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DEVELOPMENT OF MICROSTRUCTURE IN A CREAM CHEESE BASED ON QUESO BLANCO CHEESE

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Abstract

A Cream cheese was made by mixing cultured high-fat cream (58% fat) with Queso Blanco curd and by homogenizing the mix at 70°C. The final product contained 30.0% to 33.3% fat and 54.1% to 54.5% moisture. The Queso Blanco curd was obtained by precipitating the casein and denatured whey protein from heated whole milk (62.8 to 98.0°C) by bringing the pH to 5.3-5.5 with citric acid, using a continuous process. The extent of denaturation of the whey protein portion varied from 5% to 100%, depending on the heat treatment of the milk. Scanning and transmission electron microscopy revealed the development of microstructure at individual manufacturing stages.

A characteristic core-and-lining ultrastructure of the casein matrix was observed in the Queso Blanco curd, the nature of which depended on the temperature of coagulation. Mixing of the curd with the cultured cream in a Polytron blender dispersed the curd into particles approximately 50 μm in diameter. Subsequent homogenization at 17.23 MPa (i.e., 2,500 psi or 170 atm) further reduced the dimensions of both the protein (<7 μm in diameter) and fat (<5 μm in diameter) particles and produced a uniform microstructure.

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KEY WORDS: Casein; Core-and-lining structure; Cream cheese, Fat globules; Latin American White cheese; Queso Blanco.

Introduction

The development of microstructure in Cream cheese has been the subject of several studies (4, 12, 13, 19). In general, there are four types of Cream cheese manufactured. In the traditional system of manufacture, a cream mix is ripened with a lactic bacteria culture, the curd thus formed is heated, and the whey is drained (13). In the so-called newly formulated method of manufacturing Cream cheese-type products (1), the concentration of the total solids in the cream mix is increased to correspond to the final desired composition of the cheese. This makes draining of the curd unnecessary. The third method employs ultrafiltration of milk to increase the fat/solids content in the retentate to the approximate composition of the Cream cheese (9). Recently, a manufacturing method was developed (16, 17), which consists of mixing high-fat cultured cream (60-70% milkfat) with coagulated whey protein such as Ricotta cheese, or with milk curd such as Queso Blanco cheese (Latin American White cheese), which contains both casein and denatured whey proteins (12).

The Canadian standards for Cream cheese (9) specify a minimum of 30% milkfat while the moisture must not exceed 55%. Cream cheese spread products must contain a minimum of 51% of Cream cheese, no more than 60% moisture, and a minimum of 24% milkfat (10).

The development of microstructure in a Cream cheese spread based on acid-coagulated whey protein curd (Ricotta cheese) was described earlier (12), where it was noted that Ricotta and Queso Blanco cheeses significantly differed in microstructure. The objective of this study was to describe the development of microstructure in the Cream cheese made from the latter starting material.

Materials and Methods

Preparation of milk curd.

Pooled whole milk obtained from the Central Experimental Farm herd in Ottawa (3.3% fat, 3.44% protein) was pasteurized at 62.8°C for 30 min. One part of this milk was heated to temperatures between 62.8 and 82.2°C, and coagulated with a 2.5% citric acid solution (w/v) to produce final pH between 5.3 and 5.5 (measured at 25°C). Another part of the milk was heated to 96-98°C, held for 10 min, and coagulated with the curd being produced by the same procedure. The temperature regimens (temperatures, holding times, and sampling) are shown in Fig. 1.
The coagulum was drained through a cheese cloth. The drained curd of the low temperature heat treatment (62.6 to 82.2°C) contained, on an average, 47.6% total solids, 21.8% fat, and 21.8% total protein (20), and its pH was 5.36. Composition of the curd obtained by the high temperature heat treatment is presented in Table 1.

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Milk: Queso Blanco: Cream cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein:</td>
<td>3.4±0.12</td>
</tr>
<tr>
<td>Fat:</td>
<td>3.3±0.20</td>
</tr>
<tr>
<td>Total solids:</td>
<td>12.0±0.17</td>
</tr>
<tr>
<td></td>
<td>20.3±0.08</td>
</tr>
<tr>
<td></td>
<td>45.0±0.61</td>
</tr>
</tbody>
</table>

**Table 1.**

**Manufacture of the Cream cheese.**

High-fat cultured cream (58% fat, 0.76% acidity) was blended with the Queso Blanco curd to yield a product with approximately 46–63% solids and 27–32% milkfat. The ingredients were first mixed in a Groen processing kettle (Model TDC/TA-2USP) and heated to 71–73°C. Additional fat dispersion was achieved by 20 s of mixing in a Polytron blender (Model 450h) followed by homogenization in a single-stage Hanton-Gaulin homogenizer (Model 7694341) at 17.23 kPa (i.e., 2,300 psi or 170 atm). The product was packaged directly from the homogenizer in 450-mL plastic tubes while still hot (68–70°C).

**Analyses.**

Fat was determined by the procedure of Monjonnier and Troy (18). Total solids were determined by the vacuum oven procedure (3). Nitrogen was determined by the Kjeldahl method (3) and was converted to protein using a factor of 6.28. pH was measured using a Model 26 Radiometer pH-meter equipped with a combination Jena Thalamid electrode.

**Scanning electron microscopy (SEM).**

Drained curd was cut into 1x1x10 mm samples and the samples were fixed in a 2.5% glutaraldehyde solution at 6°C for 24 h, dehydrated in a graded ethanol series, defatted in chloroform, placed in absolute alcohol, frozen in liquid Freon 12 at -150°C, and fractured under liquid nitrogen. The fragments were critical point-dried from carbon dioxide, mounted on SEM stubs, sputter-coated with gold (>20 nm), and examined in a Cambridge Stereoscan Mark II scanning electron microscope operated at 20 kV.

Cultured high-fat cream was aspirated into agar gel tubes, 1.2 mm in inner diameter, and the tubes were sealed with agar gel at both ends (2). The column of cream was 12–15 mm long. The samples were fixed in a 3.5% glutaraldehyde solution at 6°C for 2 h and divided into two parts. Samples in one part were dehydrated and processed for SEM in the same way as the curd, and samples in the other part were postfixed with an imidazole-buffered (pH 7.4) 2% osmium tetroxide solution at 6°C for 24 h to retain the fat (1). The buffer was prepared by mixing a 0.2 M imidazole solution (adjusted to pH 7.4 with 1 M HCl) with a 0.05 M veronal-acetate buffer (pH 7.4) at a ratio of 1:1 (v/v). Postfixation with imidazole-buffered Os04 was aimed at retaining the fat globules in the sample during subsequent dehydration in a graded ethanol series, freeze-fracturing, and critical point-drying from carbon dioxide. The dried fragments were sputter-coated with gold and examined as described above.

In addition, the cream was also examined by cold-stage SEM. The samples were placed in hollow SEM sample holders (11) in such a way as to protrude approximately 1 mm from them, and were immediately frozen in Freon 12 at -150°C. The protruding parts of the samples were fractured off under liquid nitrogen and the freeze-fractured samples were coated with gold by vacuum evaporation using Katon's procedure (11, 14). Cultured samples were examined at -110 to -150°C in the Cambridge Stereoscan Mark II electron microscope equipped with a home-made cold stage. The microscope was operated at 10 kV.

**Transmission electron microscopy (TEM).**

Small particles (<1mm³) of the ingredients (cream, curd) and the Cream cheese at various stages of manufacture were fixed in a 2.5% glutaraldehyde solution and postfixed with imidazole-buffered or veronal-acetate-buffered osmium tetroxide solutions. Only curd samples were fixed unprotected; cream and Cream cheese samples which had been placed on the tip of a needle were coated with agar gel by dipping them for a fraction of a second in a 2% agar sol (approx. 35°C warm), withdrawing the needle from the sample, sealing the opening in the sample left by the needle with agar gel, and placing the sample thus encapsulated into fixative. Dehydration in a graded ethanol series, embedding in a Spurr's low-viscosity medium (21), sectioning, staining, and electron microscopy in a Philips EM-300 electron microscope operated at 60 kV were routine steps (5, 7).

Structure dimensions were measured in enlarged micrographs using a Zeiss MOP-3 digital image analyzer.
The Queso Blanco cheese base for the Cream cheese was made by coagulating milk with citric acid at 63-98°C to a final pH of 5.3 to 5.5. In this respect, the procedure and the curds differed considerably from the manufacture of renneted curd. The amount of whey protein denatured increased with the increasing heat treatment. In curds A and B (obtained at 62.8 and 76.5°C, respectively, Fig. 1), 5 and 33%, respectively, of the whey proteins were denatured. In curds C and D (obtained at 82.2 and 96-98°C, respectively), a total (100%) denaturation of the whey proteins had been achieved, as was determined by the Rowland's procedure.

There was a trend toward an increased compactness of the curd as the temperature and duration of heating were increased. Smoothness of the curd as a sensory attribute increased with the increased compactness of the samples. The curd obtained by acidulating milk heated at 62.8°C for 30 min (Fig. 1, sample A), appeared to consist of fibres when broken with a spatula, and tended to be stringy. It became separated into smaller particles in the mouth when subjected to sensory evaluation. The curd obtained by acidulating milk heated at 82.2°C and held at that temperature for 10 min (sample C) as well as the curd obtained at 96-98°C (sample D) produced the feeling of smoothness and elasticity. All the whey protein had been denatured in both samples.

The microstructure of the Queso Blanco curd was related to the temperature of coagulation and the duration of heating (Figs. 2-4). SEM micrographs (Fig. 2) show the granularity of the freeze-fractured samples. The grains, which had diameters of approximately 0.5 μm in curds B and C and less than 5 μm in curd D, were apparently unrelated to the mouthfeel of the samples. Acid coagulation of heated milk gave the resulting curd a microstructure significantly different from renneted curd. This is shown particularly well in the TEM micrographs. Renneted curd consists of casein.
particles fused together in chains and clusters. In spite of the fusion, individual casein particles can still be distinguished. However, fresh Queso Blanco curd consists of relatively large protein particles (as shown in Fig. 2) composed of transformed casein micelles. The individual casein particles can no longer be distinguished. They underwent severe microstructural changes characterized by the development of a so-called core-and-lining structure (Figs. 3 and 4). This unusual structure was first observed by Harwalkar and Kaláb (7) in glucono-δ-lactone-induced skim milk gels. A solid casein micelle core was found to be surrounded by an outer lining, 30-50 nm thick. There was a void space of 50 to 80 nm between the lining and the core. Individual casein micelles were easy to distinguish. Similar structures were also observed in gels obtained by precipitating milk at 70-90°C using other acidulants such as citric and hydrochloric acids (8). The altered casein micelles were fused into large protein particles and the initial micellar outlines were no more noticeable.

The core-and-lining structure was found only in curd (milk gels) obtained by acidulating hot milk to the final pH of 5.5 or very close to it. At pH 4.5, the microstructure of the gels resembled that of renneted gels (7, 8). The presence of β-lactoglobulin was essential for the development of the core-and-lining structure. All the conditions for the development of the core-and-lining structure were met in the preparation of the Queso Blanco curd as the milk was acidulated with citric acid to final pH of 5.5 at elevated temperatures. However, the curd obtained at 62.8°C exhibited only partially developed core-and-lining structures. Ill-distinguished protein particles (approximately 0.5-1 μm in diameter) were fused and apparently formed a continuous matrix (Fig. 3A), of which SEM provided no detail (Fig. 2A). In the curd obtained at 76.5°C, the protein was found in three distinctly different forms (Fig. 3B): (a) finely dispersed protein particles, (b) somewhat more compact lobular form of uneven density, and (c) individual small, elongated residues. In micrographs B to D, dark arrows point to the core-and-lining structure.

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CREAM CHEESE BASED ON QUESO BLANCO

Fig. 4. Detail of the core-and-lining structure in the Queso Blanco curd.

In micrographs A to C, light arrows point to minute protein particles. In micrographs B to D, dark arrows point to the lining on the surface of coagulated protein. c = minute particles are compacted to varying extent; p = loosely aggregated protein; r = protein in lobular form; s = protein grains.

1 μm long), compact particles bearing signs of the core-and-lining structure. Protein in the lobular form was covered with an envelope (lining). In the curd made at 82.7°C, the occurrence of loosely aggregated protein particles and of lobular particles was lower (Fig. 3C) and the matrix was formed mostly by particles in which the core-and-lining structure was well developed. In the curd made at 96-98°C, the protein was in only two forms. Compact particles, 0.5–3 μm in diameter, predominated. They revealed a well developed core-and-lining structure with regions of different density. Loosely aggregated protein was also present. These structures are shown at greater detail in Fig. 4: In curd A, the larger poorly distinguishable fused particles appear to be composed of globules, approximately 0.1 μm in diameter, i.e. similar to casein micelle dimensions. In the curds B and C, however, considerably smaller particles are seen to form the lobular as well as the compact protein regions. In curd D, the cores of the protein particles appear to be uniform at the thickness of the sections (approximately 90 nm) examined; the linings and the annular void spaces between the linings and the cores are well developed.

The presence of fat in the whole-milk curd is noticeable in samples which had been defatted for SEM. Spherical void spaces in the protein matrix indicate the distribution and dimensions of the fat globules initially present in the curd (Fig. 2).

The microstructure of cultured high-fat cream was examined by: (a) cold-stage SEM (Fig. 5); (b) conventional SEM following postfixation with imidazole-buffered osmium tetroxide to retain fat (Fig. 6); (c) TEM after conventional postfixation (Fig. 7); and (d) TEM after postfixation with OsO4 in the presence of imidazole (Fig. 8). In spite of the limitations in the control of the extent of freeze-etching using the Katoh's (14) procedure for metal coating of freeze-fractured samples for SEM, the micrographs provide sufficient information concerning the dimensions and the distribution of fat globules in the cream (Fig. 5).
Most fat globules are 3 to 6 μm in diameter, as assessed from the fractures. In samples destined for conventional SEM, the fat had been fixed with osmium tetroxide in the presence of imidazole (1) and the aqueous phase had been removed by dehydration and critical-point drying. This step exposed the fat globules and made it possible to examine the sample in some depth (Fig. 6). The fat globules appear to be smaller when viewed by this latter technique than when examined by cold-stage SEM. There could be two reasons for this apparent difference. The samples destined for conventional SEM were first dehydrated in ethanol and freeze-fractured while impregnated with this organic solvent. It is probable that the dehydration caused the cream matrix to shrink to some extent. However, no measurements were carried out to support this assumption. Another reason can be differences in fracturing of the samples with the aqueous phase present or replaced with ethanol, as was reported with other products (11).

Experiments on Cottage cheese revealed that the fracture plane ran preferably between the corpuscles (casein micelles and lactic acid bacteria) in samples impregnated with ethanol. In samples with the aqueous phase present, the fracture plane ran across such corpuscles. The micrographs in Figs. 5 and 6 apparently provide support for this phenomenon occurring also in high-fat cream.

TEM micrographs of cream fixed conventionally (Fig. 7) and cream postfixed with imidazole-buffered osmium tetroxide (Fig. 8) show the fat globules and, in particular, the fat globule membranes, with different degrees of contrast. The latter technique makes it easier to distinguish between fat and the aqueous phase. Small fat globules (<1 μm), such as those seen to surround the central fat globule in Fig. 8, were rare in the high-fat cream used.

Mixing of the Queso Blanco curd with cultured cream in the Polytron blender resulted in an
Fig. 9-13. Microstructure of a mixture prepared in a Polytron blender from high-fat cream and a high-temperature Queso Blanco curd.

9 = Sample, from which fat had been extracted before SEM. Large void spaces (dark arrows) indicate fat globule clusters from the added cream. Compact protein granules (c, light arrows) are part of the initial Queso Blanco curd; small void spaces in the granules developed due to the extraction of the fat present in the whole-milk Queso Blanco curd.

10 = Void spaces (v) in the granular protein matrix (p) developed as the result of fat extraction during sample preparation; fat globule membranes residues have been retained in the void spaces.

11 = SEM of a sample with the fat retained by fixation with imidazole-buffered CeSO₄. Dark arrows point to individual fat globules. f = fat; p = protein.

12 = Detail of fat globules (f) with fat retained in the protein matrix which has compact (c) and loose (p) areas.

13 = TEM shows fat globules and large fat (f) particles in a protein matrix consisting of grains having a core- and-lining structure (dark arrows) and of chains and clusters of loosely aggregated protein particles (p). b = bacteria.
Figs. 14-18. Microstructure of the Cream cheese prepared by homogenization of a mixture of high-temperature Queso Blanco curd and high-fat cream.

14 = Conventional SEM showing the reduction in size of the compact protein particles (c) as well as of the fat globules as evident from the void spaces (v) in the finished product.

15 = Detail of the protein matrix consisting partly of compact protein particles (c) and partly of finely dispersed protein (p); void spaces (v) indicate the presence of small fat globules.

16 = SEM of a sample with the fat retained. The image appears flat and distinction between the protein (dark arrow) and fat components (light arrow) is difficult.

17 = TEM showing the reduction in size and uniform distribution of fat globules (f) and protein grains (p). Protein is also in the form of short chains cementing the fat globules.

18 = Detail of protein particles cementing minute fat globules (f). Large dark arrows point to the lining and light arrows point to minute particles in the protein cores (p). Short protein chains (small dark arrows) are also evident.
intermediate product, which was slightly gritty when tasted, irrespective of the temperature at which the milk proteins had been coagulated. This product consisted of relatively large protein and fat particles (Figs. 9-13). The dimensions of the protein particles varied and their diameters were as large as 50 μm. Conventional SEM of defatted samples was particularly useful in easily distinguishing between the protein and fat particles (Fig. 9). Small spherical void spaces in the large protein particles indicated that fat globules were present in the initial Queso Blanco curd made with whole milk as the starting material. The void spaces observed in the micrographs were initially occupied by fat which was later removed during sample preparation (Figs. 2 and 9). The proof that the void spaces had indeed been occupied by fat is provided by SEM of samples in which residues of fat globule membranes have been retained (Fig. 10). Figs. 11 and 12 show fat retained in the protein matrix and confirm, by another preparatory technique, that the protein was indeed in a corpuscular form. Sections of this product examined by TEM (Fig. 13) reveal that the core-and-lining structure has persisted. Homogenization of the protein-cream mixture at approximately 70°C resulted in the disintegration of the protein particles and in a uniform distribution of the fat globules from the cream (Fig. 14). The dimensions of the compact protein particles were reduced to less than 7 μm in diameter. A considerable amount of the protein was reduced to particles having dimensions smaller than those of casein micelles in milk (Figs. 15-18). Fixation of fat and its retention in the matrix produced micrographs showing a product in which the aqueous phase is not apparent (Fig. 16). In this respect, formulated Cream cheese made according to the traditional method of manufacture (13). The protein particles and the fat globules were evenly dispersed in the aqueous phase as is evident in Figs. 17 and 18. The small dimensions of the fat globules and their relative uniformity in the finished product are apparent from the micrograph. Most fat globules were 0.5-1.5 μm in diameter and a small number of the largest fat globules were less than 5 μm in diameter.

The development of microstructure in the Cream cheese made from the Queso Blanco curd resembles the Cream cheese spread made from the Ricotta cheese curd (12) but differs from the traditional Cream cheese as well as from the newly formulated and imitation Cream cheese spreads. There is one important difference, however, between the Cream cheese made from the Queso Blanco and the Cream cheese spread made from the Ricotta cheese. This difference is caused by differences in the composition and microstructure of the respective protein bases used. Queso Blanco curd is more compact than the whey protein curd; the latter curd consists of small whey protein particles, approximately 100 nm in diameter (12). This difference is reflected in the spreadability of the product. The Cream cheese made from Queso Blanco does not spread as easily as the spread made from the whey protein base (17). A higher total protein content in the Queso Blanco-based Cream cheese (10,743) than in the Ricotta-based spread (8,45%) may have been the main cause of the poorer spreadability in case of the product made from the low-temperature curd (17) but not in the case of the high-temperature curd, where the protein content was comparable (8,222) to that in the Ricotta-based spread. Rather, the more compact protein matrix of the Queso Blanco curd, mostly composed of casein, may be the main contributing factor.

The emphasis of the manufacture of the Cream cheese was on the simplicity of the procedure and its rapidity. For this reason, the relatively long heating of the milk was replaced with heating to a higher temperature.

The procedure based on blending Queso Blanco and Ricotta cheeses with cultured high-fat cream provides flexibility in adjusting the composition of the final product concerning the total solids as well as fat contents. Cultured buttermilk can be used to standardize the fat content and provide additional flavour (17). The manufacturer also has the option of using different bacterial cultures in the high-fat sour cream and also buttermilk to thereby tailor the flavour profile of the finished product to meet various product specifications.

Acknowledgments

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Contribution 637 from the Food Research Institute.

References

18. Mojonnier T, Troy R. T. Marshall: Postfixation with imidazole-buffered osmium tetroxide does not seem to go with the second and third treatments, i.e. freeze-fracturing and critical-point drying. Please comment.
Authors: Yes, the pH of the cream was 4.4.
R. T. Marshall: Postfixation with imidazole-buffered osmium tetroxide does not seem to go with the second and third treatments, i.e. freeze-fracturing and critical-point drying were dehydrated in ethanol. Critical-point drying was carried out from liquid carbon dioxide. Both solvents are lipophilic and would partially extract fat unless it is fixed. This fixation was achieved by imidazole-buffered osmium tetroxide (1).

Discussion with Reviewers

F. Resmini: Do the authors know the cream pH at the moment of preparation for electron microscopy?
Authors: Yes, the pH of the cream was 4.4.

M. V. Taranto: Does the manufacturing procedure devised by the authors have any advantage over the others?
Authors: The procedure devised by the authors has several advantages. (a) It is cost-effective because the total solids and butterfat can be accurately standardized by the addition of cultured buttermilk. (b) The high-fat sour cream and cultured buttermilk can be made with different cultures to produce desired flavours. For example, the high-fat cream can be cultured with diacetyl-producing bacteria whereas the buttermilk can be cultured with high lactic acid-producing bacteria. (c) There is no syneresis in the product and this precludes the necessity to add stabilizers. (d) This Cream cheese can be produced even by a small manufacturer who does not own Cream cheese manufacturing equipment and who buys the ingredients (protein base, high-fat sour cream, buttermilk), combines them, and tailors the product to meet specific texture and flavour requirements.

M. V. Taranto: On what basis did the authors draw the conclusion that the differences in the dimensions of the protein grains in the curds B, C, and D were unrelated to the mouthfeel of the samples?
Authors: Sensory evaluation, reported elsewhere (17), indicated that both the sample C, obtained at 82.7°C, and sample D, obtained at 96-98°C, produced the feeling of smoothness and elasticity. The protein grains in sample C were approximately 0.5 μm in diameter whereas the grains in sample D were almost 10 times larger (<5 μm in diameter). Thus the difference in the grain dimensions had apparently no effect on the mouthfeel.

M. V. Taranto: The authors have not discussed the effect of the fat globule size distribution. I would suspect that this structural difference would have played a role (at least in part) in controlling the spreadability of the final product. Please comment. Would the authors also speculate on (discuss) the correlation between the observed microstructure and the mouthfeel of the final Cream cheeses? What is the controlling factor: the protein matrix or the fat globule size distribution? This point would be important in providing manufacturers sound technical means for accurately controlling the desired final product attributes.
Authors: There are many factors which may affect the spreadability of the product. The relationship between the fat globule dimensions and spreadability was not studied. We agree that this relationship would be interesting and should be the subject of a separate study.

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