Effect of Heating of Cream on the Properties of Milk Fat Globule Membrane Isolates

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Buttermilk contains large amounts of proteins derived from skim milk and from the milk fat globule membrane (MFGM). Heating the cream before churning, even at temperatures as low as 65 °C, strongly affected the functional properties of the membrane fraction and caused whey proteins to associate with the MFGM. The extent of this interaction depended on temperature. Pasteurization temperature of the cream had no effect on the emulsifying properties of whole buttermilk isolates, the casein content of which masked changes in the MFGM fraction. The stability of oil-in-water emulsions made using isolated MFGM material depended on the heat treatment of the cream. The solubility, color, iron content, amount of whey protein present in the MFGM isloate, and emulsifying properties of this material were all affected in parallel by the heat treatment.

Keywords: *Heating of cream; emulsifying properties; milk fat globule membrane isolate; buttermilk*

INTRODUCTION

In whole milk, fat globules are surrounded by a membrane, originally derived from the apical plasma membrane of the milk fat globule (McPherson and Kitchen, 1983). This milk fat globule membrane (MFGM) is a complex mixture of proteins and phospholipids and acts as a natural emulsifier, preventing coalescence of the fat globules. In the conventional process of butter-making, after pasteurization, destabilization of the fat globules occurs in cream during churning, and a water phase (buttermilk) is released. The protein content of buttermilk resembles that of skim milk (~30 g/L of protein). However, buttermilk contains not only skim milk proteins (caseins and whey proteins) but also a large fraction of MFGM material.

MFGM proteins represent only a small portion of the total milk proteins, but they are fundamental for the stability of the fat globules (McPherson and Kitchen, 1983; Keenan and Dylewski, 1995). The major proteins present in the MFGM have been isolated and studied (Greenwalt and Mather, 1985; Mather and Jack, 1993; Berglund et al., 1996). In a recent review, Keenan and Dylewski (1995) described the difficulty inherent in the isolation of the individual proteins in the MFGM, because of the strong association between butyrophilin (67 000 Da), xanthine oxidase (~150 000 Da), and other minor MFGM proteins. Most MFGM proteins are characterized by high hydrophobicity and are covalently bound to fatty acids and to carbohydrate residues (Shimizu et al., 1976; Mather and Jack, 1993).

Because of their origin and amphiphilic nature, MFGM proteins are expected to be good emulsifying agents. Recently, the functionality of MFGM proteins in emulsions has been investigated by homogenizing milk fat in the presence of MFGM (Kanno et al., 1991; Oehlmann et al., 1994). These studies did not clarify which components of the MFGM play a role in the stabilization of the reconstituted fat globules. These emulsions were also prepared with MFGM from non-pasteurized fresh cream (Kanno et al., 1991; Oehlmann et al., 1994).

Because it may be possible to utilize these fractions from buttermilk as functional ingredients in foods, an understanding of the effect of heating of the cream on the MFGM is needed, because in the manufacturing process of butter-making an initial heat treatment of the cream is generally employed. It has been shown that during heating of whole milk, even at low temperatures (i.e., 65 °C), strong interactions occur between α-lactalbumin, β-lactoglobulin, and MFGM (Corredig and Dalgleish, 1996). These interactions result in a change in the electrophoretic mobility with very little change in the diameter of the fat globules (Dalgleish and Banks, 1991). A new membrane, more resistant to coalescence, develops around the fat globules (Dalgleish and Banks, 1991; van Boekel and Folkerts, 1991). The precise nature of these interactions is not yet clear; various hypotheses have been formulated (Dalgleish and Banks, 1991; van Boekel and Folkerts, 1991) that (i) skim milk proteins might displace the original membrane material; (ii) proteins may fill in gaps in the membrane surface, which become exposed after heating; or (iii) skim milk proteins may bind to the MFGM proteins via disulfide bridging and/or hydrophobic interactions. The latter mechanism agrees with the results of Kim and Jimenez-Flores (1995); the extent of reaction of whey proteins with the MFGM proteins is strongly dependent on temperature.

Recently, we have studied the properties of MFGM material from industrial buttermilk (Corredig and Dalgleish, 1997). These MFGM isolates contained considerable amounts of associated whey proteins, and their emulsifying properties were found to be poor compared to those of MFGM material isolated from unpasteurized cream (Corredig and Dalgleish, 1998). The observed difference in functionality between the two MFGM

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isolates led to the present study, in which we investigated the changes occurring in the MFGM when cream is heated. Protein solubility, protein adsorption, and emulsifying properties of the MFGM isolates as functions of temperature were determined. The cream used was heated either by industrial pasteurization [high-temperature short time (HTST)] or by batch heating at controlled temperatures (60−85 °C).

MATERIALS AND METHODS

Heat Treatment of the Cream. Cream (∼40% w/w fat) was obtained from a local dairy after separation from fresh skim milk. The centrifugal separation was performed at 50 °C so that our "untreated cream" had actually been heated at this temperature. Cream was further heat treated in the laboratory by batch heating for 10 min at temperatures between 60 and 85 °C in a water bath in 250 mL aliquots. Samples were heated in a water bath at 95 °C to reach the required temperature in <1 min and then transferred to the water bath at the required holding temperature. Cream was also collected at the dairy plant after different HTST pasteurization steps (76−85 °C for 16 s). After heating, the creams were cooled to room temperature in an ice bath and then, after addition of sodium azide (0.02%), were kept refrigerated (4 °C) overnight.

Production of Buttermilk. To assess the effect of heating temperature on the functionality of the MFGM fractions present in buttermilk, cream destabilization was achieved by churning in a small scale churn (1 L capacity). The samples were churned at 10 °C until phase separation occurred. Buttermilk was also collected at the dairy plant, after churning of pasteurized creams (85 °C for 16 s), and used for gel permeation chromatography experiments.

Preparation of the Isolates. Buttermilk was released from the butter granules and filtered through a cheesecloth and then through a qualitative P8 filter (Fisher Scientific, Mississauga, ON). The MFGM isolates were prepared as described by Corredig and Dalgleish (1997). After addition of sodium citrate (2% w/v) to dissociate the casein micelles, buttermilk was kept at refrigeration temperature (4 °C) overnight. The samples were then centrifuged at 15 °C for 50 min at 100000*g* in a preparative ultracentrifuge (Beckman, model L8-70 M, Palo Alto, CA). Buttermilk isolates containing casein micelles as well as the membrane fraction were also prepared as control samples by centrifugation of buttermilk without addition of sodium citrate. The sedimented pellets of the buttermilk and MFGM isolates were drained on filter paper (Whatman No. 4, Fisher Scientific), resuspended in Milli-Q water using a tissue homogenizer (Polytron PT 2000, Kinematica AG, Switzerland), and then freeze-dried. The freeze-dried samples were stored at −20 °C. The isolates contained MFGM material consisting of a mixture of phospholipid and protein, the composition of which has been described previously (Corredig and Dalgleish, 1997). The protein concentration of the freeze-dried MFGM isolates was ∼60% w/w.

Emulsion Preparation. Aliquots of freeze-dried isolates were resuspended in buffer (20 mM Tris-HCl) at pH 7. Soybean oil (10% w/v) was added, and the mixture was homogenized using a microfluidizer (model 110S, Microfluidics Inc., Newton, MA) with an input pressure of 56 MPa. Each sample was circulated for 10 strokes of the pump and then collected; this procedure was repeated 4 times.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis. SDS−PAGE was performed as described in detail elsewhere (Corredig and Dalgleish, 1997, 1998). The protein composition of the isolates was determined by suspending aliquots (3 mg) of freeze-dried samples in 200 μL of 10 mM Tris, 1 mM EDTA, and 20 mM imidazole buffer, pH 8. SDS (300 μL, 20% w/v solution), bromophenol blue (100 μL, 0.05% w/v), and 2-mercaptoethanol (100 μL) were added before sample denaturation.

Emulsions were separated by centrifugation at 10000*g* for 50 min (Beckman, model L8-70M); the oil droplets were collected by draining them on a filter paper and then suspended in buffer to a final concentration corresponding to that of the original oil-in-water emulsion. The samples were loaded onto a 20% acrylamide homogeneous gel (Pharmacia Biotech, Baie D'Urfé, PQ) and run in a rapid electrophoresis system (Pharmacia Biotech). The protein bands were fixed and stained using a solution of Coomassie blue (1% w/v) in methanol/acetic acid/water. Destaining was achieved by washing the gel in the same mixture of methanol/acetic acid/water. The dried gels were scanned with a Sharp JX330 scanner. The images were analyzed with Imagemaster software (Pharmacia Biotech) for quantitative analysis. The amounts of α-lactalbumin and β-lactoglobulin were quantified as percentages of the total protein present in the sample. The individual protein bands were identified by comparison with known standards; the caseins and serum proteins were prepared in the laboratory, and the membrane proteins were compared with the patterns shown by Keenan and Dylewski (1995).

Fat Globule Size Distribution. The fat globule size distribution of the emulsions was determined by integrated light scattering using a Mastersizer X (Malvern Instruments, Southoboro, MA). The samples were dispersed in Milli-Q water with a dilution factor of approximately 1:200 before the measurement. The droplet size distribution and the average diameter, $d_{3,2}$ [$\Sigma nd^3/\Sigma nd^2$], of the emulsions were used to characterize the emulsifying behavior of the different isolates. The size distributions were generally measured within 2 h of the preparation of the emulsions. Preparations that had monomodal size distributions were stable for times of several weeks.

Size Exclusion Chromatography. To obtain information on the aggregation of the MFGM in buttermilk from heated cream, size exclusion chromatography was performed (Superose 12, Pharmacia LKB) on samples of industrial buttermilk. The separation range of Superose 12, as declared by the manufacturer, is between 1 and 300 kDa. Before analyses, samples were filtered through a 0.45 μm filter (Millipore, Fisher Scientific). Two different elution buffers, pH 7.0, were used: (i) a nondissociating buffer containing 20 mM imidazole, 5 mM $CaCl_2$, and 50 mM NaCl; and (ii) a dissociating buffer containing 6 M urea and 20 mM imidazole. A Pharmacia LKB optical unit UV-1 (Pharmacia Biotech) at 280 nm was employed to follow the elution peaks. To determine which aggregates were mainly held together by disulfide bonds, 5 mM 2-mercaptoethanol was added prior to chromatography. The elution patterns of reduced samples were compared with those of the same samples, eluted in the absence of 2-mercaptoethanol. After collection of the fractions separated by chromatography, SDS−PAGE was carried out to determine the protein composition of the eluted peaks. To 0.5 mL of sample were added SDS (200 μL), 2-mercaptoethanol (50 μL), and bromophenol blue (100 μL).

Solubility Studies. Freeze-dried protein isolates (∼60% w/w protein) obtained from differently heated creams were resuspended (0.8% w/v) in one of three buffers: (i) 20 mM Tris, pH 7.0, with or without added Triton X-100 (1% w/v); (ii) 100 mM Na_2CO_3, pH 11.5; or (iii) 20 mM Tris, 4 M urea, pH 7.0. The nonionic detergent Triton X-100 and the carbonate buffer at high pH were chosen because of their common use in removing proteins from membrane material (Yanagita and Kagawa, 1986; Fujiki et al., 1982), and the urea buffer was used because of the well-known tendency of urea to dissociate protein complexes. After continuous stirring for 3 h, the samples were centrifuged at 10000*g* for 30 min. Protein analysis on the soluble phase was carried out according to a Lowry procedure as modified by Markwell et al. (1978) for membrane protein and lipoprotein preparations. Bovine serum albumin (Sigma Chemical, St. Louis, MO) was used as the protein standard. The protein composition of the soluble fractions was also determined by SDS−PAGE. The electrophoresis was performed as described above, with aliquots (200 μL) of soluble phase.

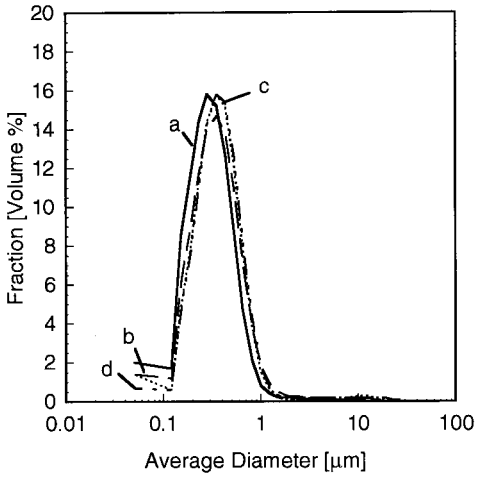

Figure 1. Typical droplet size distribution of 10% (w/v) soybean oil-in-water emulsions measured by integrated light scattering. Emulsions were prepared with 2% (w/v) buttermilk isolate derived from (a) untreated cream or (b−d) cream heated by HTST for 16 s at temperatures of (b) 76, (c) 78, and (d) 85 °C.

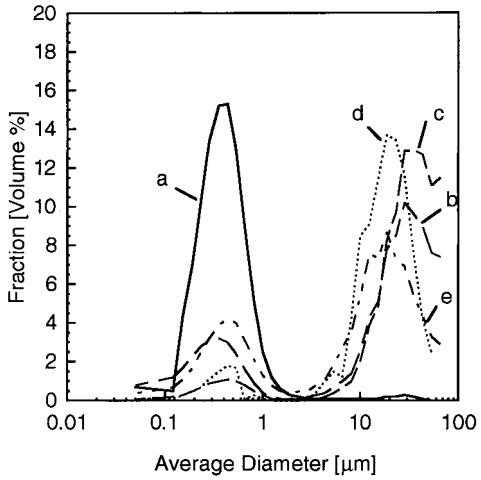

Figure 2. Typical droplet size distribution of 10% (w/w) soybean oil-in-water emulsions measured by integrated light scattering. Emulsions were prepared with 1% (w/v) of MFGM isolate from buttermilk derived from (a) untreated cream or (b−e) cream heated by HTST for 16 s at temperatures of (b) 76, (c) 78, (d) 80, and (e) 85 °C.

Determination of Iron Content of the Samples. The amount of Fe contained in the MFGM isolates derived from cream heated at various temperatures was quantified by flame absorption spectrometry. Approximately 0.25 g of freeze-dried MFGM isolate was digested with nitric acid and analyzed with a Perkin-Elmer 5100 ZL atomic absorption spectrometer. The analyses were performed by the Laboratory Services Division of the University of Guelph (Guelph, ON).

Statistical Analysis. Analysis of variance of the effect of heating temperature on the amount of whey protein associated with the MFGM fraction, the average diameter of the emulsions prepared, and the solubility of the MFGM isolates was performed (SAS/STAT software, SAS Institute, NC). Results were considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

Studies on Industrial Cream and Buttermilk. *Emulsifying Properties of Isolates from Buttermilk Prepared with Cream Heated by HTST (76−85 °C).* Two different isolates were used to prepare oil-in-water emulsions: buttermilk isolates and MFGM isolates. Buttermilk isolates contained considerable amounts of skim milk proteins, as they were prepared simply by centrifuging buttermilk. Figure 1 shows the droplet size distribution of 10% (w/v) soybean oil-in-water emulsions prepared with buttermilk isolates originating from creams pasteurized by HTST at different temperatures. The diameter distribution of emulsions prepared with 2% (w/v) buttermilk isolate from unpasteurized cream was not different from that of emulsions prepared with the same amounts of buttermilk isolates from creams heated at 76, 78, or 85 °C. Caseins, present in the buttermilk isolates, were the main proteins adsorbed to the oil−water interface and determined the behavior of the emulsions. These results were in agreement with those previously reported for isolates derived from industrial buttermilk (Corredig and Dalgleish, 1997).

To study changes caused by the effects of temperature of the HTST pasteurization of the cream on the emulsifying properties of the MFGM material, we determined the droplet size distributions in emulsions prepared with MFGM isolates. These isolates contained a much higher concentration of MFGM material than buttermilk isolates and much smaller amounts of skim milk proteins. The droplet size distributions of MFGM isolate emulsions containing 10% (w/w) soybean oil are shown in Figure 2. All of the MFGM isolates from creams heated by HTST at temperatures between 76 and 85 °C showed emulsifying properties that were much worse than those of MFGM isolates from unpasteurized cream. Emulsions prepared with 1% (w/v) MFGM isolate from unheated cream had a narrow distribution of droplet sizes, with an average diameter of ~0.4 μm. All of the emulsions made from the MFGM of HTST creams contained a large number of droplets with diameter >10 μm and relatively small amounts with diameter <1 μm (Figure 2).

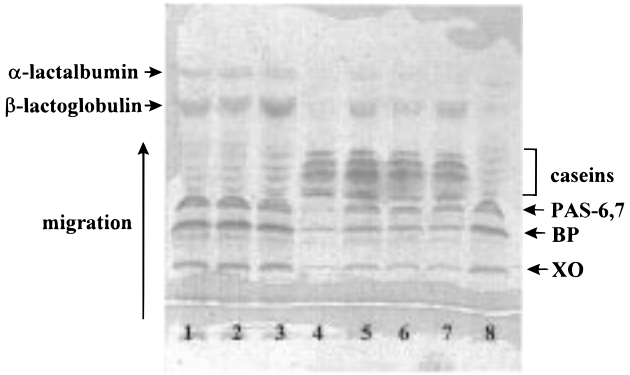

Figure 3. SDS−PAGE of MFGM and buttermilk isolates prepared by ultracentrifugation of buttermilk from HTST creams: (lane 1) MFGM isolate from cream heated at 78 °C for 16 s; (lane 2) MFGM isolate from cream heated at 76 °C for 16 s; (lane 3) MFGM isolate derived from industrial buttermilk; (lane 4) buttermilk isolate derived from unheated cream; (lane 5) buttermilk isolate derived from cream heated at 78 °C for 16 s; (lane 6) buttermilk isolate derived from cream heated at 76 °C for 16 s; (lane 7) buttermilk isolate derived from industrial buttermilk; (lane 8) MFGM isolate derived from unheated cream. XO, xanthine oxidase; BP, butyrophylin.

When resuspended in Tris/urea buffer, the MFGM isolates from HTST cream−buttermilk were not soluble. In addition, SDS−PAGE indicated large amounts of whey proteins in these isolates. Figure 3 shows the electrophoretic pattern of MFGM isolates derived from cream heated at 76 and 78 °C and also from industrial buttermilk. In the latter, cream destabilization occurred in a continuous churn after pasteurization at 85

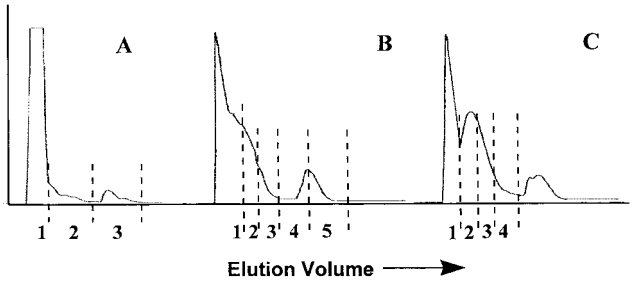

Figure 4. Gel permeation chromatography patterns of industrial buttermilk, using different buffers, showing the fractions collected for analysis by SDS−PAGE: (A) chromatography in imidazole buffer; (B) chromatography in imidazole/urea buffer; (C) chromatography in imidazole/urea buffer containing 5 mM 2-mercaptoethanol.

°C for 16 s. The presence of whey protein in the MFGM of all of these fractions was caused by heat-induced interactions between α-lactalbumin, β-lactoglobulin, and the membrane of the fat globules occurring in cream during heating (Dalgleish and Banks, 1991; Corredig and Dalgleish, 1996). Figure 3 also shows the electrophoretic separation of buttermilk isolates. Whey proteins were absent only in the isolates (both buttermilk and MFGM) that had been obtained from nonpasteurized cream.

Size Exclusion Chromatography. The association of skim milk proteins with the MFGM was investigated by gel permeation chromatography using non-denaturing and denaturing buffers. Buttermilk was collected after industrial churning of cream pasteurized at 85 °C for 16 s. When these buttermilk samples were eluted on a column of Superose 12, most protein was found in the excluded volume (molecular size > 300 kDa). In nonreducing conditions, three fractions could be separated: one at the exclusion limit of the column and two others. Large MFGM aggregates were eluted together with casein micelles in the excluded volume (fraction 1, Figure 4A; lane 1, Figure 5A); however, this fraction also contained β-lactoglobulin in an appreciable amount (∼50% of the total β-lactoglobulin). The remaining β-lactoglobulin was isolated in the second fraction, which contained no other protein, and the third fraction contained only α-lactalbumin, which was absent from the other two peaks (Figure 5A, lanes 2 and 3). Previous research on heating of whole milk has shown that both α-lactalbumin and β-lactoglobulin interact with the membranes of fat globules (Dalgleish and Banks, 1991; Corredig and Dalgleish, 1996). In the aggregates smaller than 0.45 μm present in buttermilk (the samples were filtered before chromatography), only β-lactoglobulin was associated with the MFGM fraction. Therefore, the reactions between proteins in heated cream and whole milk may differ.

Urea buffer disrupts the interactions that hold together casein micelles and aggregates. Chromatography of the buttermilk samples in urea allowed collection of five samples (Figure 4B), the protein compositions of which are shown in Figure 5B, lanes 1−5. The fraction eluting at the void volume contained MFGM proteins, κ-casein, and β-lactoglobulin. Progressive elution provided fractions containing less MFGM and more caseins but also amounts of β-lactoglobulin and α-lactalbumin (Figure 5B, lanes 1−4). The final peak in the elution pattern contained no protein. When 2-mercaptoethanol was added to the sample (Figure 4C), the β-lactoglobulin and κ-casein were lost from the void volume peak and appeared at longer elution times, but the membrane proteins remained in this fraction (Figure 5B, lanes 6−9).

These results suggest that there are interactions between cysteine-containing proteins of milk and those of MFGM. The β-lactoglobulin and κ-casein in the void volume fraction must be there because they are linked by disulfide bonds to other proteins, but these experiments do not permit us to determine whether, for example, the κ-casein is specifically linked to β-lactoglobulin or to membrane proteins or if it is simply polymeric. Because the MFGM isolate does not appear to contain κ-casein (Figure 3), it is likely that it is present in the void volume peak because of its polymeric nature (Yaguchi et al., 1967). At least part of the β-lactoglobulin in the void volume peak is, however, bound to the MFGM.

MFGM Isolates Derived from Cream Heated at Temperatures of 60−85 °C. No differences were observed in the emulsifying properties of MFGM isolates from creams heated by HTST at 76 or 85 °C. All of the emulsions prepared with these MFGM isolates showed a bimodal droplet size distribution with a large proportion of droplets >10 μm. On the other hand, the emulsions prepared with MFGM isolates from buttermilk obtained from nonpasteurized cream were stable; they were characterized by a monomodal droplet size distribution with an average diameter of ∼0.4 μm.

Therefore, we studied the effect of heating at lower temperature than those used for HTST on the properties of the MFGM. Cream was heated by batch heating for 10 min at temperatures ranging between 60 and 85 °C. Unpasteurized cream was collected after centrifugal separation at 50 °C. MFGM isolates were prepared from all of the heated creams.

Solubility Studies. Solubility is an important index for the use of proteins in food systems. MFGM isolates, obtained from creams heated at different temperatures, were resuspended in various buffers (0.8% w/v), and after centrifugation, the soluble phases were analyzed for protein concentration as well as protein composition (Figures 6 and 7). When MFGM isolates were resuspended in Tris/urea buffer, the solubility was affected by the temperature of heating of the cream (Figure 6). The three major bands that characterize the MFGM proteins in SDS−PAGE (xanthine oxidase, butyrophilin, and PAS-6 and -7) were identified in samples from untreated creams (heated at 50 °C, during centrifugal separation at the dairy) and from creams heated at 60, 62, and 65 °C. MFGM isolates of cream heated at 70 °C (Lane 4) contained in their soluble fraction only one of these three MFGM protein bands. These residual proteins have been identified as PAS-6 and PAS-7, which are known to be on the surface of the membrane, not tightly associated, and soluble in urea (Kim et al., 1992). MFGM isolate suspensions derived from cream heated at temperatures >70 °C were not soluble in 4 M urea buffer. In addition, no whey protein was present in the soluble material. In the MFGM isolates derived from cream heated at temperatures >70 °C, there was a large amount of whey protein, but it was covalently linked to the membrane material and was insoluble.

The solubility of the MFGM fraction, not only in urea buffer but also in Tris/Triton X-100 or sodium carbonate buffer, was strongly affected by the temperature of heating of the cream (Figure 7). The MFGM isolates were not soluble in Tris buffer, whereas with the

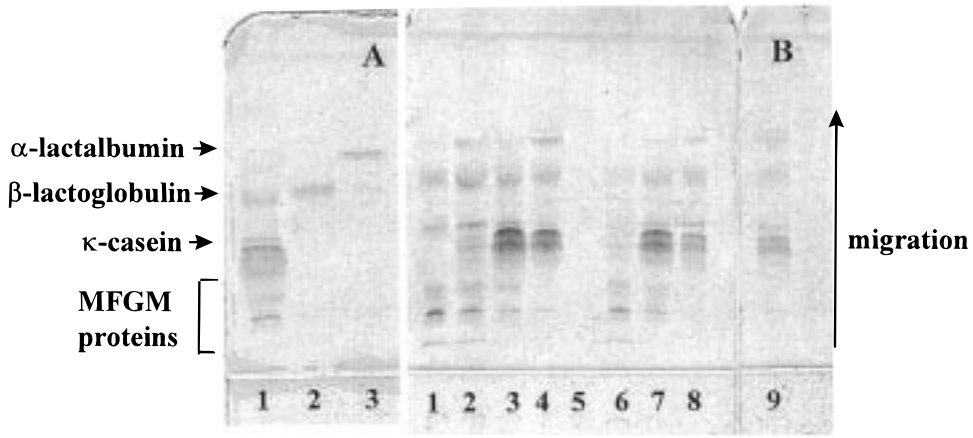

Figure 5. Electrophoretic migration of the protein fractions eluted by gel permeation chromatography: (A) industrial buttermilk eluted with imidazole buffer, chromatography fractions 1−3; (B) chromatography fractions 1−5 from industrial buttermilk eluted with imidazole/urea buffer, without (lanes 1−5) and with (lanes 6−10) 5 mM 2-mercaptoethanol. SDS−PAGE was performed in reducing conditions.

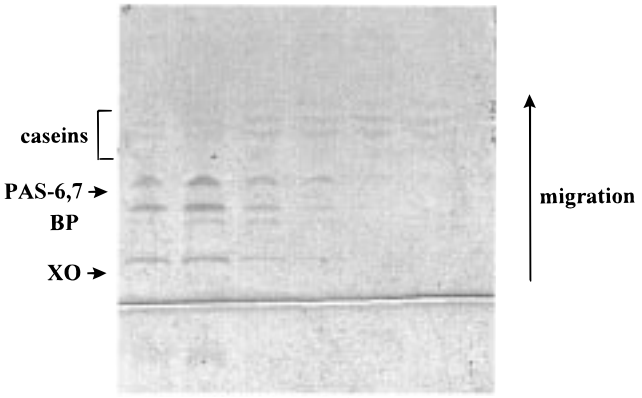

Figure 6. SDS−PAGE of the soluble fractions (after centrifugation) of MFGM isolate suspensions (0.8% w/v) in 4 M urea buffer: (lane 1) MFGM isolate from buttermilk derived from unheated (50 °C) cream; (lanes 2−6) MFGM isolates from creams heated at temperatures of (lane 2) 60, (lane 3) 65, (lane 4) 70, (lane 5) 75, and (lane 6) 80 °C. SDS−PAGE was performed in reducing conditions. XO, xanthine oxidase; BP, butyrophylin.

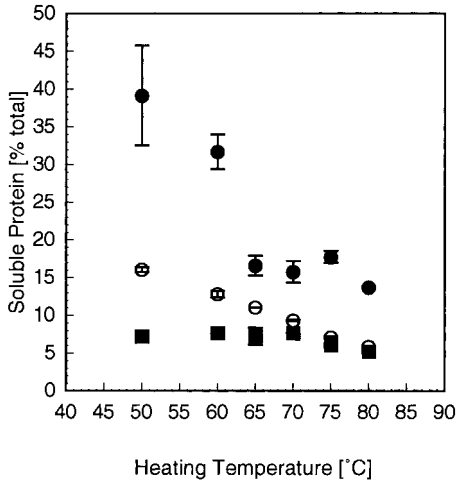

Figure 7. Concentration of soluble protein (percent of total isolate weight) after centrifugation of suspensions (0.8% w/v) of different MFGM isolates (60% w/w protein), as a function of temperature of heating of the cream: (■) MFGM resuspended in 20 mM Tris buffer, pH 7.0; (○) MFGM resuspended in 20 mM Tris buffer and 1% Triton X-100, pH 7.0; (●) MFGM resuspended in 0.1 M Na_2CO_3 buffer, pH 11.5.

addition of Triton X-100 their solubility was increased but depended significantly ($p < 0.05$) on the temperature of heating. The amount of protein in the soluble phase of Tris/Triton X-100 buffer differed from that present in the soluble phase of Tris buffer for temperatures <70 °C. This effective solubilization of some MFGM protein with Triton X-100 agreed with the results reported by Houlihan et al. (1992) for MFGM from pasteurized cream and milk. Sodium carbonate, pH 11.5, solubilized larger amounts of protein than Triton X-100, in samples of MFGM isolates from cream heated at temperatures <65 °C. In this case also, there was a significant ($p < 0.05$) effect of temperature of heating on the amount of residual soluble protein. However, in no case was >50% of the total protein solubilized.

Emulsifying Properties of the MFGM Isolates. Emulsions were prepared with MFGM isolates from buttermilk obtained from differently heated creams. Whereas 1% (w/v) MFGM from unheated cream had good emulsifying ability (Figure 2), emulsions with 1% (w/v) MFGM isolates from heated creams contained large particles, and for cream heated at 75−85 °C, obvious phase separation was observed after homogenization. The droplet size distribution of 10% (w/w) oil-in-water emulsions prepared with 2 and 3% (w/v) MFGM isolates is shown in Figure 8. Emulsions prepared with MFGM isolates from creams heated at 60 and 62 °C had monomodal distributions similar to those of emulsions prepared with MFGM isolates from unheated cream. When MFGM isolates obtained from creams heated at higher temperatures were used, the emulsion droplets increased in size and the number of large droplets seemed to depend on the heating temperature of the cream. The overall size distribution improved when emulsions were prepared with larger amounts of MFGM isolates (3% w/v) (Figure 8B), but even at this concentration a significant shift of the average droplet size to a larger diameter occurred as MFGM from increasingly heated cream was used.

Analysis of variance showed a significant ($p < 0.05$) effect of temperature on the average diameter of the droplets for emulsions prepared with 2 and 3% (w/v) MFGM isolates. Figure 9 shows the increase in $d_{3,2}$ for 2% (w/v) MFGM isolate in 10% (w/w) soybean oil emulsions, as a function of the heating temperature. In this case also, as already shown for the solubility of the

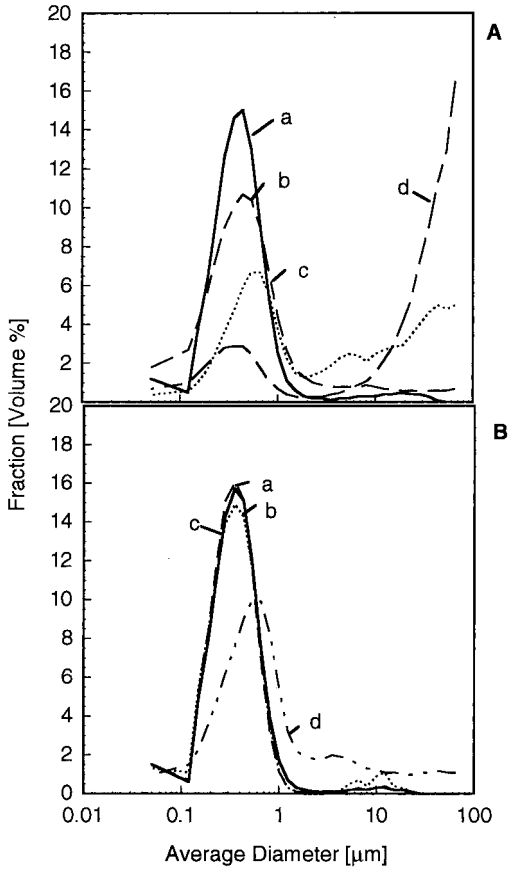

Figure 8. Typical droplet size distribution of 10% (w/w) soybean oil-in-water emulsion measured by integrated light scattering. Emulsions were prepared with 2% (A) and 3% (w/v) (B) MFGM isolate from buttermilk obtained from cream heated for 10 min at (a) 60, (b) 65, (c) 70, and (d) 80 °C.

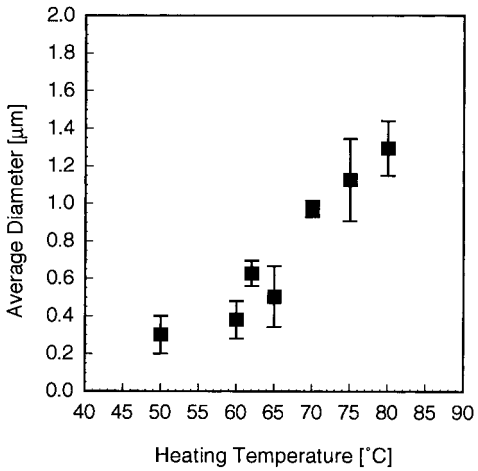

Figure 9. Average diameter as determined by integrated light scattering of oil-in-water emulsions containing 10% (w/w) soybean oil and 2% (w/v) MFGM isolate. Values of $d_{3,2}$ are plotted versus temperature of heating of the cream.

different MFGM isolates, up to 65 °C there was no significant difference in the average diameter size.

Whey Proteins Associated with the MFGM. The protein compositions of the MFGM isolates prepared from creams heated at different temperatures were analyzed by SDS−PAGE, which showed that the amount of whey protein associated with the MFGM increased with increasing temperature. These results were in agreement with those reported for MFGM obtained from

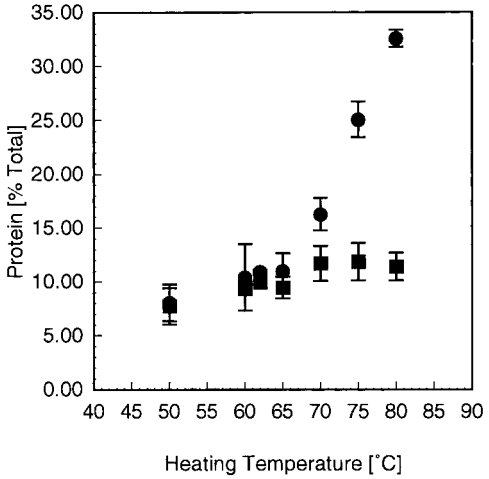

Figure 10. Amounts of (■) α-lactalbumin and (●) β-lactoglobulin (percent of the total MFGM protein in SDS−PAGE) as a function of temperature of heating of the cream. Results are the averages of at least three independent experiments.

HTST creams (Figure 3). The association of β-lactoglobulin increased at temperatures >65 °C. SDS−PAGE gels were analyzed by image analysis, and the amounts of α-lactalbumin and β-lactoglobulin were quantified as percent of the total protein present (Figure 10). For temperatures up to 85 °C, the amount of α-lactalbumin was not significantly affected by temperature. On the other hand, the amount of β-lactoglobulin associated with the MFGM increased significantly ($p < 0.05$) with temperature of heating, especially at temperatures >65 °C. The presence of significant amounts of β-lactoglobulin and very little α-lactalbumin in the heated membranes was in agreement with the findings reported by McPherson et al. (1984) on pasteurized creams. Moreover, the higher amounts of β-lactoglobulin associated with the MFGM at high temperature agreed with the differences reported by Kim and Jimenez-Flores (1995) in the binding of β-lactoglobulin to proteins from the MFGM at different temperatures. A temperature >65 °C seemed to be necessary for β-lactoglobulin to react with the MFGM. Jang and Swaisgood (1990) reported very little disulfide interchange between β-lactoglobulin and κ-casein in skim milk during heating at temperatures <75 °C. Noncovalent protein−protein interactions seem to play an important role in skim milk under those conditions. Recently, the formation of disulfide aggregates at 65 °C in β-lactoglobulin in solution has been reported (Hoffmann and van Mil, 1997). At the lowest temperature of heating of the cream, the formation of β-lactoglobulin complexes with MFGM might occur via both noncovalent interactions and disulfide interchange, and these reactions might occur simultaneously or sequentially.

Iron Content of the MFGM Material. The amount of iron found in the MFGM isolates decreased with increasing temperature of heating, from 110 ppm in the MFGM from unheated cream (50 °C) to 20 ppm in MFGM from cream heated at 80 °C (Figure 11). A change in the color of the MFGM isolates with temperature of heating was also observed, from brown-red in the unheated isolates to white-yellow in the material derived from cream heated at 85 °C. In contrast with the other observations we made, there did not appear to be a threshold temperature in the region of 65 °C. It was evident that loss of iron was significant even at 62 °C.

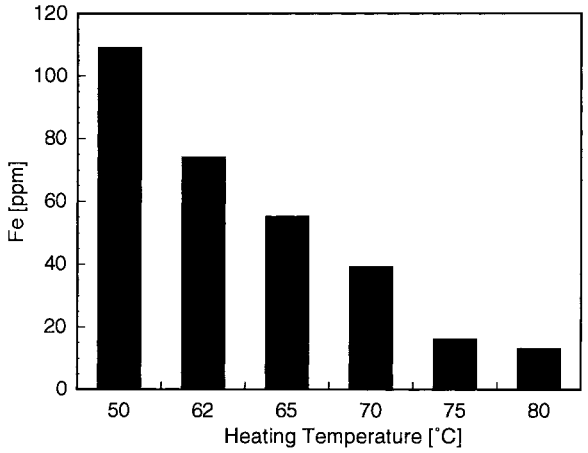

Figure 11. Amount of iron (parts per million) in the MFGM isolates as a function of heating temperature of the cream. Analyses were performed by atomic absorption spectroscopy.

One of the major proteins in the MFGM (8−10% of the total protein in the membrane), xanthine oxidase, is a complex metalloflavoprotein (Mangino and Brunner, 1977). This enzyme is present in dehydrogenase and oxidase forms. Conversion of the dehydrogenase to the oxidase form can be achieved by proteolysis, heating, or incubation with sulfhydryl modifying reagents (Coughlan, 1980). The decrease in the amount of iron in the MFGM with increased heating temperature of the cream might be related to a heat-induced release of metal from xanthine oxidase. In fact, four cysteines bind the iron−sulfur cluster of xanthine oxidase (Berglund et al., 1996). The structural change of the protein and the release of iron would render the cysteines of the membrane protein available for disulfide exchange with other cysteine-containing proteins present in the cream (i.e., β-lactoglobulin, κ-casein).

CONCLUSIONS

Two main effects occurring during heating of fat globules in milk and cream have been previously reported: the loss of membrane-constituent material and the formation of a new protein-polymerized surface (Dalgleish and Banks, 1991; van Boekel and Folkerts, 1994; Houlihan et al., 1992). During heating, an increasing amount of β-lactoglobulin associated with MFGM. This was in agreement with the reported disulfide interaction occurring between β-lactoglobulin and some MFGM proteins (Kim and Jimenez-Flores, 1995).

Heat treatment of the cream not only affected the formation of MFGM aggregates with the cysteine-containing proteins present in skim milk but also affected the iron content, the solubility, and the emulsifying properties of the MFGM isolates. Analysis of variance of the data showed a significant effect of temperature on (i) the solubility of the MFGM with urea, Tris/Triton X-100, or sodium carbonate; (ii) the average droplet diameter of 2 and 3% (w/v) MFGM isolate emulsions; and (iii) the amount of β-lactoglobulin associated with the MFGM. In general, apart from loss of iron, temperatures up to 65 °C did not result in significant changes in the functional properties of the MFGM isolates.

Figure 12 shows a synthesis of our results, relating the amount of β-lactoglobulin associated with the MFGM and the emulsifying properties of the MFGM fractions

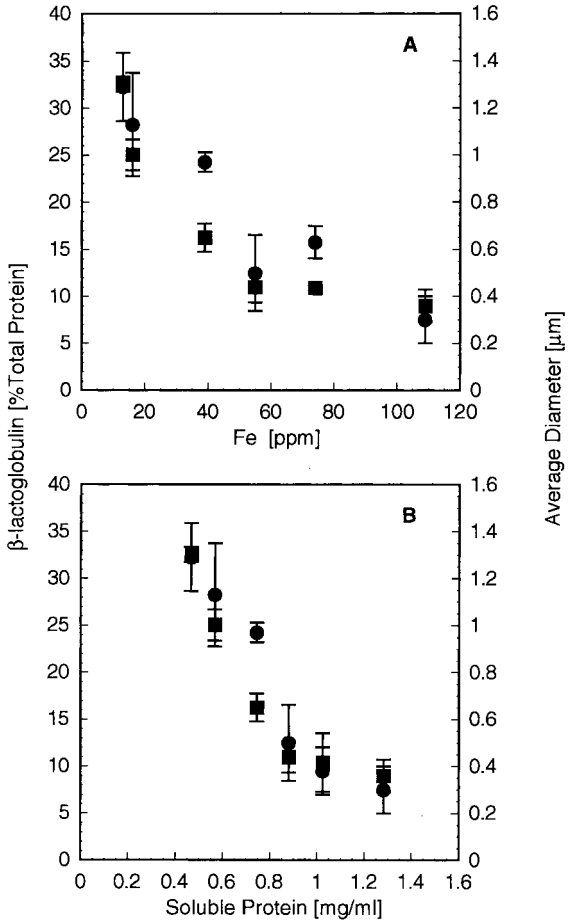

Figure 12. Amount of β-lactoglobulin present in the MFGM isolates (■ and left-hand scale) and average diameter of emulsions prepared with 2% (w/v) MFGM isolates and 10% (w/w) oil (● and right-hand scale) plotted versus the amount of iron present in the MFGM (A) and the solubility of the MFGM isolates in Tris/Triton X-100 buffer (B).

[$d_{3,2}$ of a 2% (w/v) MFGM isolate 10% (w/w) oil emulsion] as functions of the amount of iron and soluble protein in Tris/Triton X-100 buffer. It is evident that significant correlations exist between all of these properties, and this demonstrates the importance of heating on structure, composition, and functionality of the membrane isolate.

Heating of cream before butter-making is therefore a critical step that may limit the utilization of MFGM fractions isolated from buttermilk. High temperatures caused extensive denaturation of the MFGM isolates and interaction with skim milk proteins. Pasteurization of the cream affects the composition of the complex MFGM isolates by a combination of effects of heat treatment on the MFGM itself and association of skim milk proteins with the membrane. Thus, in considering the functional properties of the MFGM fraction in buttermilk, it is necessary to know the heat treatment given to (i) the milk before cream separation, (ii) the cream before churning, and (iii) the buttermilk after separation.

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