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The Influence of Mincing and Temperature of Storage on the Oxidation of Pork Back Fat and Its Effect on Water- and Fat-Binding in Finely Comminuted Batters

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ABSTRACT

Besides the ability of swollen and dissolved meat proteins to form a heat-stable network in finely comminuted sausage batters, the fat in the batter is an additional factor of batter stability. Fat cell integrity and the melting point of fat are regarded in the literature as essential for fat retention. In practice, however, butchers report that slaughterfresh pork back fat, whilst within a few hours post mortem *and still hot, enhances batter stability compared with chilled and stored back fat. Experiments confirmed this observation. There could be several reasons for this behaviour. One would be the oxidation processes in pork fat which occur on storage. The experiments reported here show that rancidity of fat has little or no influence on batter stability. Disintegration of fat cells by mincing and/or fat softness by increased temperature, enhance batter stability. The conclusion drawn emphasizes the importance of the early formation of small fat particles in the batter for batter stability.*

INTRODUCTION

For many years butchers in central European countries have reported that the use of slaughterfresh pork back fat, within a few hours *post mortem*,

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enhances batter stability by reducing jelly and fat separation in cooked (Frankfurter type) sausages. Chilling of the fat and/or storage for some days eliminates this effect.

We were able to prove this observation in our laboratory in a number of preliminary experiments. The reason for this behaviour of fat on batter stability could have various causes:

(1) the structure of fat cells and the connective tissue in the fatty tissue could change with time after slaughter;
(2) the molecular association of fat in fat crystals could change with temperature and time of storage;
(3) oxidation of pork back fat (about 60% unsaturated fatty acids) could increase with time *post mortem* and influence batter stability.

The influence of fat cells and fat softness (Causes (1) and (2)) on the cooking stability of batters or minces has been discussed in the literature (Evans and Ranken, 1975; Lee *et al.*, 1981; Tornberg & Persson, 1987). However, the papers by Evans & Ranken and Tornberg & Persson did not consider finely comminuted meat products. Evans & Ranken (1975) concluded, from their experiments, that lipid cookout on heating of non-emulsified meat products depends on the connective tissue present and the extent to which fat cells are broken during comminution; but they conclude that in sausages (emulsified meat products) other mechanisms may exist. Tornberg & Persson (1987) draw similar conclusions, stating that cell disintegration and connective shrinkage during heating causes higher cooking losses on heating of animal fat compared with tissue which has not been disintegrated. Lee *et al.* (1981) concluded that appropriate melting properties of fat, in addition to the properties of the protein matrix, are responsible for a heat-stable meat 'emulsion'.

Swift *et al.* (1968) and Tinbergen & Olsman (1979) investigated the effect of temperature of comminution and fat cell rupture on batter stability. Swift *et al.* (1968) found that meat emulsions with a fat content of 22% (finely comminuted sausages contain 18–40% fat) became stable with high-melting fats and remained stable with increasing temperatures and liquefaction of fat; on the other hand, oily fats formed unstable emulsions. These results are contradictory to those of Tinbergen & Olsman (1979) who concluded that the integrity of fat cells rather than the availability of released fat determines the heat stability of the sausage batters.

With regard to batter stability and the state of oxidation of the fat used (Cause (3)) no studies are reported in the literature, whereas studies on fat rancidity and on the flavour of meat products are well established (Greene & Cumuze, 1981; Melton, 1983; Poste *et al.*, 1986). In meat proteins, especially

in myosin, the availability of free SH-groups of the cysteine side chains are necessary for the formation of gels of myosin (Samejima *et al.*, 1988). Blocking the SH-groups by oxidation reduces the gelling capacity of myosin.

In our studies on the mode of action of the additive citrate in cooked sausage batters (Denk & Honikel, 1987) we found that citrate sequesters the ions of transition elements and increases the number of available surface SH-groups of meat proteins in unheated sausage batters. The increase in free SH-groups in the batters ran parallel to the batter stability (Bloukas & Honikel, paper in preparation).

Furthermore we observed that monoglycerides, when used as additives in cooked sausages, influenced the batter stability according to the structure of the fatty acid in the molecule. Saturated fatty acids (palmitic or stearic acid) lowered jelly separation on heating, whereas increasing amounts of oleic acid increased jelly separation considerably (Honikel, in preparation). One possible explanation was the oxidation of the oleic acid in the monoglyceride. Another possibility could be the difference in the interaction of the unsaturated fatty acid part of the molecule with other ingredients of the batter. The hydrophobic part of the emulsifier may interact differently with the meat proteins or the fat globules according to the different molecular structure of the fatty acid chains.

These observations need clarification. We have therefore studied the influence of mincing, temperature and time of storage of pork back fat on fat oxidation and batter stability in this paper. A further paper (Bloukas & Honikel, 1990) investigates the role of antioxidative agents on fat oxidation and batter stability.

MATERIALS AND METHODS

Preparation of pork back fat samples

Fresh pork back fat was taken from a local slaughterhouse about 1–2 h *post mortem*. After removing the skin, the fatty tissue was cut into large pieces, the pieces were mixed and then divided into two approximately equal portions. The first portion of the fat (referred to as 'unminced') was further divided into samples. Each sample, containing one or two pieces and weighing about 480–500 g, was put on a glass tray. From the second portion (referred to as 'minced') the pieces of fat were minced once in a mincer with a 3 mm mincer plate. The minced fat was divided into samples of 480–500 g and each sample was put in a plastic pouch during incubation at 37°C; on storage in the cooler it was put on a glass tray.

An equal number of samples from unminced and minced fatty tissue were treated as follows:

(a) stored immediately in a chill room at 0°C;
(b) incubated at 37°C for 24 h and then stored in the chill room;
(c) incubated at 37°C for 48 h and then stored in the chill room.

Samples from unminced and minced fat of each treatment were removed at random on days 0, 1, 2, 8 and 15 and were used in batters. The samples of unminced fat were minced just before their use in the same way as described above.

Preparation of meat samples

Lean slaughterfresh beef (less than 5% fat) was purchased fresh from a local meat market 5–6 days before the start of each series of experiments. At this time the visible fat and connective tissue was removed, the meat was cut into small pieces (3 by 3 by 8 cm), the pieces were mixed and then minced once in a mincer with a 3 mm mincer plate. The pH of the meat was 5·5–5·7. The minced meat was divided into 450 g unit packs, placed in moisture-proof plastic bags, closed under vacuum and stored at −25°C. When needed the samples were thawed for 24 h at +4°C.

Preparation of batter

One 450 g pack of thawed lean beef was comminuted for 20 s in a 2 litre bowl cutter equipped with eight blades. Then 450 g crushed ice and 22 g curing salt (table salt, containing 0·1 g $NaNO_2$) was added and the mixture was comminuted for another 160 s. The temperature of the batter was monitored continuously with a thermometer (Pt 82) which was placed permanently at the center of the bowl, 3 mm over its surface. The maximum temperature was 6°C.

After the addition of 380 g of ground fat the comminution was continued for a further 180 s. The maximum temperature of the batter at this point was about 12°C.

At the end of the comminution process six preweighed cans (size 99/36 mm) were filled with about 200 g of the fat-containing batter. All cans were closed and heated for 35 min in a boiling water bath (core temperature about 90°C). After cooling in running tap water the cans were stored at 0°C for 24 h. The batters contained about 10 ± 1·5% protein, 30 ± 3% fat and 1·7 ± 0·1% salt. All experiments were repeated three times.

Determination of jelly and fat separation

After warming up the cans in a water bath at 45°C for 1 h they were opened and the fluid of each can was collected in a 100 ml volumetric cylinder. The fluid jelly and fat, separated well in the volumetric cylinder, was measured in milliliters and calculated as a percent of the original weight of batter. The mean value of the six cans was taken for each treatment.

Fat extraction

About 100 g of fat were taken from each sample just before its use in the batter. The fat was mixed with an equal quantity of purified sea sand and about 20 g of anhydrous sodium sulfate. After intensive mixing, about 80 ml of diethylether were added and the mixture was stirred for about 10 min. The liquid phase was separated through a fine plastic sieve and was filtered through glass wool. The ether phase was removed by the use of a rotation evaporator. The separated fat was used immediately for determination of lipid oxidation.

Measurement of lipid oxidation

The 2-thiobarbituric acid (TBA) and the peroxide-value tests were used to assess lipid oxidation. The TBA test was carried out in duplicate samples according to the method described by Schmidt (1959). Results are expressed as TBA number, i.e. mg malondialdehyde/kg of fat. The peroxide-value test was carried out in duplicate samples according to the method described by Wheeler (1932). Results are expressed as peroxide value, i.e. moles O_2/kg of fat.

pH measurement of fat

About 3 g of fat were homogenized with 10 ml of distilled water in a 25 ml flask using a homogenizer (E. Bühler, Tübingen). The homogenized lard was filtered through a filter paper. The pH was measured in the filtrate with a digital pH-meter equipped with a combined glass electrode. The electrode remained in the filtrate until the pH value became constant.

Statistical analysis

Three replicas were made for every experiment. The experimental design consisted of a $2 \times 3 \times 5$ factorial arrangement, the factors being: (A) mincing (unminced, minced); (B) storage temperature (0°C, 37°C for 24 h; 0°C, 37°C

for 48 h; 0°C); and (C) storage time (0, 1, 2, 8 and 15 days). Data collected from this study for jelly and fat separation were analyzed by three-way analysis of variance using the MSTAT program. Means were compared by using the $LSD_{0.05}$ test. Correlation analysis was also carried out between TBA and jelly and fat separation.

RESULTS AND DISCUSSION

pH of fat

The effect of mincing and temperature on the pH value of fat is given in Fig. 1. The pH value of unminced and minced samples of fat which were stored immediately at 0°C remained almost constant at pH 6·5 during the 15 days of storage. Contrary to muscular tissue, the pH of fatty tissue does not fall very much *post mortem* and the pH values of 6·0–6·5 are normal. Incubation of fat at 37°C resulted in a decrease of pH. The longer the time of incubation at 37°C the lower the pH value of the fat (pH 6·1–5·9).

During storage of incubated fat at 0°C the pH value increased to 6·1–6·35. The pH of the fat in the meat batters did not influence the pH of the batter, which was always between pH 5·6 and 5·8.

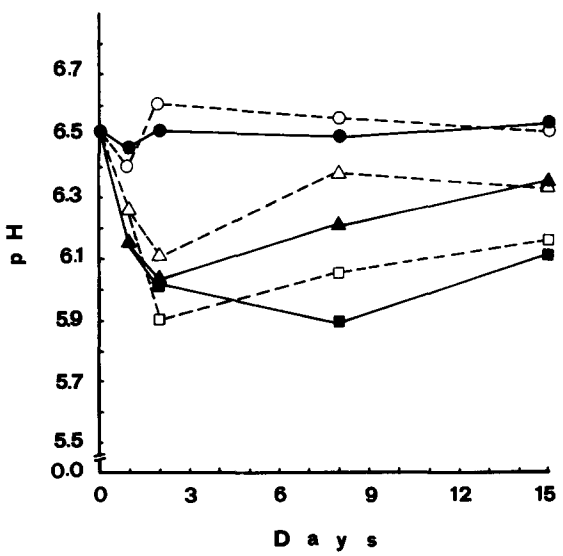

Fig. 1. Effect of mincing and temperature on the pH value of pork back fat. (●▲■) Unminced fat; (○△□) minced fat; (○●) stored immediately at 0°C, (△▲) incubated at 37°C for 24 h and then stored at 0°C (□■) incubated at 37°C for 48 h and then stored at 0°C.

Oxidation of fat

The effect of mincing and temperature on the oxidation of fat is given in Figs 2 and 3. The TBA number (Fig. 2) increased in all treatments after the second day of storage. The unminced and minced samples of fat stored at 0°C had about equal values of TBA numbers from Days 2 to 8, increasing considerably in the minced sample at Day 15. The minced samples showed an increase in TBA number after Day 2, which was very fast after storage for 24 or 48 h at 37°C. Mincing increased TBA values during storage considerably.

The peroxide value of unminced and minced samples of fat which was stored immediately at 0°C remained more or less constant and low during the 15 days of storage. The peroxide value of all incubated samples of fat, minced or unminced, increased after Day 2 of storage. The longer the incubation time the higher was peroxide value. The minced and incubated samples showed higher peroxide values than the unminced and incubated samples.

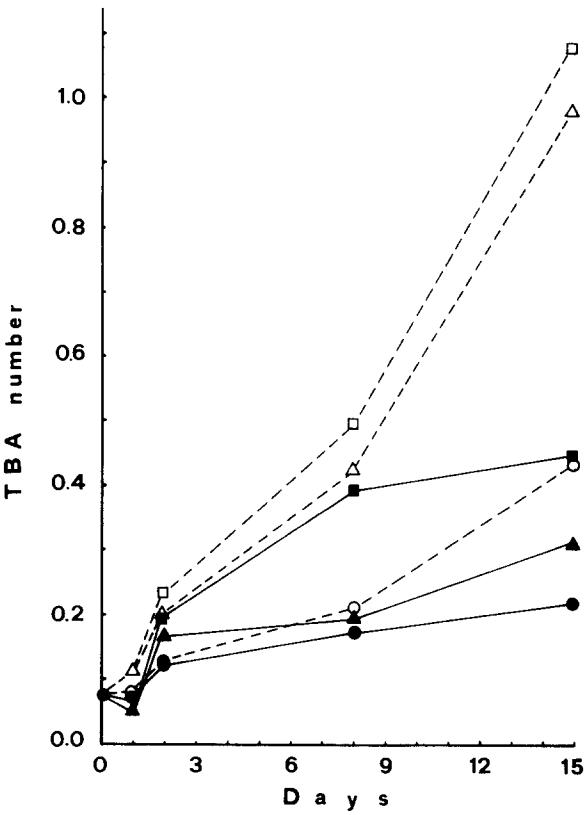

Fig. 2. Effect of mincing and temperature on lipid oxidation (thiobarbituric acid); for details and symbols see Fig. 1 and Materials and methods.

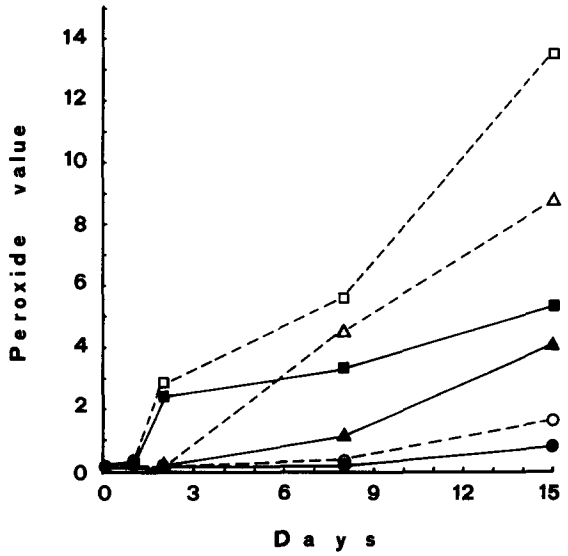

Fig. 3. Effect of mincing and temperature on lipid oxidation (peroxides); symbols as in Fig. 1.

The unminced samples of fat which were incubated at 37°C for 48 h showed, up to day 8, the same pattern of change and about the same values in TBA number and peroxide value as the minced and incubated samples. A strongly significant ($P < 0{\cdot}01$) correlation ($r = 0{\cdot}77$) was found between the TBA number and the peroxide values of all fat samples.

Jelly and fat separation

As it was our intention to observe an eventual increase or decrease in jelly and, especially, in fat separation by the various treatments, we chose a rather extreme batter composition which we heated above the normal temperature treatment of fresh cooked sausage batters (90° versus about 75°C).

The effects of mincing, storage temperature and storage time on jelly and fat separation of batters are given in Figs 4–6 and in Table 1.

Jelly separation (Fig. 4) increased significantly ($P < 0{\cdot}001$) in all treatments on the first day of storage, as reported by the butchers (see Introduction), and then remained more or less constant until Day 15. Only the unminced samples which had been incubated for 48 h at 37°C, showed a decrease in jelly separation by the second day, and this remained smaller than all the other treatments up to Day 8.

The analysis of variance showed no significant differences ($P > 0{\cdot}05$) in jelly separation between the unminced and minced treatments.

In contrast to jelly separation, which is significantly affected only by

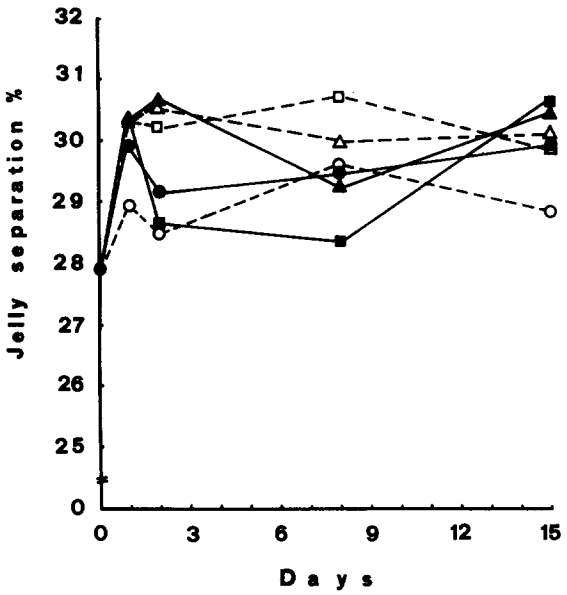

Fig. 4. Effect of mincing and temperature on jelly separation of batter; symbols as in Fig. 1.

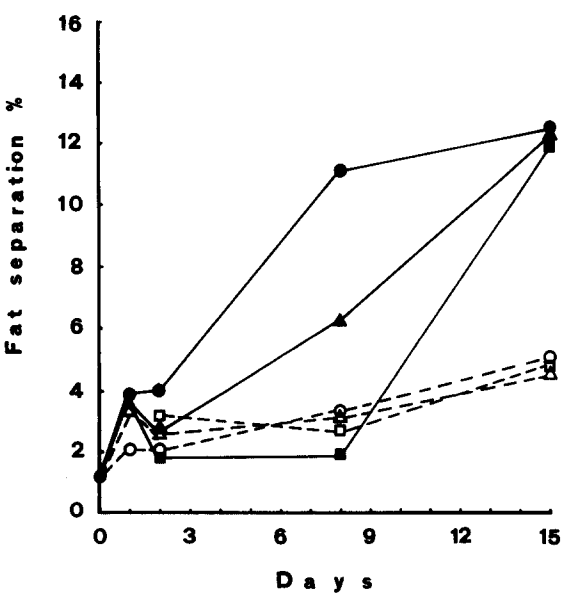

Fig. 5. Effect of mincing and temperature on fat separation of batter; symbols as in Fig. 1.

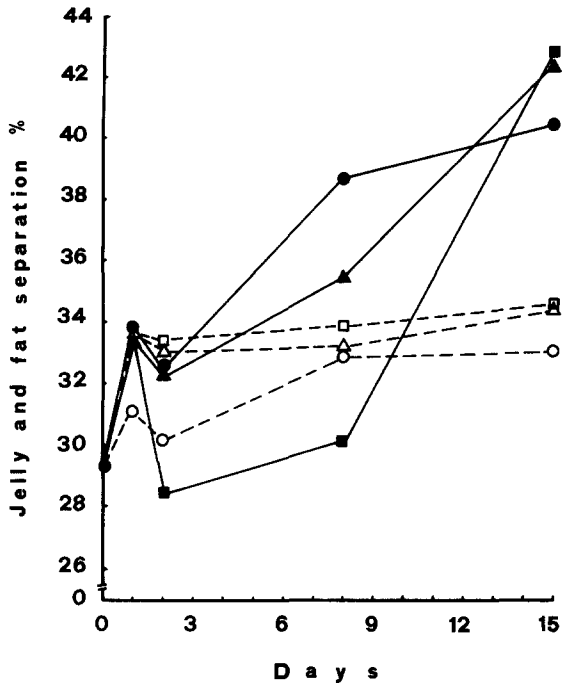

Fig. 6. Effect of mincing and temperature on jelly and fat separation of batter; symbols as in Fig. 1.

TABLE 1
Mean Squares from the Analysis of Variance of the Effect of Mincing, Storage Temperature and Storage Time of Fatty Tissue on Jelly and Fat Separation of Batter

Source of variance	*Degree of freedom*	*Mean square*	
		Jelly separation	*Fat separation*
Mincing	1	0·487	112·426***
Storage temperature	2	3·306	5·590*
Mincing × Storage temperature	2	4·210	11·190**
Storage time	4	14·908***	133·901***
Mincing × Storage time	4	2·524	47·177***
Storage temperature × Storage time	8	1·371	8·415***
Mincing × Storage temperature × Storage time	8	1·375	4·020*
Error	60	3·081	1·875

Significant at level * $P = 0{\cdot}05$; ** $P = 0{\cdot}01$; *** $P = 0{\cdot}001$.

storage time, fat separation was affected significantly by all the factors and their interaction (Table 1).

Fat separation (Fig. 5) increased with all treatments after the first day of storage. Between Days 1 and 15 all the treatments of minced fat showed similar rather low values in fat separation, and these had increased slightly by Day 15 of storage.

The unminced fat which was stored immediately at 0°C showed the highest values in fat separation at Day 2 up to Day 15. The difference in fat separation between the unminced and minced fat, stored at 0°C, when compared on Days 8 and 15, it amounted to about 7·5%. This difference was found to be statistically significant ($P < 0{\cdot}001$).

The unminced but incubated samples showed lower values for fat separation on Days 2 and 8 than the minced samples stored at 0°C. The longer the time of incubation the lower the fat separation on Days 2 and 8. By Day 15 all the treatments of unminced fat were associated with more or less equal extents of fat separation.

It is remarkable, as shown in Fig. 6, that the unminced pork back fat, which was incubated for 48 h, showed the lowest value in jelly plus fat separation up to Day 8. With this treatment the fat became very soft after incubation, showed the lowest value of pH (Fig. 1) and medium values for TBA number and peroxide value in comparison with all other treatments of the fat.

It seems from these results, and observations following the use of additives (Bloukas & Honikel, 1992), that there is no correlation between lipid oxidation and the separation of jelly and fat in batter. Indeed the correlation coefficient between the TBA number and fat separation was found to be very low; and that between the TBA number and jelly separation negligible (Table 2).

It seems that the difference in fat separation between the unminced and minced samples of fat is related more to the structure of the fat than to its

TABLE 2
Correlation Coefficients between TBA Number and Jelly and Fat Separation

	Unminced fat (No. = 39)		*Minced fat (No. = 34)*		*Unminced fat minced fat (No. = 73)*	
	TBA	*Jelly*	*TBA*	*Jelly*	*TBA*	*Jelly*
Jelly	0·04	—	0·12	—	0·08	—
Fat	0·35*	0·22	0·51*	0·06	0·09	0·14

* Significant at $P < 0{\cdot}1$ level (Student's *t*-test).

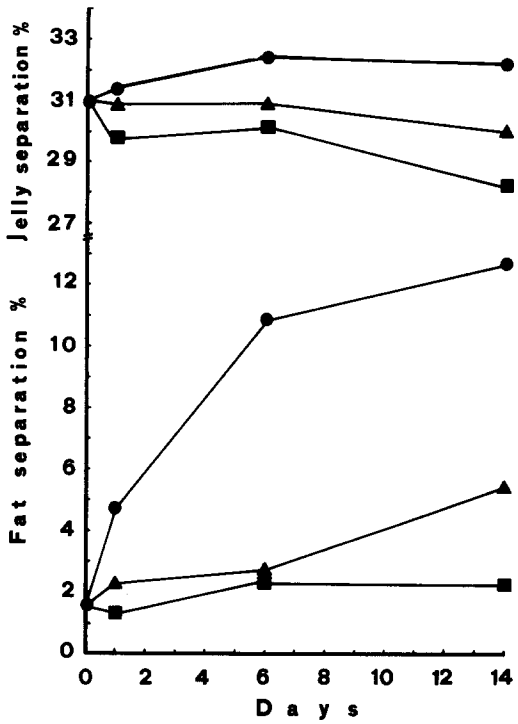

Fig. 7. Effects of mincing and temperature on fat and jelly separation of batter. ● unminced fat stored immediately at 0°C; ▲ minced fat stored immediately at 0°C; ■ minced fat stored immediately at 0°C and warmed up at 50°C for 1 h before use.

oxidation. Mincing of the fat, and the storage which follows, destroys this structure. It enhances the softness of the fat as observed in the experiments during handling.

In a further experiment (carried out in duplicate) we found that fat which became more soft, when warmed up to 50°C for 1 h before use, reduced the fat and the jelly separation of batter (Fig. 7).

CONCLUSION

Rancidity of the fat in meat reduces its sensory qualities but it has apparently no influence on its technological properties with regard to batter stability. The expected disintegration of fatty tissue structures caused by mincing, or the softness of the fatty tissue caused by increasing high temperature storage or warming up before use in the batter, reduces fat separation. Jelly release is also reduced by the same treatment, but to a minor extent. From these experiments we draw the conclusion that the early

formation of small fat particles during comminution, and the subsequent formation of a stable colloidal system with finely distributed fat particles in the batter, is essential for batter stability. The influence of meat proteins, and their swelling and solubility changes during comminution is an additional important factor for batter stability. This factor was not investigated in the present paper since it has been well established by others (e.g. Hamm, 1986). The role of pro-oxidative and anti-oxidative additives (Bloukas & Honikel, 1992) seems to influence the meat proteins more than the fat batters.

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