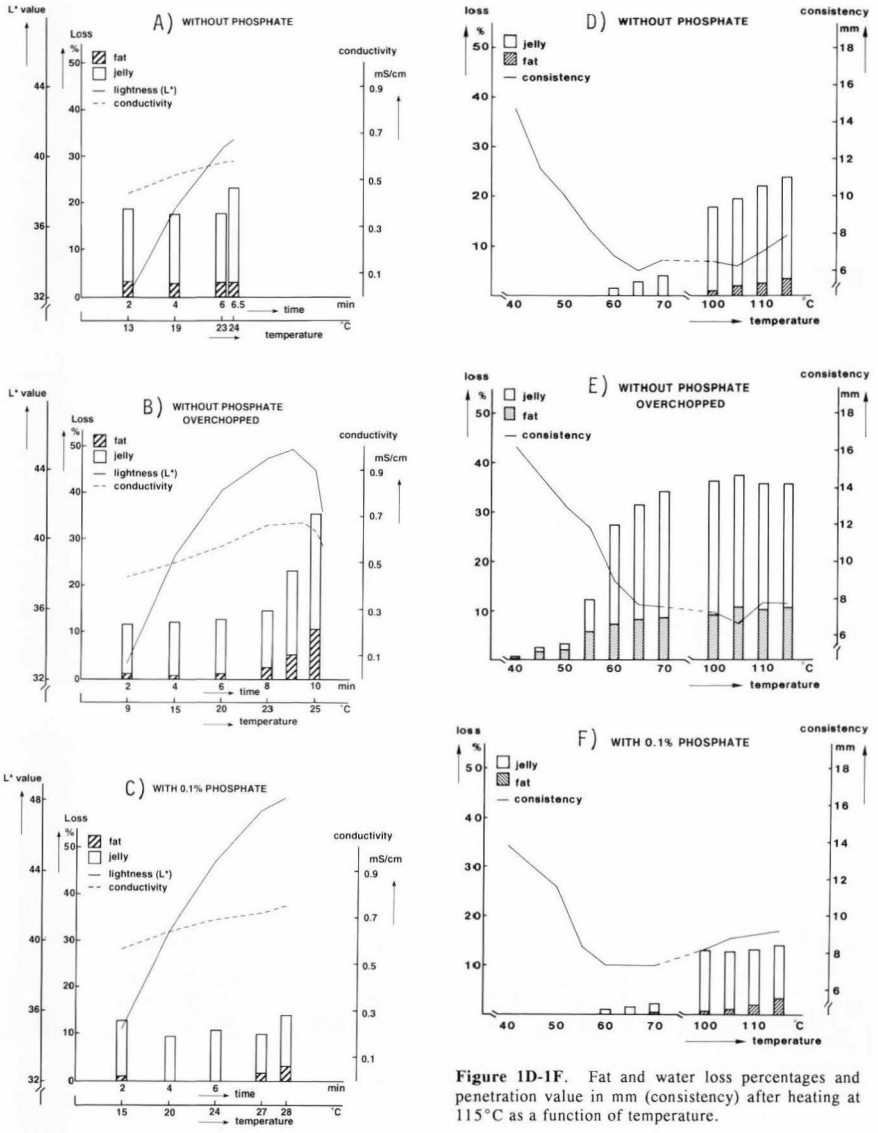
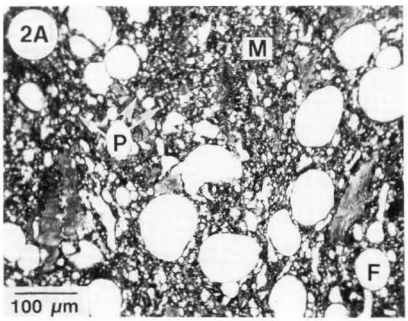
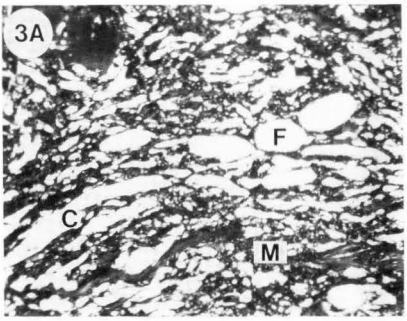
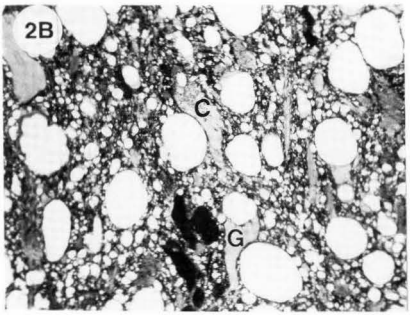
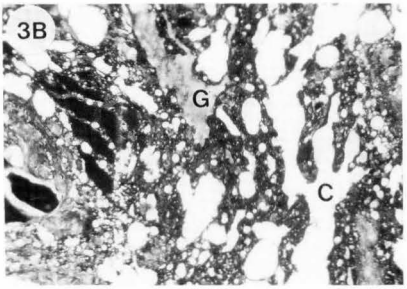


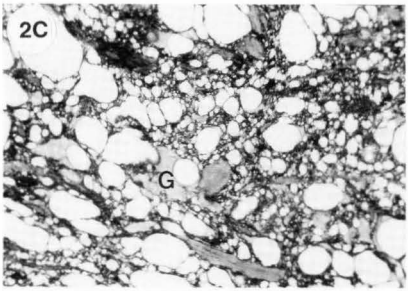
Figure 1D-1F. Fat and water loss percentages and penetration value in mm (consistency) after heating at 115°C as a function of temperature.











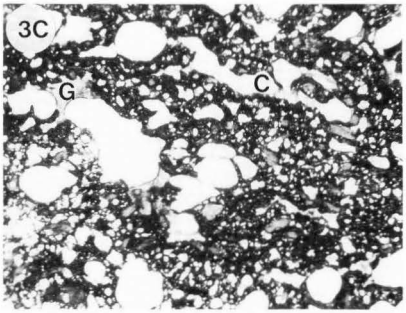
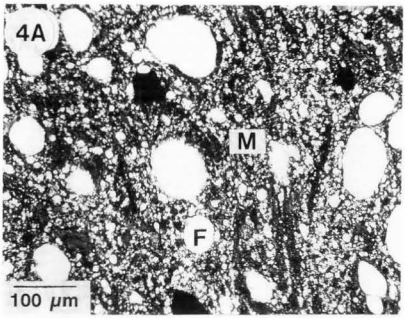
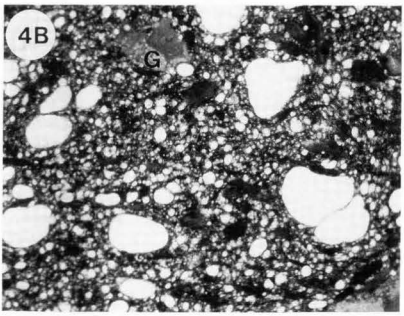


Figure 2. Micrographs of meat batter A after chopping (**A**), and after heating at 70°C (**B**) and 100°C (**C**).
Figure 3. Micrographs of meat batter B after chopping (**A**), and after heating at 50°C (**B**) and 100°C (**C**).
P = pores, M = matrix, C = channels, G = gelatine, F = intact fat cells. All figures at identical magnifications.





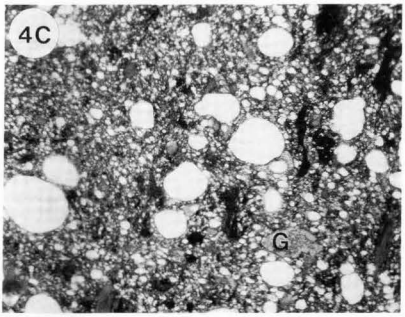


Figure 4. Micrographs of meat batter C after chopping (**A**), and after heating at 70°C (**B**) and 100°C (**C**). M = matrix, G = gelatine, F = fat cells.

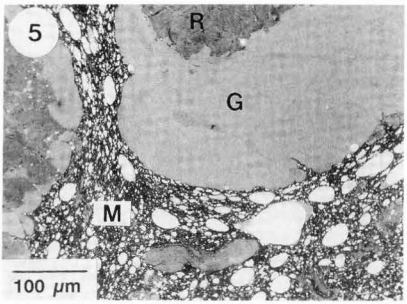


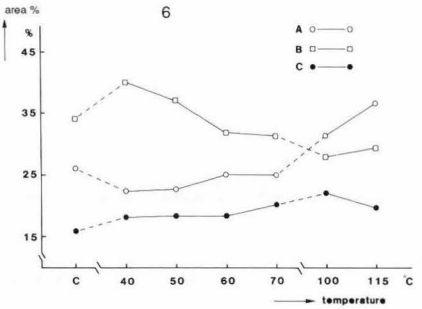
Figure 5. Micrograph of precooked pork rind in meat batter A after heating at 40°C. M = matrix, R = rind, G = gelatine.

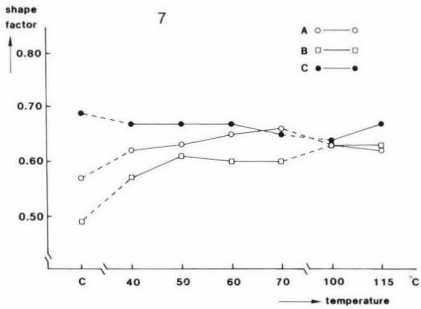
(Schmidt *et al.*, 1981; Siegel and Schmidt, 1979). Between 60 and 115°C, batter B showed a similar microstructure. After heating at 100°C, the porosity decreased slightly due to further shrinkage of the protein matrix (Figure 3C).

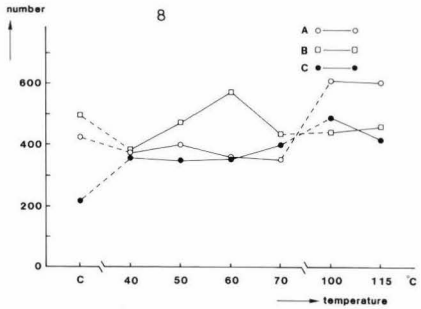
The microstructure of the unheated control of batch C was characterized by a very dense and uniform protein matrix in which the fat was finely dispersed (Figure 4A). The effect of polyphosphate on the protein matrix and the fat dispersion is obvious and in agreement with earlier observations (Koolmees *et al.*, 1989). Compared with batches A and B, the microstructure of batch C did not change to a great extent between 40 and 70°C. In batch C, some aggregation of the finer pores occurred between these temperatures. The matrix was not disturbed by the swelling of the pork rind particles. After heating at 70°C, the matrix was still intact (Figure 4B). At higher temperatures ($\geq$ 100°C) the area percentage had slightly increased. This coincided with a fat and jelly release of 13%. Between 100 and 115°C, this release remained at the same level and no significant microstructural changes were observed (Figure 4C).

Image analysis

The results of the porosity measurements which refer to the mean total area of all pores in percentages of the total area measured, are presented in Figure 6. These results are largely in agreement with the observations by light microscopy. Batches A and B had a higher area percentage than batch C after all temperature treatments. In batch C, the area percentage of the matrix was affected by the addition of polyphosphate; the area percentage of pores remained relatively low at the same level with an average of 19.1 $\pm$ 1.9% after all temperature treatments. Partly, the finer pores of batch C, which can be observed in Figures 4B and 4C, were smaller than the lower measuring level used in image analysis. Apparently, a sufficient amount of extracted







Figures 6, 7 and 8. Mean total area of pores in percentages (**Figure 6**), mean shape factor of pores (**Figure 7**) and mean density of pores expressed as number per 2.74 mm² (**Figure 8**) measured in meat batters A, B and C after chopping (c) and after heating at different temperatures.

myosin was available to form a dense protein matrix in which most of the fat was stabilised (Barbut, 1989). Until heating at 70°C, the batters of batch A showed a constant area percentage of 24.3 ± 1.6% and the matrix was capable of binding fat and jelly. After heating at higher temperatures, this batter became more porous (average of 34.1%). At these higher temperatures, the protein network was no longer able to resist the pressure differences and disintegration occurred. This resulted in a considerable fat and jelly release. In batch B, the pattern was reversed; a high area percentage at low temperatures and a more dense matrix at higher temperatures. Most of the jelly and fat release had already occurred at relatively low temperatures, while after heating at temperatures from 60°C onwards, the matrix became more rigid and dense. On average, the area percentage of pores was high at all temperatures (33.2 ± 4.2%).

The relation between jelly and fat release on the one side and porosity on the other side, which we had observed in the batches microscopically, was confirmed by the determination of the area percentage, the mean density and the size distribution of pores by means of image analysis. The correlation coefficients between area percentage and fat and jelly losses were significant for batches A and B (Table 2). In batch C, the correlation between fat release and area percentage was not significant (0.23). This was caused by the fine pores which were below the detection level and therefore not measured by image analysis. The fine pores retain fat very well. Due to these fine pores, the water loss is somewhat lower than in batches A and B. Fat and water release in batches A and C occurred from 60°C onwards and in B from 40°C onwards. This also affected the correlation coefficients between area percentage and fat and water losses.

The results of the shape factor measurements, which are presented in Figure 7, revealed that a high value was associated with a high batter stability. In all batches, higher shape factors were measured for the smaller fat droplets. Theoretically, a low surface/volume ratio is obtained with a spherical form. This phenomenon is probably due to the Laplace pressure by which emulsion droplets will always assume a spherical shape, since any deformation causes the local radius of curvature to decrease, hence the pressure to increase (Walstra, 1985).

The differences in shape factor can be observed when batches B and C are compared. When the amount of extracted protein was low or when the batter was overchopped, aggregation occurred and the integrity of the matrix decreased resulting in lower shape factors of the pores. The shape factor of pores in the control sample of batch B, which was overchopped, was very low. When sufficient extracted protein was available to surround the fat globules, the shape factor was high. Batch C had a compact and uniform matrix which was reflected by a high mean shape factor of the pores (0.67). Furthermore, intact fat cells with an average

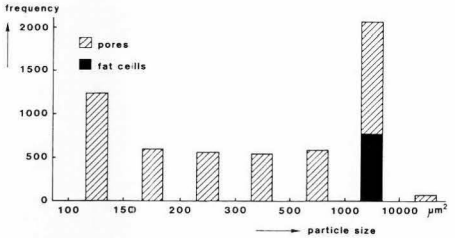


Figure 9. Size distribution of pores and frequency of intact fat cells in the total number of pores present in batch A after heating at 70°C.

shape factor of 0.75 ± 0.07 (Koolmees *et al.*, 1989) contributed to this higher mean value of the shape factor at all temperatures. At the end of the chopping process, the fat was finely dispersed in a tightly interwoven matrix with a high integrity. This microstructure only slightly changed during the heating process.

The results of the density measurements of pores in the three batches are presented in Figure 8. At the moment where the highest fat and jelly release had occurred, also a significant ($p < 0.01$) increase in the number of pores was noticed. In batters A and C, this was the case after heating at 100°C, and in batch B, after heating at 60°C. A significant ($p < 0.01$) increase of pores between the unheated batters and after heating at 40°C was also measured in batter C. However, no fat and jelly release occurred upon heating at 40°C, and hence no correlation between the two. This increase was due to aggregation of the protein matrix resulting in more pores larger than the detection limit. In batches A and C, there was also a strong correlation between the area percentage and the density; 0.90 and 0.97 respectively. After heating at temperatures above 100°C, these batters were more porous, which was associated by an increased number of pores. This 'increase' was caused by coagulation of pores which were originally too small to be included on the image analysis system before coagulation but which were recognised after coagulation. In batch B, this pattern was different. Upon heating at 40°C, the area percentage of pores in the batter increased, however, the number of pores decreased. This can be explained by the formation of large pores and channels. Between 40 and 60°C, the area percentage decreased while the number of pores increased. After heating at 60°C, the protein matrix was more dense and the large channels had disappeared. Most of the fat and water losses have occurred by 60°C which suggests that the unstable system collapses during cooking up to 60°C and at around 60°C a more stable, if somewhat coagulated structure has been formed. Between 60 and 70°C, the number of pores decreased significantly ($p < 0.01$) by shrinkage of the matrix. At higher temperatures, the density and area percentage remained at the same level.

The pore size distribution provided information about the contribution of each of the eight size classes and of intact fat cells to the total distribution. In all batches, the largest contribution in the total size distribution consisted of pores from size class 1 ranging from 100-150 μm^2 and class 6 ranging from 1,000-10,000 μm^2. In batches A and C, a considerable increase of pores in all classes was observed between 70 and 100°C. In batch B, a decrease in all classes was noticed between 60 and 70°C. Size class 6 had the highest contribution in batch A at all temperatures. In batch C, this was size class 1. Batch C with the highest stability had a uniform matrix characterised by many small and round pores. This was revealed by the significant correlations between density and shape factor ($p < 0.05$) and between area percentage and shape factor ($p < 0.01$). Apparently, the microstructure of batch C was able to limit the fat and jelly release during the heating process. The stability was determined by the physical properties of the protein matrix and the fat distribution pattern at the end of the comminution process. During the subsequent heating process, the microstructure of this batter did not substantially change. This observation is in agreement with research findings of Carroll and Lee (1981) and Lee *et al.*, (1981).

By comparing the different sizes of the pores with the average fat cell area (3,400 μm^2, diameter 66 μm) and shape factor (0.75), it was possible to assess the contribution of intact fat cells to the total area occupied by pores (Koolmees *et al.*, 1989). In Figure 9, the size distribution of pores and the frequency of intact fat cells in the total number of pores present in meat batter A heated at 70°C is shown. The area contribution of intact fat cells to the total area of pores present in the three different batches, calculated as the mean average of controls and samples heated at different temperatures, was 24.5% for batter A, 5.2% for B, and 18.9% for C. The latter results confirm the fact that the integrity of fat cells plays an important role in the stabilization mechanism of fat during the heating process.

The amount of intact fat cells in batches A, B and C also indicate the different effect of chopping time and shear force in these batters. Batter A contained a relatively high amount of intact fat cells due to the shorter chopping time. Batters B and C were both chopped longer, but showed a considerable difference in percentage intact fat cells. In batch B, the longer chopping time resulted in the formation of a coarse aggregated protein matrix with many disrupted fat cells and coalesced fat in large channels. In batch C, the addition of polyphosphate provided a uniform protein matrix with many fine pores. This matrix with a high consistency and toughness protected intact fat cells from being coalesced or damaged. It seems that extended chopping times of a meat batter with a high amount of released muscle protein involve more mixing

than comminution of the components during the last stages of the chopping process.

Conclusions

The microstructure of the three types of meat batters at the end of the comminution process differed. These types of meat batters also revealed a different pattern of fat and jelly release after the different temperature treatments. These changes coincided with microstructural changes of the protein matrix and the fat distribution as observed by light microscopy and determined by image analysis at a magnification of 25x. In each batter, the highest mobility of fat and jelly was associated with the highest area percentage (A and C) and number of pores (A, B and C). The structure of the protein matrix and the fat distribution pattern at the end of chopping were crucial for the thermal stability. Upon heating, the more dense protein matrices without disruptions could better withstand pressure differences caused by swelling and shrinkage of the different constituents of the batters during the transformation of a viscous mass into a solid product. Further, the integrity of fat cell membranes represented an important factor in the mechanism of fat stabilization.

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Discussion with Reviewers

F.W. Comer: Not only fat globules, but also water and air, produce the pores which were observed and measured. Indeed, more water than fat is lost in cooking. The batters were manually filled incorporating air. Were you able to determine the contributions of water and air to the pore area and frequency?

A. Gordon: How can you differentiate holes in the matrix caused by fat removal from spaces normally present in the matrix?

Authors: We are aware of the fact that not only fat but also air and water contributed to the area and frequency of the pores which were observed and measured in protein-stained paraffin sections. In spite of this limitation, we prefer to conduct measurements by image analysis in comminuted meat systems with paraffin sections and to use fat-stained cryosections as controls. Compared with paraffin sections, the morphology of cryosections of comminuted meats is rather poor due to disruption during sectioning, and dislocation and slippage of smaller fat globules by the staining and mounting procedure. Moreover, paraffin sections of 5 μm thickness allow more accurate image analysis measurements at higher magnifications than 8 μm thick cryosections.

To evaluate the presence of air pockets in the sections, we applied dark-field microscopy and polariscopy to unstained frozen sections. In this way, it was possible to distinguish between fat, protein and empty areas in the sections. From these observations, we concluded that the contribution of air-produced pores to the total surface area, measured in protein stained sections, was negligible. Palombo (1990) observed that almost all entrapped small air bubbles in a comminuted meat system formed rather large round bubbles (diameter $\geq$ 400 μm) by disproportionation within 36 minutes after the end of chopping. In our experiments, we did not measure such large round pores. The amount of entrapped air in a well defined porcine lean meat batter was estimated by Palombo at 5 to 8 volume %.

The aim of our investigation was to determine any distinct microstructural changes of the meat batters upon heating. The three different batters used in our experiment all underwent the same preparation treatment. Hence, the presence of only a few smaller air bubbles could not have had a significant effect on the differences between the meat batters during heating that we determined by image analysis.

Water holding by capillary forces mainly occurs in small pores of 0.1 to 2.0 μm (Hermansson, 1986). These pore sizes are well below our detection limit. Therefore, the contribution of water to the pore area and distribution could not be determined in our experiments. In order to study water holding in meat batters, high resolution micrographs such as those from transmission electron microscopy (TEM) are required. The aim of our experiments, however, was not to study this phenomenon, but to quantify microstructural changes that could be visualized by light microscopy. The contribution of the small water-produced pores to the pore area and distribution did not effect the measurements by image analysis at a low magnification nor the results concerning microstructural changes upon heating which are based on these measurements.

E. Tornberg: Only pores larger than 100 μm^2 could be measured accurately. According to Hermansson (1986), pore sizes of 0.1-2.0 μm are of great importance for the water-holding capacity of many foods. Is the magnification used in your investigation too small to study pores of that size in the batters?

Authors: In our experiment, we conducted image analysis in paraffin sections of 5 μm thickness at a magnification of 25x. The pore size mentioned by Hermansson (1986) lay well below our detection limit of 11.3 μm diameter. At a magnification of 25x, the number of

pixels per pore are too small to allow accurate measurements of pores $\leq 100\ \mu m^2$. The aim of our investigation was not to study the water holding capacity but to study the possibilities to quantify microstructural differences observed by light microscopy. The main changes in the microstructure of heated meat batters which we observed in the experiments reported here refer to fat-holding. We are planning to use light microscopy at higher magnifications and TEM to further investigate the relation between porosity and water-holding in meat batters.

D.F. Lewis: In all microscopical observations features which are smaller than the level of detection can have a profound influence on the interpretation of results. In image analysis, this effect can be more sinister since once the results are presented as numbers the original image becomes insignificant. If I have understood the text correctly, then pores smaller than $100\ \mu m^2$ will not be measured. Let us consider the hypothetical size distribution (number distribution) for samples A and C presented in Table 3. If the image analysis ignores those pores below $100\ \mu m^2$, then A and C raw will appear to be identical, on cooking A will appear to get coarser (which it does) whilst C will appear to get finer (which it doesn't). This effect seems to be operating in your samples; Figure 4A clearly shows a structure with many very fine pores and this is not reflected in the image analysis data. How do you propose to overcome this problem?

Authors: We agree with the reviewer that the interpretation of image analysis figures without taking the pictorial evidence into account may lead to misunderstanding. Microscopical results obtained by image analysis and presented in numbers should always be evaluated together with the original image. However, qualitative microscopy alone is likely to involve subjectivity of the observations due to the limitations of the human eye. Image analysis provides the possibility to reinforce descriptive qualitative microscopy by quantitation and by characterizing microstructural features in figures. Furthermore, these figures allow comparisons with physical-chemical results and statistical analysis. For example, image analysis was successfully applied to characterize the different microstructure of meat batters which were heated either by a continuous process in a radio-frequency field or conventionally in a water bath, and to compare microstructural features with rheological properties of these batters (Van Roon *et al.*, 1994).

Of course, there are also limitations in the application of image analysis when evaluating meat batters. One of the problems involved, is the magnification used and the large variation in size of certain microstructural features to be studied. When image analysis is applied in TEM, the samples taken at random are relatively very small and the size of microstructural features like pores may well be larger than the field of view. The same goes for light microscopy at higher magnifications. In other words, there is a risk that microstructural changes occurring at low magnification levels may be overlooked. On the other hand, however, a low level of magnification could also miss some of the key changes. Hence, each magnification has its own limitations and therefore, the chosen magnification is often a compromise. It is our opinion that depending on the morphological features under investigation, microstructural studies including image analysis should begin with light microscopy at lower magnification levels. Depending on the results, this may be followed by a study of specific features at higher magnification levels.

As for the hypothetical size distribution given by the reviewer, we do not consider this as being realistic, especially the one given for C (70°C). It is our experience that also at higher magnifications, the lowest size range always contains the largest number of pores. In earlier research (Koolmees *et al.*, 1989) we used two magnifications, i.e., 40x and 250x, the latter with a lower limit of sizing of $3.5\ \mu m^2$, and observed the same size distribution. The setting of the lower detection limit is determined by the section thickness and the magnification used. In a pilot study, in which we determined the size distribution of pores in plastic embedded sections of $1.0\ \mu m$ thickness at a magnification of 400x, the lowest size range also contained the highest numerical density. In other words, the thinner the sections, the higher the detection level and hence, the more smaller pores become visible and detectable. The contribution of these very fine pores to the total area percentage of pores is relatively small. So the number of pores should always be evaluated together with the size range of the pores. At light microscopy levels, the presence of a large number of large pores provides more information about the thermal stability of a batter than the exact number of very fine pores which indicate the amount of emulsified smaller fat droplets. In other words, the different morphological parameters should be taken into account separately as well as together when the relation between microstructure and fat and water losses are evaluated.

We are aware of the fact that at the magnification level used in the experiments described here, pores $< 100\ \mu m^2$ were disregarded and hence, not all fine pores were reflected in the image analysis data. As described above, this limitation is inherent to any chosen lower detection level. However, using image analysis at a higher magnification would have made it impossible to properly measure the number and area percentage of the larger fat channels and pools of coalesced fat which were present in batch A and particularly in B. Therefore, we chose the magnification 25x which proved to be suitable for measuring all microstructural changes occurring at low light microscopy levels in all three different types of meat batters. In addition to this experiment, we are planning to perform more experiments to further investigate the relation between fat and water binding and porosity at higher magnifications and by TEM.

Table 3. Hypothetical size distributions mentioned in question by D.F. Lewis.

size range	< 100	100-150	150-200	200-300	300-500	500-1000	> 1000μm^2
% in A (raw)	40	20	5	5	5	5	20
% in C (raw)	70	10	2.5	2.5	2.5	2.5	10
% in A (70°C)	25	20	5	7.5	7.5	10	25
% in C (70°C)	22.5	55	5	2.5	2.5	2.5	10

D.F. Lewis: I cannot agree that the matrix of the unheated control of batch C was very dense, Figure 4A shows a matrix which is full of small holes.
Authors: We do not agree with the reviewer. We described the microstructure of the protein matrix of the unheated control of batch C as 'very dense and uniform' (Fig. 4A). We made this remark while comparing this structure with the matrices of batches A and B (Figs. 2A and 3A) at a magnification of x25. In spite of the small holes in batch C, which indeed are present, the overall impression is that of a more dense structure than A and B. Moreover, in batch C the matrix is not interrupted by channels or any other irregularly shaped holes, as is the case in batters A and B.

A. Gordon: How many times was the experiment repeated, i.e., how many sets of each batter were prepared and examined independently, as described? If only one set was done this would explain the fact that correlations between the parameters discussed were not uniform across batches (Table 2).
Authors: The main target of our investigation was to create clear differences between batches during heating. Therefore, we needed model systems which were not prepared according to conditions in practice. The experiment was not repeated. However, we disagree with the reviewer when he states that 'this would explain the fact that correlations between the parameters discussed were not uniform across batches (Table 2)'. Batches A, B and C represented three different comminuted meat systems, prepared under distinctly different conditions. Repeating the experiment would again lead to three different systems in which correlations between the parameters discussed would still be different for the three separate batches, i.e., without polyphoshate, overchopped and with polyphosphate.

A. Gordon: What are you defining as a pore (versus a fat globule/particle)?
Authors: In our experiment we defined pores as holes (not limited by any size) present in the defatted paraffin sections. Of course, all artifacts present in these sections were disregarded during image analysis measurements.

A. Gordon: I would suggest that a distinction be made between "pores" (diameter $\leq$ 12 μm) and tunnels or very large openings in the matrix which form routes out of the batter for the exist of fat and jelly (Gordon and Barbut, 1989; 1991).

Authors: Under 'Microscopical examination' we discuss the pattern of fat and jelly release. We agree that fat and jelly are squeezed out of the batters mainly through larger tunnels. However, to make a distinction between pores $\leq$ 12 μm diameter and larger tunnels is rather difficult. In our opinion, the size of 12 μm is quite arbitrary. How would you define a tunnel by means of shape and size? From what size on would a pore become a tunnel, since mobility of fat and jelly also occurs in smaller pores. Because of these reasons we see no benefit in making such a distinction.

A. Gordon: The authors keep mentioning the effect of pressure differences but I am not clear as to whether any measurements of pressure were done to justify these statements.
Authors: No pressure measurements were conducted. However, we mention pressure differences because we observed certain changes in the microstructure that implicate the occurrence of pressure. For instance, we observed a distinct swelling of precooked rind particles which consequently pushed the surrounding matrix aside (Fig. 5). On the other hand we observed mobility of fat and jelly from the inner part of the cans to the outer parts by shrinkage of the protein matrix upon heating.

A. Gordon: What are the dark areas in the protein matrix? What causes them?
Authors: These are particles of intact muscle fibres.

E. Tornberg: The number of pores or density, as expressed in the article, gives a hint of the distribution of the pore sizes. Therefore, perhaps a quotient between area% and density would be a more appropriate way of expressing porosity. Tornberg *et al.* (1989) have suggested the calculation of a mean d_{al}, where both the area of the pores and their perimeters are considered. Could some sort of a mean of pore size distribution be presented?
Authors: The mean average of pore size distribution cannot be used as a reliable figure since it may consist of extremes of a great number of very small pores and a small number of very large pores, and thus, resulting in a very large standard deviation.

Indeed, porosity may be better defined when such a quotient is used. The quotient of n/area% was determined (see Figure 10). This did not result in a more appropriate figure which can be used in correlation and ANOVA calculations. It is our opinion that

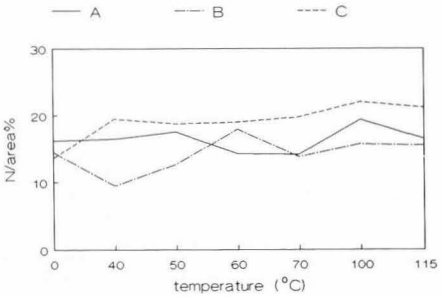

Figure 10. Quotient between number of pores (N) and area% of pores present in control samples of batches A (without phosphate), B (without phosphate, overchopped) and C (with 0.1% phosphate) and after heating these batches at different temperatures between 40 and 115°C.

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the figures for N (number), area, shape factor and size distribution should be evaluated separately. In our experiment, we did not measure perimeters, therefore we were not able to check if d_{al} would have provided for a useful expression for porosity. However, we also made some equations with pore diameters (which can be related to perimeters). These did not result in other (better) correlation coefficients than the ones we calculated based on area%, particle area and number of particles.

E. Tornberg: One is also concerned about the high heating temperatures above 100°C, which is not common practice in sausage production. Therefore, the applicability of these results is somewhat restricted. It should therefore be pointed out that it is only at these high temperatures that substantial fat and jelly release occur.
Authors: We agree with the reviewer that these temperatures are not commonly used in practice. However, we investigated model-systems under these conditions to be sure that a clear pattern of fat and jelly release should occur, and hence, we were able to study this fat and jelly release mechanisms microscopically. We disagree with the reviewer that only at high temperatures substantial fat and jelly release occurred. In batter B a substantial loss was already noticed after heating from 50°C onwards.

E. Tornberg: The authors mention 'shrinkage of the protein matrix'. How did the matrix shrink? Were there any whole fibers left that could shrink? Otherwise, what mechanism caused the shrinkage?
Authors: This shrinkage could be noticed by observing the volume of the batter in the cans. Before heating the cans were completely filled. After heating at temperatures above 60°C the volume of the protein had decreased, whereas this protein was surrounded by a layer of fat and jelly. Upon heating the protein matrix shrank because of aggregation of the muscle proteins. The shrinkage of intact muscle particles within the protein matrix upon heating is a well-known phenomena and can be seen in Fig. 3B (left side of the micrograph). About the shrinkage of muscle fibres upon heating, please see Offer *et al.* (1988).

E. Tornberg: The authors speculate on the amount of released protein, which in fact, has not been measured. Could the authors comment on this?
Authors: Although we did not measure the amount of released protein, we provided ample circumstantial evidence that more muscle protein was released in batters A and C than in B. Furthermore, the effect of polyphosphate on released muscle protein and on the microstructure of meat batters is well documented in the literature. See for example: Aguilera and Stanley (1990); Barbut (1989); Offer and Knight (1989).

F.W. Comer: Industry practice is to chop to batter temperatures in the range 12°-18°C. As expected, batter B chopped for an extended period (10 minutes) to 25°C was overchopped as shown by loss of stability. It is surprising to me that batter C, which was chopped for 9 minutes to 28°C, was stable. For various functional reasons, levels of phosphate normally used, ranged from 0.25% to 0.50%. Some phosphates decrease batter viscosity. I am interested in comments or references from the authors on the effects of phosphate types and usage levels upon batter stability, viscosity, cooked consistency and the optimum batter temperature (28°C is very high).
Authors: Batter C was more "stable" compared with the other ones, but still there were jelly and fat losses of 10.7% and 3.2%, respectively after heating at 115°C. We agree that in industry practice higher phosphate percentages are used. However, these percentages are often higher than strictly necessary to ensure batter stability under non-optimal processing conditions. In our experiments with a comminuted meat system under well defined processing conditions (recipe, temperatures etc.) we used a lower phosphate level. Concerning the effects of phosphate types and usage levels upon batter stability we refer to: Barbut (1988); Kotter and Fischer (1975); Offer and Knight (1989).

F.W. Comer: Do you have any solid evidence that fat cells were still present in the batters after chopping or after cooking? It is surprising to me that any native "fat cells" could remain in batter C since there is clear evidence that they are not present in batter B. Size of pores provides only circumstantial evidence of the possible existence of "fat cells". Invariably in chopping, fat globules of various sizes will be obtained. Extended chopping reduces fat globule size further until break-

down in the protein matrix permits fat agglomeration. I would expect phosphates to protect the protein matrix stability but to be defenseless against the chopper knives.

Authors: The presence of intact fat cells in the batters after chopping and cooking was verified by the observation of intact fat cell membranes in frozen sections stained with picro Sirius red F3BA. We observed intact fat cells in batters B and C by polariscopy of unstained cryosections. We do not understand the statement that there should be clear evidence of the absence of native fat cells in batter B. Although not very clear, some intact fat cells (F) can be seen in batter B (Fig. 3A). By the addition of polyphosphate, abundant muscle protein is released to form a very dense protein matrix with a high consistency and toughness. Such a protein matrix protected intact fat cells from being damaged. As stated in the last sentence of the **Results and Discussion** section, it is our opinion that extended chopping times of a meat batter with a high amount of released muscle protein involve more mixing than comminution of the components during the last stages of the chopping process. Furthermore, the higher end-temperature of batter C (28°C) seems to confirm this statement, because in batter C the chopping process resulted in more frictional heat. For more information on optimum chopping temperature we refer to Brown and Toledo (1975).

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