1987

Textural Properties and Microstructure of Process Cheese Food Rework

Miloslav Kalab

Joseph Yun

Suk Hing Yiu

Follow this and additional works at: https://digitalcommons.usu.edu/foodmicrostructure

Part of the Food Science Commons

Recommended Citation

Kalab, Miloslav; Yun, Joseph; and Yiu, Suk Hing (1987) "Textural Properties and Microstructure of Process Cheese Food Rework," Food Structure: Vol. 6 : No. 2 , Article 10.
Available at: https://digitalcommons.usu.edu/foodmicrostructure/vol6/iss2/10

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Food Structure by an authorized administrator of DigitalCommons@USU.
For more information, please contact rebecca.nelson@usu.edu.
TEXTURAL PROPERTIES AND MICROSTRUCTURE OF PROCESS CHEESE FOOD REWORK

Milošlav Kalab, Joseph Yun*, and Suk Hing Yiu

Food Research Centre, Research Branch, Agriculture Canada
Ottawa, Ontario, Canada KIA OC6

*Canada Packers Research Centre, Toronto, Ontario, Canada M6N 1K4
Present address: Department of Food Science, Cornell University
Ithaca, NY 14853

Abstract

Process cheese food was made using sodium citrate (2.7%) or trisodium phosphate (TSP, 2.7%) as emulsifying agents. No precooked cheese (rework) was used in some samples whereas in others the rework (20%) consisted of a cheese blend emulsified with sodium citrate (2.7%) and (a) briefly heated to 82°C, (b) heated to 82°C for 1 h, (c) heated to 82°C for 5 h, and (d) heated to 82°C for 5 h, frozen at -10°C for 24 h, and thawed at 4°C. Heating for extended periods of time produced so-called hot melt. When used as rework, hot melt considerably decreased the meltability of the product made. All samples under study were examined by light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Gradual solubilization of the emulsifying agents in the cheese blend and emulsification of fat were visualized by LM and SEM. TEM revealed considerable changes in the protein matrices of the hot melt and the process cheese food made with TSP. Small electron-dense areas having a high affinity for osmium developed in both products, but their shapes and degrees of affinity for osmium were different. It was possible to detect the presence of hot melt used as rework in the process cheese food samples under study.

Introduction

Process cheese food is an emulsion prepared by cooking one or more varieties of natural cheese with other ingredients such as water, milk solids including butter, emulsifier, salt, and colouring agents to produce a homogeneous product. According to Canadian regulations [12], process cheese food should contain no less than 51% cheese and the final product should have a maximum 46% of moisture and a minimum 23% of fat. Process cheese food can be packaged in cans or as blocks but is marketed most commonly in a slice form for convenience of use.

Processing conditions vary depending on the equipment used and the type of the cheese product to be made. A minimum temperature of 65°-70°C is desirable for processing [7]. In general, a high cooking temperature such as 80°-90°C with sufficient stirring and an appropriate holding time renders a uniform product and generally ensures its microbiological safety [9]. However, the heat treatment applied during cooking and holding varies and can become excessive at times. Excessive heating can occur, e.g., if there is a delay or stoppage in packaging lines. Consequently, the process cheese food emulsion thickens considerably and becomes difficult to extrude to form slices. The stiffened mass, which is called 'hot melt', is removed from the pipes using compressed air and is frozen until re-used. To be re-used ('reworked' or 'reprocessed' [7]), hot melt is thawed, shredded, and added as an ingredient into a fresh cheese blend. Thus, hot melt is a type of process cheese food rework, which can be defined as any process cheese food that is not packaged for sale although it meets the product specifications but is mixed with a fresh blend and processed again. Unlike regular rework (i.e., precooked cheese), however, hot melt sometimes increases the viscosity of a freshly cooked emulsion and changes the quality of the final product in an unpredictable way. It is not understood how hot melt affects the texture of the product.

The objective of this study is to examine the effect of hot melt (used as rework) on the microstructure and rheological properties of process cheese food.

Materials and Methods

Two series of experiments were carried out. In the first series, hot melt taken out from the pipes of a commercial plant was used as rework. Three types of rework were examined: (1) Regular process cheese food slice, (2) quickly frozen cooked process cheese food emulsion, and (3) hot melt.

Key Words: Electron microscopy, Hot melt, Light microscopy, Microstructure, Osmophilic protein areas, Precooked cheese, Process cheese food, Rework.
The regular process cheese food slice used in this present study was a product of the usual kind from a process cheese plant. Hot melt was produced in the plant by slowly cooling (from 82°C to 4°C in 5 h) and freezing cooked process cheese food emulsion that was too thick to extrude. The quickly frozen process cheese food emulsion was prepared by rapidly cooling (from 82°C to 4°C in 10 min) and freezing a process cheese food emulsion cooked in the laboratory using the same cheese blend.

Preparation of process cheese food on laboratory scale

Mixtures of natural cheeses were shredded in a Hobart cutter/mixer (Model N-50, The Hobart Manufacturing Company, Troy, OH) and blended with other ingredients. Water, butter, and salt were added to adjust the composition of the product to 45% water and 24% fat, and sodium citrate (2.7%) was used as an emulsifier. The blend was cooked in a stainless steel container thermostatically controlled by a boiling water bath. With the agitation provided by a Crafco stirrer (Type RZRL, Canlab, War- ton, Ontario, Canada), the internal temperature of 82°C was achieved in 5-7 min. Cooked emulsions were stored in 500 g plastic tubs at 4°C.

Hot melt was prepared on laboratory scale by holding the process cheese food emulsion made with sodium citrate at 82°C for 1 h and for 5 h to study the effect of two different heat treatments. The hot melt was then frozen at -10°C for 24 h, thawed at -4°C, shredded, and used as rework at 20% (w/w) in a fresh cheese blend. Sodium citrate or trisodium phosphate (TSP, 2.7%) was used as the emulsifying agent for the mixture.

Measurement of apparent viscosity

Immediately after the emulsion had been cooked, its apparent viscosity was measured using a Brookfield viscosimeter (Brookfield Engineering Laboratories, Stoughton, MA). The T-b type spindle was used at 4 rpm with the Helipath feature in operation. At the time of measurement, the temperature of the emulsion was 71 ± 2°C. Results are presented in poises.

Determination of firmness

The firmness of the process cheese food was determined after 24 h of storage at 4°C using a precision penetrometer (Precision Scientific Co., Chicago, IL). The cone penetrating into the cheese under a load of 150 g was 2.0 cm in diameter and was 3.2 cm high. Results are presented in depth of the cone penetration in millimeters.

Estimation of meltability

After the penetration test, samples were cut into disks 6 mm high and 40 mm in diameter and equilibrated for 2 h at 20°C in petri dishes. The equilibrated disks were then placed in an oven heated at 140°C and kept there for 6 min to melt. After cooling to 20°C for 30 min, the diameters of the melted disks were measured using a ruler. Results are expressed in millimeters.

Chemical analysis

Moisture of the process cheese food was determined by a vacuum oven method [1] and the fat content was determined using a modified Babcock test [6].

Microscopy

All process cheese food samples submitted for microscopic examination were first cooled in a refrigerator at 4°C for 48 h before vacuum packaging and shipping using a cooled container.

For light microscopy (LM), 1-2 mm cubes of unfixed process cheese food samples were frozen at -20°C and sectioned into sections 6-8 μm thick using a Cryo-Cut E microtome (Reichert-Jung Scientific Instruments, Beverly, Ontario, Canada). The sections were affixed to glass slides, air-dried, stained, and examined under a Zeiss Universal Research Photomicroscope (Carl Zeiss Ltd., Montreal, Quebec, Canada). The microscope was equipped with both a conventional brightfield illumination system and a III epi RS epi-illuminating condenser combined with an HBO 100 W mercury-arc illuminator for fluorescence analysis. An exciter/barrier filter set for maximum transmission at 450-490 nm/520 nm was used for fluorescence examination of sections stained with Acri- dine Orange (BDH Chem. Ltd., Poole, England), or Nile Blue A (Eastman Kodak Co., Rochester, NY) according to the methods described by Yiu [13]. Unstained or stained sections were also viewed under crossed polarizers to examine the birefringence of crystalline structures. Photomicrographs were taken with Ektachrome 400 Daylight film and were processed as black-and-white prints.

For scanning electron microscopy (SEM), the process cheese food and the hot melt samples were cut into prisms, 1 x 1 x 10 mm. The prisms were fixed in a 3.5% aqueous glutaraldehyde solution at 20°C for 2 h, washed in water, dehydrated in a graded ethanol series, and defatted in chloroform. Then the samples were returned into absolute ethanol, frozen in Freon 12 cooled to -150°C with liquid nitrogen, and fractured under liquid nitrogen. The fragments were melted in absolute ethanol at 20°C and were critical point-dried in a modified Sandri PVT-3 critical-point drier (Tousimis Research Corp., Rockville, MD) using carbon dioxide. Dry fragments were mounted on aluminum stubs, sputter-coated with gold (a layer of gold approximately 20 nm thick) using a Technics Hummer II sputter coater (Soquelec Ltd., Mont­ real, Quebec, Canada) and examined in an AMR-1000A scanning electron microscope (AMRay Inc., Bedford, MA) operated at 10 kV. Micrographs were taken on 35-mm Kodak Plus-X film.

For transmission electron microscopy (TEM), the samples initially fixed in the 3.5% glutaraldehyde solution for SEM examination were cut into particles approximately 1 mm in diameter and these were postfixed at 20°C for 2 h in a 2% osmium tetroxide solution in a 0.05 M veronal-acetate buffer, pH 6.75, washed with the buffer, and dehydrated in a graded ethanol series. The dehydrated samples were embedded in a Spurr's low-viscosity medium (J. B. EM Service, Inc., Pointe Claire-Dorval, Quebec, Canada). Sections, approximately 90 nm thick, (obtained using a diamond knife mounted in an OM U2 microtome, Reichert Ophthalmische Werke AG, Vienna, Austria) were stained with uranyl acetate and lead citrate solutions [4, 5] and examined in a Philips EM-300 electron microscope (N. V. Philips, Eindhoven, the Netherlands) operated at 60 kV. Micrographs were taken on 35-mm Eastman Kodak Fine Grain Release Positive Film 5392.

Fat globule dimensions were evaluated from micrographs using an MOP-3 Digital Image Analyzer (Carl Zeiss, Inc., Don Mills, Ontario, Canada). The fat globules were grouped into size classes at 1.0 μm intervals. Particles in each range were assigned the mean diameter of that range which was used to calculate the total area of the sections. Corrections for true diameters were not used.

Results and Discussion

The commercially made regular process cheese food slice and the quickly frozen process cheese food emulsion were homogeneous soft products with a smooth texture. The hot melt sample was dry and crumbly, especially in the core region. Physical and chemical changes may take place in the hot melt before it is used as rework. Firstly, the core receives the most heat treatment before cooling due to the difference in the cooling rate depending on the
position in the container into which hot melt is placed. Secondly, solutes are known to migrate towards the centre of hydrated materials upon freezing [3]. The changes thus induced may contribute to the characteristics of the hot melt. When the hot melt is added to a fresh batch, it may lead to the formation of a thick and undesirable texture of the finished product.

Textural properties

Using the three types of rework of commercial origin mentioned above, five kinds of process cheese food were prepared in the laboratory (first column in Table 1). All the process cheese food contained 43 ± 1% moisture and 24 ± 1% fat, and its pH ranged from 5.5 to 5.7. The results of the apparent viscosity, firmness, and meltability tests are summarized in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Composition of process cheese food with rework</th>
<th>Apparent viscosity (poise)</th>
<th>Firmness (pen read in mm)</th>
<th>Meltability (melted diameter in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular PCF* (no rework)</td>
<td>200</td>
<td>12.9</td>
<td>62.8</td>
</tr>
<tr>
<td>Regular PCF*, containing:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% quickly frozen PCF*</td>
<td>400</td>
<td>11.4</td>
<td>48.5</td>
</tr>
<tr>
<td>20% PCF* slice</td>
<td>490</td>
<td>12.0</td>
<td>46.5</td>
</tr>
<tr>
<td>10% hot melt</td>
<td>750</td>
<td>11.3</td>
<td>45.9</td>
</tr>
<tr>
<td>20% hot melt</td>
<td>840</td>
<td>10.2</td>
<td>40.0</td>
</tr>
</tbody>
</table>

* Process cheese food

The apparent viscosity of cooked process cheese food emulsion was increased in general when any rework was added; the increase depended on its type and amount. The process cheese food slice and the quickly frozen process cheese food emulsion used as rework increased the apparent viscosity of fresh batches. The increase was considerably greater with the hot melt used as rework and was directly related to the amount of the hot melt added. At 71°C, the cooked process cheese food emulsion was easily pourable whereas the emulsion made with hot melt was too thick to pour.

Results of the penetrometer test show that rework in general decreased the depth of penetration, indicating that the firmness of the product was increased. The sample with the greatest amount of hot melt was the firmest whereas the regular process cheese food with no rework, used as a control, was the softest. There is a positive correlation between apparent viscosity of the cooked emulsion and firmness of the product.

Products that are easy to melt, such as the regular (control) process cheese food containing no rework, have large melted diameters (Table 1). The finished product containing 20% hot melt almost did not melt at all under experimental conditions and, hence, had the smallest melted diameter. In the samples under study, the melted diameter showed a negative correlation with apparent viscosity and firmness of the process cheese food.

Microstructure

The process cheese food samples examined by microscopy are listed in Table 2.

Light microscopy. Clearly distinguishable mixtures of cheeses were revealed by LM in samples #11 and #20. Differences were found in the structure and chemical composition of both samples. A relatively dense protein matrix of hot melt (sample #4) and a less dense protein matrix with thin protein strands of the regular process cheese food blend (sample #1) were demonstrated using Acridine Orange (Figs. 1 - 4). Staining with Nile Blue A showed that hot melt contained an apparently lower concentration of fat globules than the regular process cheese food blend (Figs. 5 and 6). An absence of crystalline structures in the hot melt component and their abundance in the regular process cheese food base was noticeable when both samples were examined under polarized light (Figs. 7 and 8). It is evident that sodium citrate crystals used to make the hot melt component had been dissolved in the aqueous phase of the cheese during the preceding processing treatment. Crystals of the emulsifying salts added to the fresh cheese blend, i.e., sodium citrate in sample #1 and TSP in sample #17, were still noticeable at the beginning of processing when the temperature of the cheese blend reached 71°C.

Table 2.

<table>
<thead>
<tr>
<th>Number: Composition:</th>
<th>Temperature:</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 PCF* + NaCit**</td>
<td>cooked to 82°C</td>
</tr>
<tr>
<td>#2 PCF + NaCit</td>
<td>held at 82°C for 1 h</td>
</tr>
<tr>
<td>#3 PCF + NaCit</td>
<td>held at 82°C for 5 h</td>
</tr>
<tr>
<td>#4 #3</td>
<td>frozen at -10°C for 24 h, thawed at 4°C</td>
</tr>
<tr>
<td>#5 PCF + NaCit</td>
<td>71°C</td>
</tr>
<tr>
<td>#6 #5</td>
<td>76°C</td>
</tr>
<tr>
<td>#7 #5</td>
<td>82°C</td>
</tr>
<tr>
<td>#8 PCF + NaCit + 20% of #1</td>
<td>71°C</td>
</tr>
<tr>
<td>#9 #8</td>
<td>76°C</td>
</tr>
<tr>
<td>#10 #8</td>
<td>82°C</td>
</tr>
<tr>
<td>#11 PCF + NaCit + 20% of #4</td>
<td>71°C</td>
</tr>
<tr>
<td>#12 #11</td>
<td>76°C</td>
</tr>
<tr>
<td>#13 #11</td>
<td>82°C</td>
</tr>
<tr>
<td>#14 PCF + TSP***</td>
<td>71°C</td>
</tr>
<tr>
<td>#15 #14</td>
<td>76°C</td>
</tr>
<tr>
<td>#16 #14</td>
<td>82°C</td>
</tr>
<tr>
<td>#17 PCF + TSP + 20% of #1</td>
<td>71°C</td>
</tr>
<tr>
<td>#18 #17</td>
<td>76°C</td>
</tr>
<tr>
<td>#19 #17</td>
<td>82°C</td>
</tr>
<tr>
<td>#20 PCF + TSP + 20% of #4</td>
<td>71°C</td>
</tr>
<tr>
<td>#21 #20</td>
<td>76°C</td>
</tr>
<tr>
<td>#22 #20</td>
<td>82°C</td>
</tr>
</tbody>
</table>

* Process cheese food
** Sodium citrate
*** Trisodium phosphate

Both the fluorescence and polarizing microscopy studies revealed a distinct boundary between the hot melt component and the regular process cheese food base in samples that had been heated to 71°C. However, no distinct boundary was observed in samples heated at higher temperatures. Apparently, hot melt became uniformly dispersed in the blend soon after the temperature of 71°C had been exceeded.

Scanning electron microscopy. Process cheese food samples examined by SEM were considerably smaller than those examined by LM. This difference in size is
stipulated by the technique used. Dehydration in ethanol, freezing, and critical point-drying of samples destined for SEM require that the samples be small. In small samples, however, it was difficult to find borders between the two kinds of material, i.e., the regular cheese food blend and hot melt.

The starting material, which was obtained by cooking a fresh cheese blend to 92°C (sample #1, Table 2) contained a great number of crystals of sodium citrate [8] used as the emulsifying agent (Fig. 9). By holding the blend at this temperature for 1 h (sample #2) and also for 5 h to give it an excessive heat treatment and to produce hot melt (sample #3), the occurrence of the sodium citrate crystals was markedly reduced (Figs. 10 and 11). Following freezing to -10°C for 24 h and thawing, there was almost a complete absence of sodium citrate crystals in sample #4 (Fig. 12). No structural difference between the base and the reswork was detected in samples #8, #9, and #10, all of which consisted of a regular cheese blend containing 20% of sample #1, but differed slightly in terms of the degree of processing. A similar finding was made in samples #17, #18, and #19, all of which contained the same ingredients as those present in the samples mentioned above except the emulsifying agent which was TSP.

There was a marked difference, however, in the processing conditions to which the two components were exposed in samples #11 to #13 and in samples #20 to #22. Although the small samples (c mm³) made it difficult to find sites which would show both components side by side, minute compact areas were occasionally found in some of the samples under study, e.g., in sample #12 (Fig. 13). The process cheese food blend emulsified with TSP (sample #14, Fig. 14) resembled in general the cheese blend emulsified with sodium citrate (sample #1, Fig. 9) except that the structure was coarser, e.g., the fat globules and the crystals of the emulsifying agent were larger. In addition, calcium phosphate crystals [2, 4, 8] were abundant (Fig. 14) apparently as a result of using a phosphate-based emulsifying agent. Compact areas were present in this product (Fig. 15) although no reswork was used. It is doubtful, therefore, that compact areas noticeably by SEM also in other samples of this experimental series could be related to the presence of hot melt. Mixtures of the base cheese blend and hot melt emulsified with TSP, i.e., samples #20 to #22, contained a higher concentration of the compact areas, some of which were quite large (Fig. 16), than samples #11 to #13, where such areas were very rare. Gradual dissolution of TSP in these process cheese food samples was also evident.

It was assumed that using different emulsifying agents would make it easier to distinguish hot melt from the process cheese food base. However, LM showed (Figs. 7 and 8) that there was a structural difference between a briefly processed cheese blend and hot melt even if both products were made using the same emulsifying agent. The occurrence of sodium citrate crystals was high in the briefly processed cheese and was low in the hot melt because most of the crystals had dissolved during the extended period of heating. This finding was confirmed by SEM.

LM studies showed that hot melt dispersed readily in the cheese blend emulsified with either sodium citrate or TSP. Hence, LM appears to be more suitable for detecting the dispersion of hot melt in the process cheese food blend than SEM. However, neither technique helped explain why physical properties such as firmness and meltability were changed in the finished product if it contained hot melt.

Transmission electron microscopy. The higher resolution provided by TEM, as compared with LM and SEM, made it possible to examine the structures of the process cheese food and hot melt samples in greater detail. At a low magnification, TEM was used to study the nature and the size distribution of emulsified fat particles whereas structural details of the protein matrix were examined at higher magnifications.

Fat particles were counted and measured in composite micrographs of total areas of approximately 6250 μm² using a Zeiss MOP-3 digital image analyzer. The fat particles, enlarged 4000X, were classified (class intervals of 1 μm) for diameters. Because the areas slightly differed from each other, the data were adjusted to the standard area of 6250 μm², related to the dimensions of the sections and the 60-mesh hexagonal grids used to support them.

In the hot melt (sample #4), emulsification appeared to be more advanced than in the regular process cheese food (sample #1) and the number of the fat particles in the hot melt was higher by almost 25% (Fig. 17). The mean diameters were converted into mean radii (shown in Figs. 17 and 18) and these were used to calculate the circular areas occupied by the fat particles. The latter distribution is presented in Fig. 18. Assuming that there was no change in the total volume of the fat present in the process cheese foods samples, the total area occupied by the fat particles in the micrographs should have remained the same; the empirical difference of approximately 11% must, therefore, be considered as an acceptable experimental error. There was a higher number of

Fig. 1. Process cheese food blend containing 20% of hot melt, emulsified with sodium citrate and heated to 71°C (sample #11). Section stained with Acidine Orange reveals the boundary between freshly processed cheese (dark area) and hot melt (light area). Dark circles (arrows) are fat globules.

Fig. 2. The same sample as in Fig. 1 reveals structural difference between the two components at a higher magnification. Light dots (small arrows) are lactic acid bacteria, dark circles (large arrows) are fat globules.

Fig. 3. Process cheese food blend containing 20% of hot melt, emulsified with trisodium phosphate and heated to 71°C (sample #20). Section stained with Acidine Orange reveals the boundary between freshly processed cheese (dark area) and hot melt (light area). Dark circles (arrows) are fat globules.

Fig. 4. The same sample as in Fig. 3 reveals structural difference between the two components at a higher magnification. Light dots (small arrows) are lactic acid bacteria, dark circles (large arrows) are fat globules.

Fig. 5. Staining of a section of sample #11 with Nile Blue A shows the distribution of fat particles (light circles, arrows) in the hot melt (H) and in the surrounding freshly processed cheese food (S).

Fig. 6. Section of sample #20 stained with Nile Blue A demonstrates a similar observation as in Fig. 5. Arrows: fat particles, H: hot melt, S: freshly processed cheese food.

Fig. 7. An unstained section of sample #11 viewed in polarized light shows crystallite inclusions (arrows) in the freshly processed cheese food component (S) and their absence in the hot melt (H).

Fig. 8. An unstained section of sample #20 viewed in polarized light shows crystallite inclusions (arrows) in the freshly processed cheese food component (S) and their absence in the hot melt (H).
TEXTURE AND STRUCTURE OF PROCESS CHEESE REWORK

[Eight images showing different textures and structures of cheese rework with scale bars indicating measurements in micrometers.]
TEXTURE AND STRUCTURE OF PROCESS CHEESE REWORK

coalescing fat particles in the hot melt (samples #3 and #4) than in sample #1. The large composite micrographs used to determine the fat particle size distribution showed this phenomenon better than the relatively small areas of individual micrographs presented in Figs. 19 and 22. It is probable that the fat particles coalesced in the process cheese food during the prolonged heating without stirring.

There were also structural differences in the appearance of the fat particles. In the initial process cheese food (sample #1), most fat globules appeared to be lined with a material that was electron-dense than the surrounding protein matrix (Figs. 19 and 20). The occurrence of this material was lower in sample #2 (Fig. 21) heated for 1 h at 82°C and was almost completely absent in hot melt (samples #3 and #4, Figs. 22 and 25) heated for 5 h. The nature of this material is not known. It may be hypothesized that its incidence was associated with the addition of water to the initial cheese blend.

Prolonged heating of the process cheese food made with sodium citrate led to the development of characteristic electron-dense areas (Figs. 22 and 25). Repetition of the experiments confirmed that these areas developed in the process cheese food in the presence of sodium citrate as a result of prolonged heating and were not artefacts. These electron-dense areas had sharp outlines even at a higher magnification (Fig. 23) and were distinctly different from fat globule membrane fragments which were present in small quantities in the process cheese food samples. It is possible that the protein in the electron-dense areas was changed in such a way that it formed compact clusters or that its affinity for osmium, uranyl, and/or lead was increased. The increased local concentration of the heavy metals resulting from their more intense binding may have simulated a more compact structure. To establish whether there were differences in the intensity with which the clusters would bind the individual heavy metals, sections postfixed with osmium tetroxide but not stained, and sections stained with uranyl acetate, with lead citrate, and with both reagents were examined. The dark areas are clearly visible in an unstained section (Fig. 24) indicating that their affinity for osmium was high. Other strongly osmophilic structures were found to form a part of the lining at the fat particles. Sections stained with only uranyl acetate or lead citrate did not differ in contrast from unstained sections and are therefore not shown.

Fig. 9. Process cheese food blend briefly heated to 82°C (sample #1). Round void spaces in the cheese matrix indicate the distribution and dimensions of fat globules.

Fig. 10. Process cheese food blend heated at 82°C for 1 h (sample #2). Sodium citrate crystals (C) are smaller and less numerous than in sample #1.

Fig. 11. Process cheese food blend heated at 82°C for 5 h (hot melt, sample #3). Most sodium citrate crystals are dissolved, arrows point to a few crystal residues.

Fig. 12. Process cheese food blend heated at 82°C for 5 h, frozen at -10°C for 24 h, and thawed at 4°C (hot melt, sample #4). In comparison to the preceding samples, the structure is characterized by the absence of crystals of the sodium citrate emulsifying agent.

Fig. 13. Sample #12 consisting of process cheese food blend (sample #1) to which 20% of rework (hot melt, sample #4) was added and the mixture was briefly heated to 76°C; R: compact area, arrows: residues of the emulsifying agent (TSP) crystals.

Fig. 14. Process cheese food blend emulsified with 2.7% trisodium phosphate (TSP). No rework was used (sample #14). C: crystals of the emulsifying agent, TSP. Arrows: calcium phosphate crystals are abundant.

Fig. 15. Sample #14 contains compact areas (R) although no rework was used. C: gradually dissolving crystals of the emulsifying agent, TSP.

Fig. 16. Sample #20 consisting of process cheese food emulsified with 2.7% TSP and 20% of rework (sample #4). The mixture was heated at 76°C; R: compact area, arrows point to residues of the emulsifying agent (TSP) crystals.

Fig. 17. Histograms of the size distribution (radius in μm) of fat particle sections in areas of 6250 μm² in process cheese food samples #1 (basic process cheese food) and #4 (hot melt).

Fig. 18. Histograms showing the distribution of combined areas (in μm²) occupied by fat particles classified by their radius (in μm) in areas of 6250 μm² in process cheese food samples #1 (basic process cheese food) and #4 (hot melt), calculated from the mean radius shown in Fig. 17.
Fig. 19. Process cheese food blend heated to 82°C (sample #1). m: lactic acid bacteria, light arrows: fat globule membrane fragments, dark arrows: fat particle (F) lining.

Fig. 20. Detail of fat particle (F) osmiophilic lining (arrow) in sample #1. C: calcium salt crystal.

Fig. 21. Process cheese food heated at 82°C for 1 h (sample #2). m: microorganisms, light arrows: fat globule membrane fragments, dark arrows: fat particle (F) lining.

Fig. 22. Process cheese food heated at 82°C for 5 h (hot melt, sample #3) contains many electron-dense areas (dark arrows). Light arrow: fat globule membrane fragments.

Fig. 23. Detail of electron-dense areas developed in hot melt (process cheese food heated at 82°C for 5 h, sample #3).

Fig. 24. Unstained section of hot melt (sample #3). Electron-dense areas (o) developed in the protein matrix of process cheese food heated at 82°C for 5 h and fat particle (F) lining (dark arrows) have strong affinity for osmium. C: emulsifying agent (sodium citrate) crystals, F: fat particles, m: microorganisms.
TEXTURE AND STRUCTURE OF PROCESS CHEESE REWORK

Process cheese food which contained rework in the form of the cheese blend briefly heated to 82°C (sample #10) had the structure (Fig. 26) similar to regular process cheese food blend emulsified with sodium citrate which was free of rework (samples #1 and #2, Figs. 19 and 21). The protein matrices in all three samples were uniformly stained and contained only a small number of fat globule membrane fragments.

It was possible to detect the presence of 20% of hot melt as rework in the process cheese food (emulsified at 82°C with stirring, sample #13) based on the presence of the characteristic dark areas (Fig. 27).

Interestingly, the process cheese food made with 2.7% TSP also had a characteristic, though different, appearance under the TEM. Although the blend had been heated only briefly to 82°C (sample #16), there was a high concentration of dark areas in the protein matrix (Fig. 28). These areas (Figs. 26-35) were larger but not as dark as the areas that developed in hot melt made with sodium citrate (Figs. 23 and 25). The use of 20% rework in the form of regular process cheese food, sample #1, led to a product which contained one component emulsified with sodium citrate and the other component emulsified with TSP (sample #19). The microstructure of this process cheese food (Fig. 29) resembled that of process cheese food emulsified with TSP containing no rework, i.e., sample #16 (Fig. 28). However, the presence of rework used in the form of hot melt (sample #22) resulted in a product which contained both kinds of dark areas in the protein matrix (Figs. 30-32, 35). Examination of unstained thin sections confirmed that the dark areas that had developed in the hot melt were either more compact or had a higher affinity for osmium than the less dark areas that had developed in the process cheese food in the presence of TSP (Fig. 32). This was confirmed by an examination of thin sections stained with uranyl
Rework (20%) consisting of process cheese food heated with 2.7% sodium citrate to 82°C (sample #1) used in process cheese food made with 2.7% TSP did not affect the structure of the product (sample #19) compared to process cheese food made without rework (sample #16 shown in Fig. 24). C: melting salt crystals, F: fat particles, M: microorganisms. Fig. 29. Detail of two kinds of dark areas in process cheese food sample #22: darker areas (a) originate from hot melt (sample #4) used as rework and less dark areas (t) originate from the use of TSP as the melting salt. M: microorganisms, dark arrows point to the the highly osmophilic lining of fat particles (F).

Fig. 32. Unstained section of sample #22 showing the osmophilic nature of dark areas (a) which originated in the hot melt (sample #4). Areas (t) which originated in process cheese made with TSP have a lower affinity for osmium. Lining (dark arrows) in the fat particles (F) is highly osmophilic. Light arrow points to a fat globule membrane fragment.

The distinction between the dark areas and fat globule membrane fragments at high magnification (Fig. 34) was as clear as was the distinction between the two kinds of dark areas (Fig. 35).

The causes for the development of the dark areas in the hot melt, i.e., in a cheese blend heated at 82°C for 5 h in the presence of 2.7% sodium citrate, and in a similar blend briefly heated at the same temperature in the presence of 2.7% TSP, are not known. It is possible that the protein underwent some changes [10] which either increased its affinity for osmium or made it interact in a way that would result in areas more compact than the rest of the protein matrix. It is probable that the increased affinity for osmium may also be related to detectable structural changes as revealed by LM (Figs. 1-4). It may be suggested that the development of the dark areas in hot melt as observed by TEM is related to a decreased meltability of the process cheese food which contains hot melt as rework.

Additional experiments are needed to explain the phenomena described in this paper. TEM of freeze-fractured process cheese samples, which shows the microstructure of unstained protein matrices [11], should be used in addition to the examination of stained thin sections. Ion etching followed by SEM may be useful for characterizing the nature of the electron-dense areas. Gel filtration and electrophoresis is suitable to characterize changes in the physical nature of the proteins constituting the matrices of the hot melt as well as other process cheese food samples. Amino acid analysis will elucidate changes in the chemical composition of the process cheese food proteins resulting from prolonged heating.
Fig. 33. Detail of dark areas (t) developing in process cheese food in the presence of TSP. F: fat particle.

Fig. 34. Greater detail of the dark areas (t) developing in process cheese food in the presence of TSP. Dark arrows: fat globule membrane fragments.

Fig. 35. Detail of two dark areas present in process cheese food made with 2.7% TSP using 20% rework consisting of hot melt (sample #4) made with sodium citrate. o: area developed in hot melt, t: area developed in the presence of TSP.

Acknowledgements

Skillful technical assistance with electron microscopy provided by Mrs. Paula Allan-Wojtas and Mr. J.A.G. Larose is acknowledged. Electron Microscope Unit, Research Branch, Agriculture Canada in Ottawa provided facilities. Effects of the rework on apparent viscosity, firmness, and meltability of the process cheese food were studied at the Canada Packers Research Centre in Toronto. The authors thank Mr. C. Farnum and Dr. H.W. Modler for useful suggestions. Contribution 735 from the Food Research Centre.

References


Discussion with Reviewers

D.N. Holcomb: I find the authors' distinction between 'hot melt' and 'rework' a little confusing. Can this be explained?

Authors: The product that had been once processed but not packaged for sale and is to be reprocessed, is called 'rework'. 'Hot melt' is defined in this study as the process cheese which solidified in the pipes due to excessive heat treatment. If the 'hot melt' is reusable, it becomes 'rework'.

D.N. Holcomb: The authors use a more-or-less 'standard' test for cheese meltability. Would additional useful
information be obtained by monitoring the actual internal temperature of the melting cheese?

Authors: The internal temperature of the melting cheese was not monitored. Separate experiments would be required to study the heat conductivity of the cheeses.

M. Carlić: What were the types of cheese used to make the process cheese blend?

Authors: Part skim milk cheese and stirred curd were used in this study.

D.N. Holcomb: Could the authors explain why it is necessary to use osmium tetroxide when fixing samples for TEM but not for SEM? Presumably, fat is extracted for SEM, but is not extracted for TEM. Can the authors estimate how complete are the extraction and retention?

Authors: For order to obtain the most information from milk product samples, fat should either be completely removed or completely retained when preparing these samples for electron microscopy. Partial removal or retention constitutes an artefact [14]. Because TEM is used to demonstrate relationships between components in samples, it is important that the fat component be completely retained. Postfixation with OsO₄ is employed for this purpose. The reaction of unsaturated fatty acids with OsO₄ is accelerated in the presence of imidazole [15]. For SEM, the microstructure of the protein matrix can be studied using samples in which fat has been completely removed (extracted with chloroform after ethanol dehydration). The distribution of fat in the matrix is revealed by freeze-fracturing and is apparent from the distribution of void spaces which had initially been occupied by the fat. Postfixation with imidazole-buffered OsO₄ can be used to retain fat in samples destined for SEM. However, the micrographs of freeze-fractured samples show topographically flat fracture planes, occasional void spaces in the protein matrix indicate the presence of whey or air pockets.

R.E. Cartwright: The heat treatment of the product featured in Table 1 has not been revealed. Please clarify.

Authors: Process cheese food featured in Table 1 was made in the laboratory. After the internal temperature of the cheese reached 82°C (in 5 to 7 min of heating), the cooked cheese was cooled and stored. The hot melt used as rework at 10% and 20% levels was taken out from the pipes of a commercial plant. The amount of heat received by this batch of hot melt varied but is not known. Table 1 is used to demonstrate the considerable effects of hot melt on textural properties of the process cheese food. For the subsequent experiments, another batch of hot melt was made in the laboratory using a known heat treatment.

R.E. Cartwright: The rate of freezing for the laboratory samples must be very fast compared to industrial applications where the bulk quantity to be frozen is much greater. How do you feel a reduced rate of freezing would affect the microstructure of processed cheese and how would its re-use at a 10% or 20% level affect the finished product?

Authors: The rate of freezing was not determined, but it may be assumed that the rate for a small laboratory sample would be higher than the rate for bulk quantity. Slow freezing of cheese may result in the development of ice crystals. Should any ice be formed in slowly frozen 'hot melt', the melted water would be absorbed during processing and the void spaces would vanish as the 'hot melt' is dispersed in the cheese blend rapidly. This hypothesis should be confirmed by experiments. However, the rate of freezing probably does not have as much effect as the rate of cooling. The slowly cooled 'hot melt' rework receives more heat (more heat denaturation), particularly in the high-temperature region, than a rapidly cooled rework and would make the fresh batch of process cheese firmer and less melttable.

R.E. Cartwright: How do you determine that the phenomenon seen in TEM micrographs of the hot melt samples is fat coalescing? Earlier you reported that the emulsification in the hot melt appeared to be more advanced with 25% more fat droplets and a smaller mean radius. The data seem to suggest just the opposite.

Authors: The fat particles in the hot melt were smaller and, thus, more numerous than in the control process cheese sample. This would suggest that additional dispersion of the fat took place during the excessive heat treatment. However, as the hot melt had been heated quiescently, i.e., without stirring as a form of mechanical energy required to break the fat particles [16], it is difficult to perceive that the emulsification would continue. The term 'coalescing' has been used based on the appearance of fat particle clusters. This comment is valuable as it may stimulate interest in studying the effects of quiescent heating and/or cooling on the dispersion of fat in process cheese.

M. Carlić: Amino acid analysis of cheese proteins is carried out after acid hydrolysis of the peptide bonds, so all amino acids present in the system are simply quantitatively determined giving no information about their mutual interactions or interactions with other components. Chemical analysis of the protein fractions (protein nitrogen, soluble nitrogen) and Millard's reaction products such as 5-hydroxyethylfurural would provide more information about the amino acid interactions than the amino acid analysis.

Authors: We agree that a variety of analytical methods should be used to study the changes in the process cheese proteins. As excessive heat treatment may induce changes in the amino acid composition of the proteins (loss of individual amino acids and/or their racemization), amino acid analysis including the detection of D-amino acids should be one of the analytical procedures used.

Additional references