Isolates from Industrial Buttermilk: Emulsifying Properties of Materials Derived from the Milk Fat Globule Membrane

Milena Corredig and Douglas G. Dalgleish*

Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Two isolates were produced from commercial buttermilk: (i) an isolate containing whey proteins, caseins, and proteins of the milk fat globule membrane (MFGM); (ii) an isolate of MFGM only. The MFGM isolate contained a large quantity of membrane fragments from the milk fat globule. The MFGM fraction was a poor emulsifier, compared to the whole isolate. Emulsions prepared with 10% soybean oil needed a much larger amount of MFGM isolate to produce a droplet size distribution similar to that found for the emulsion prepared using 1−2% (w/v) whole isolate. Heat treatment of the cream and the industrial churning process appeared to affect the emulsifying behavior of MFGM proteins. A large amount of β-lactoglobulin was present in the MFGM isolate as a result of heat-induced interactions during pasteurization of the cream.

Keywords: *Buttermilk; food emulsions; food proteins; protein adsorption*

INTRODUCTION

Buttermilk, which is the aqueous phase released during the manufacture of butter, contains not only skim milk proteins but material, mainly composed of proteins and phospholipids, derived from the original milk fat globule membrane (MFGM). This characteristic composition makes buttermilk an interesting source of ingredients with unique functional properties among milk-derived products. The growing interest in the dairy industry for new products has led to an increase in the number of studies on buttermilk (Ramachandra Rao et al., 1995; Mistry et al., 1996); however, the full potential of this byproduct has yet to be exploited.

In untreated milk and cream, the MFGM is characterized by a complex mixture of proteins, phospholipids, and glycoproteins and acts as a natural emulsifier that keeps the milk fat in suspension (Keenan et al., 1983). Although little is known about their functional properties, much progress has been made in the purification of the proteins from the membrane (McPherson and Kitchen, 1983; Basch et al., 1985; Kim et al., 1992). The proteins from MFGM have been identified according to their migration during sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) (Keenan and Dylewski, 1995). Two polypeptides, xanthine oxidase (MW 150 000 Da) and butyrophylin (MW ∼67 000 Da), account for nearly 50% of the total protein in the MFGM material, as detected by Coomassie blue staining of electrophoresis gels (Greenwalt and Mather, 1985).

Most studies on MFGM proteins have been carried out with membranes released and collected from washed, untreated milk fat globules (Keenan and Dylewski, 1995). It has been reported that, with pretreatment of the cream, losses of membrane material may occur during the washing steps (McPherson and Kitchen, 1983; Basch et al., 1985); however, at present there appears to be no alternative procedure for obtaining MFGM proteins free from skim milk proteins. These MFGM proteins play a very important role in stabilizing fat globules in cream, and they have been used to reconstitute milk fat in emulsions (Kanno et al., 1991; Oehlmann et al., 1994). It has been reported that MFGM proteins contained in buttermilk enhance the functional properties of this byproduct (Kanno, 1989; Mistry et al., 1996).

The increasing interest in MFGM proteins as food ingredients and in their functional properties makes it important to study membrane-derived material isolated from industrial buttermilk rather than from buttermilk produced by laboratory procedures. The research described here aimed at isolating membrane-derived material from an industrial source of buttermilk and investigating the potential utilization of the byproduct as an ingredient of oil-in-water emulsions.

MATERIALS AND METHODS

Buttermilk was collected from the butter production line of a local dairy, and 0.2 g L^{-1} of sodium azide was added as a preservative. When freshly collected buttermilk was centrifuged (Beckman, Model L8-70M, Palo Alto, CA) at 15 °C for 50 min at 100000*g*, the sedimented pellet contained an isolate ("buttermilk isolate") with a protein composition identical to that of buttermilk.

Preparation of MFGM Isolate. Calcium is essential for maintaining structure and stability of the casein micelles (Holt et al., 1986), and consequently they can be disrupted by the addition of the calcium-chelating agent sodium citrate. In buttermilk, citrate added at concentrations between 2 and 5% (w/v) provided the required breakdown of the micellar structure. The addition of sodium citrate did not substantially alter the pH of buttermilk (in most cases the increase in pH was <0.5 unit). In practice, 2% (w/v) trisodium citrate was added to the buttermilk, and the mixture was incubated overnight at 4 °C. The treated samples were then centrifuged at 100000*g* for 50 min at 15 °C, and the resulting small pellets were collected, and dried on a filter paper (Whatman No. 4), resuspended in a minimum amount of Milli-Q water with a tissue homogenizer (Polytron PT 2000, Kinematica AG, Switzerland), and freeze-dried. The freeze-dried samples were stored at −20 °C; these are the MFGM isolates. By analysis of the serum after centrifugation, it was established that virtually all of the membrane material from the buttermilk was collected in the pellet.

Transmission Electron Microscopy. The pellet fractions isolated by ultracentrifugation were dried on filter paper (Whatman No. 4) and treated overnight with 1.4% glutaraldehyde in 0.05 M veronal acetate buffer (pH 7.0). The preparations were then washed in buffer and fixed for 24 h in

* Author to whom correspondence should be addressed [fax (519) 824-6631; e-mail ddalglei@uoguelph.ca].

S0021-8561(97)00531-1 CCC: $14.00

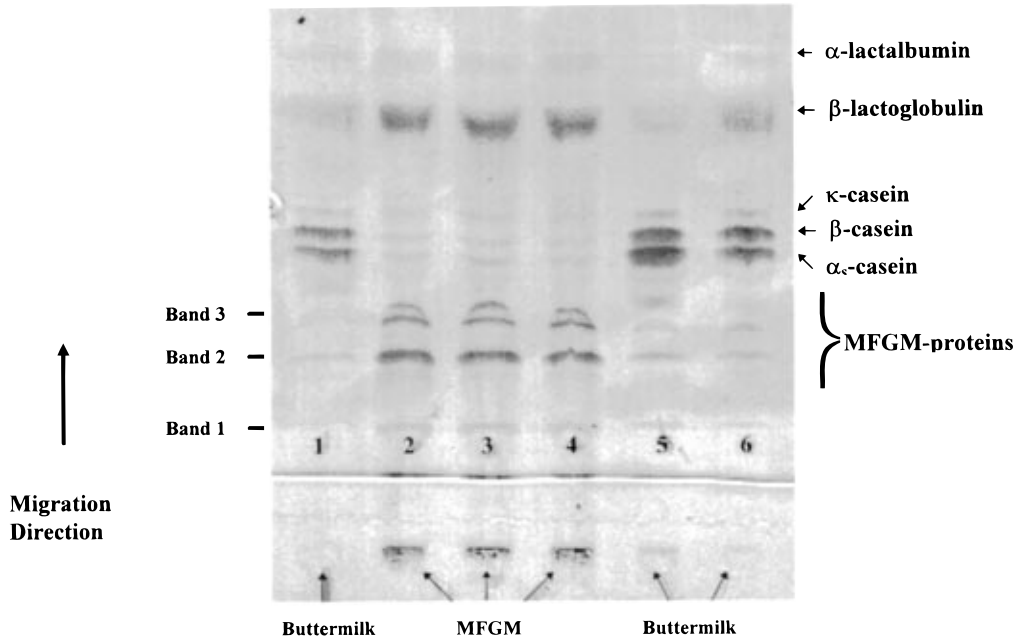

Figure 1. Typical SDS−PAGE of freeze-dried material from buttermilk: (lane 1) buttermilk; (lanes 2−4) MFGM isolate; (lanes 5 and 6) buttermilk isolate. Aliquots (3 mg) of material were resuspended in 200 μL of electrophoresis buffer, 300 μL of SDS, and 100 μL of mercaptoethanol and bromophenol blue. Analysis was performed on 20% homogeneous SDS gel.

imidazole and OsO_4 (0.5%) solution. The samples were then dehydrated in a graded alcohol series (Kalab, 1980) and embedded with Epon resin (Luft, 1961). Ultrathin sections were poststained with 4% uranyl acetate and 50% ethanol followed by lead citrate solution in 1 N NaOH. The micrographs were produced by a Hitachi transmission electron microscope with a measuring voltage of 75 kV.

Phospholipid Determination. Freeze-dried samples of the pellet fractions (30 mg) were resuspended in 6 mL of 0.5% KCl solution and extracted with 30 mL of chloroform/methanol in a 2:1 ratio. The two phases were separated by low-speed centrifugation (2000*g* for 5 min), and the lipid was dried by rotary evaporation. The dry sample was then resuspended in 0.5 mL of chloroform/methanol (2:1) and filtered through a 0.2 μm filter (Gelman, Ann Arbor, MI). Samples were spotted at the bottom of chromatorods (Iatron Laboratories, Tokyo, Japan). A two-step solvent development was carried out: (1) chloroform/methanol/water (50:20:2.5 v/v) up to 5 cm migration; (2) hexane/diethyl ether/acetic acid (65:5:0.5 v/v) for a further 10 cm migration. After the two development steps, the lipid migration was determined by scanning with an Iatroscan (Iatron Laboratories) equipped with a flame ionization detector (Shantha, 1992; Tvrzická and Votruba, 1994). The lipids present in the sample were identified by comparison with the migration of standard phospholipid samples (Avanti Polar Lipids Inc., Alabaster, AL) and standard samples of triolein and cholesterol (Sigma, St. Louis, MO).

Emulsion Preparation. Aliquots of freeze-dried isolate (either buttermilk or MFGM isolates), soybean oil, and buffer (20 mM Tris-HCl buffer at pH 7.00) were mixed in different proportions. The mixtures were homogenized using a Microfluidizer Model 110S (Microfluidics Inc., Newton, MA) using an input pressure of 56 MPa. Each sample was circulated for 10 strokes of the pump and then collected; this procedure was repeated four times.

Determination of Particle Size Distribution. The diameter distribution of the fat globules in the emulsions and their specific surface areas were determined by integrated light scattering using a Mastersizer X (Malvern Instruments Inc., Southboro, MA). The samples were diluted ~1:200 in Milli-Q water and were measured at ambient temperature (22 °C). The presentation factor used was 0303 corresponding to a refractive index of 1.41.

SDS−PAGE Electrophoresis. The protein compositions of the isolates were determined by SDS−PAGE. Aliquots of freeze-dried samples (~3 mg) were resuspended in 200 μL of electrophoresis buffer (10 mM Tris, 1 mM EDTA, 20 mM imidazole, pH 8.0), mixed with 300 μL of 20% (w/v) SDS and 100 μL of 2-mercaptoethanol and bromophenol blue (0.05% w/v). The emulsions prepared with the isolates and the corresponding creams and serum phases were separated by ultracentrifugation (Beckman, Palo Alto, CA) at 15 °C for 40 min at 10000*g* and analyzed by SDS−PAGE, as described earlier (Hunt and Dalgleish, 1994). The samples were run on a 20% homogeneous gel in a rapid electrophoresis system (Phastsystem, Pharmacia Biotech, Baie d'Urfé, PQ, Canada) at 15 °C. The gels were stained with Coomassie blue. After the separation, scanning densitometry was performed using a Sharp JX330 scanner. The scanned images were evaluated using ImageMaster software (Pharmacia Biotech).

RESULTS AND DISCUSSION

Production of Isolates from Buttermilk and Their Composition. Both of the isolates contained ~40% lipid, in agreement with the reports of other authors (Kanno and Kim, 1990). The moisture contents of the freeze-dried isolates were low (~4% w/w). After high-speed centrifugation of buttermilk, SDS−PAGE showed that the pellet of buttermilk isolate had a protein composition similar to that of the whole buttermilk. The isolate contained the four caseins, α-lactalbumin, β-lactoglobulin, and proteins derived from the fat globule membrane, with the caseins making up ~50% of the total protein. This agrees with previous results; it has been reported that skim milk and buttermilk have very similar protein compositions (Basch et al., 1985; Keenan and Dylewski, 1995). In our study, the presence of skim milk proteins hindered the determination of the physical and chemical properties of MFGM proteins. In cream, most of the skim milk proteins can be removed by pretreating the milk fat globules prior to extraction of the MFGM proteins (Basch et al., 1985), but in the industrial buttermilk, the presence of caseins and whey proteins was unavoidable. We therefore used the method described above to separate the MFGM fraction from the caseins.

Figure 1 shows the SDS−PAGE of the two different isolates. The pellet from the buttermilk after treatment with citrate contained a large quantity of MFGM proteins, and in this isolate caseins were present in only small amounts. The MFGM isolate differed substantially in composition from the buttermilk isolate. It

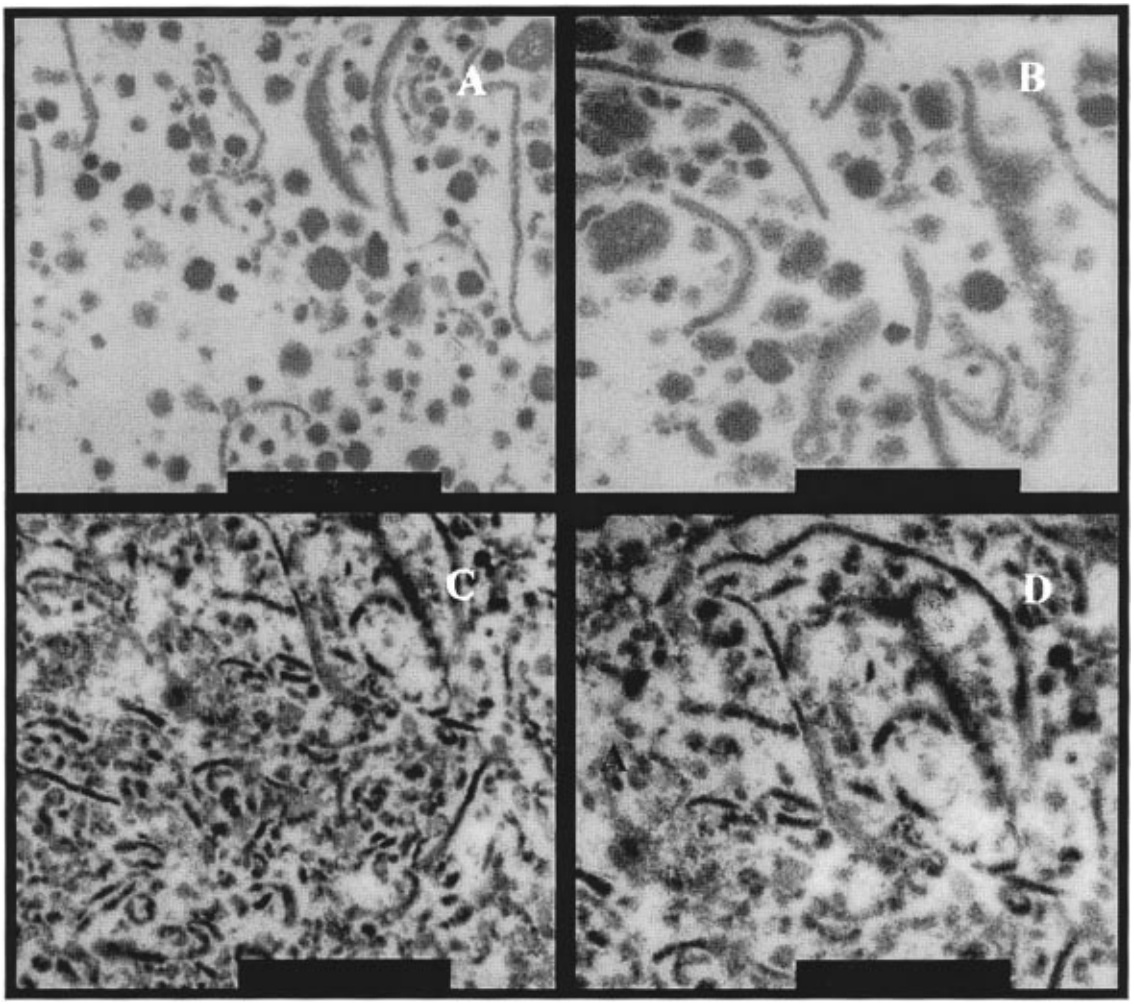

Figure 2. Transmission electron micrograph of pellet isolated by centrifugation of buttermilk: (A and B) pellet from buttermilk isolate; (C and D) pellet of MFGM isolate. The specimen was fixed with glutaraldehyde and postfixed with OsO_4. Bar size: 1.13 μm (A, C); 0.68 μm (B, D).

contained three major polypeptides migrating in the high molecular weight range of the SDS−PAGE gel (MW > 45 000): band I, identifiable as xanthine oxidase (MW 150 000); band II, at ~70 000, butyrophylin; and band III, migrating at ~50 000 (Keenan and Dylewski, 1995). Another band, of mobility higher than that of band III, was also detected and has not been identified. The electrophoretic migration of the MFGM isolate was similar to that reported for MFGM proteins derived from native fat globules (Kanno and Kim, 1990; Malin et al., 1994). In addition, the MFGM isolate from buttermilk contained large amounts of β-lactoglobulin, which appeared to be covalently linked by disulfide bonds with the MFGM. This association of β-lactoglobulin with MFGM proteins was almost certainly the result of the heat treatment of the cream prior to buttermaking. Several studies have been carried out on the interactions of whey proteins with fat globule membranes during heating of whole milk and cream; however, these interactions are not completely understood (Dalgleish and Banks, 1991; Kim and Jiménez Flores, 1995; Corredig and Dalgleish, 1996). The presence of β-lactoglobulin and the almost complete absence of α-lactalbumin in the MFGM isolate from industrial buttermilk confirmed the hypothesis that a major interaction occurring during heating of cream is the reaction between β-lactoglobulin and the MFGM proteins. There were also in the MFGM isolate quantities of aggregated proteins that did not enter the gel. These are probably large complexes formed when the cream is heat-treated and do not break down in the presence of SDS and mercaptoethanol. These complexes were also present, but to a smaller extent, in the buttermilk isolate.

The two isolates produced from industrial buttermilk were also investigated using transmission electron microscopy (TEM). These observations confirmed the results obtained by SDS−PAGE. The buttermilk isolate contained a large quantity of spherical particles, which are assumed to be casein micelles (Figure 2A,B). The polydisperse population of micelles coexisted with fragments of membrane material, which were characterized by a size varying from 0.4 to 1.5 μm. On the other hand, the MFGM isolate contained a large number of the sheetlike membrane fragments and many fewer casein micelles than did the buttermilk isolate (Figure 2C,D). The sections obtained from the MFGM isolate were very similar to those reported in the literature for purified MFGM material from washed native fat globules (Keenan et al., 1977; Keenan and Dylewski, 1995). The membranes were characterized by a densely staining material that was oriented toward the interior of the milk fat globule in native cream (Figure 2C,D) (Keenan et al., 1977). Previous work had demonstrated that this coat material retained its structure even after washing of the membrane fractions with salt and various detergents (Keenan et al., 1977).

The lipid compositions of the two isolates were determined by thin layer chromatography (Figure 3). The buttermilk isolate contained mainly neutral lipids

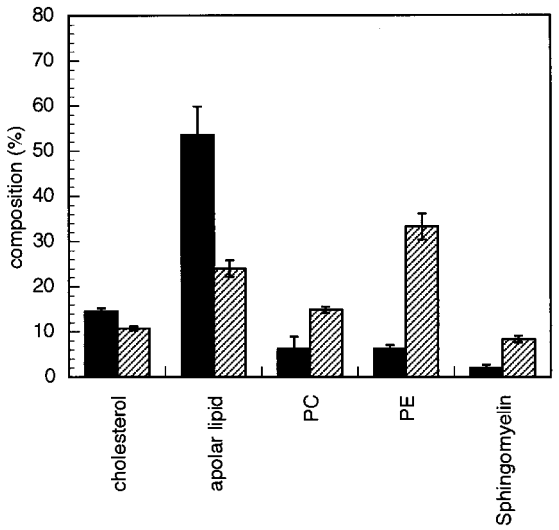

Figure 3. Lipid composition (percent) as determined by thin layer chromatography (Iatroscan, Iatron Lab., Japan): samples of buttermilk isolate (solid) and MFGM isolate (dotted). Values are the average of three independent experiments; error bars represent the standard deviation.

with a smaller amount of phospholipids; ~65% of the total lipids consisted of triglycerides and cholesterol, with the remaining lipid fraction containing mainly phosphatidylethanolamine, phosphatidylcholine and sphingomyelin (Figure 3). In contrast, the MFGM isolate contained a significantly lower amount (40%) of apolar lipids than the buttermilk isolate. These results agree with the reported phospholipid composition of the fat globule membrane (Kanno, 1990; Malmsten et al., 1994). The selective centrifugation of membrane fragments occurring during the preparation of MFGM isolate decreased the amount of apolar lipids present, possibly because in the original buttermilk fraction small fat globules were complexed with casein micelles and cosedimented with them. In the MFGM isolate phospholipids constituted ~60% of the total lipids with the following composition: 33% phosphatidylethanolamine, 16% phosphatidylcholine, and 9% sphingomyelin.

Emulsions Prepared with Isolates from Buttermilk. Figure 4 illustrates the size distribution of emulsion droplets prepared with 10% (w/w) soybean oil. When buttermilk isolate was used, the emulsions were characterized by a monomodal size distribution, at fairly small concentration of protein (1−2% w/v of isolate) (Figure 4A). On the other hand, the 10% oil-in-water emulsions prepared with MFGM isolate showed aggregation behavior and a bimodal distribution of droplet size up to 4% (w/v) of the isolate. Only by using a much higher concentration of MFGM isolate (8%) was a monomodal distribution of droplet size achieved (Figure 4B). Figure 5 illustrates the change in average droplet size, expressed as $D_{3,2}$, with increasing concentration of MFGM isolate in emulsions with 5 and 10% (w/w) soybean oil. While at least 2% (w/v) of MFGM isolate was needed to obtain an average droplet size of 0.4 μm in 5% oil emulsions, 4.5% (w/v) of MFGM isolate was necessary to obtain an average size of 0.25 μm in 10% oil emulsions (i.e., as the amount of emulsion surface was increased, the amount of protein necessary was also increased). The amount of protein necessary to produce emulsions with small droplet size was higher than the 0.5−1% required for emulsions prepared with isolated milk proteins (Fang and Dalgleish, 1993a; Hunt and Dalgleish, 1994). The different behaviors observed between the whole emulsions made with protein isolate

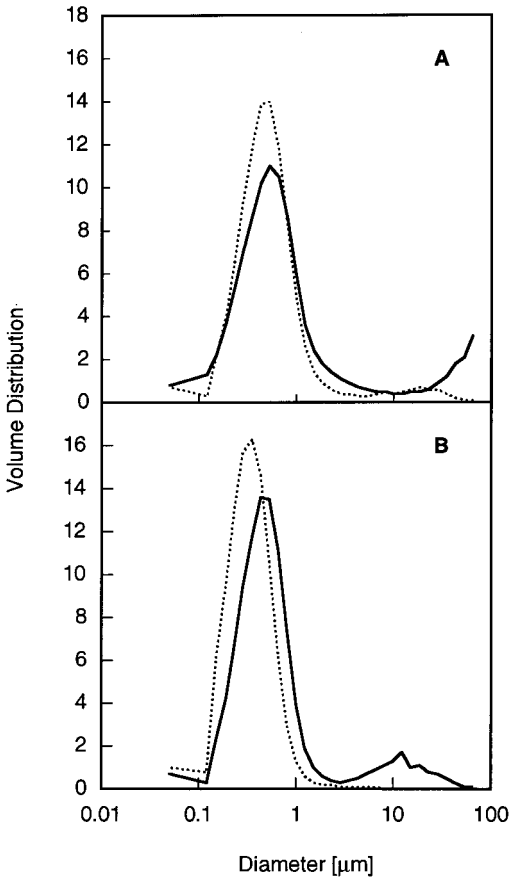

Figure 4. Typical particle size distribution of 10% soybean oil-in-water emulsions: (A) emulsions prepared with buttermilk isolate at 1% (w/v) (solid line) and 2% (w/v) (broken line); (B) emulsions prepared with MFGM isolate at 4% (w/v) (solid line) and 8% (w/v) (broken line). Analysis was carried out by integrated light scattering (Mastersizer X).

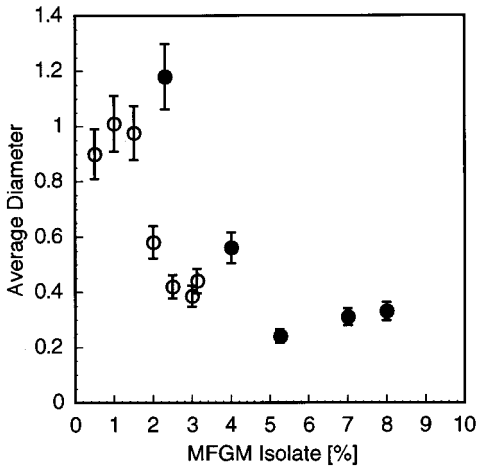

Figure 5. Average droplet size of emulsions as a function of MFGM isolate concentration. Emulsions prepared with (○) 5% (w/w) soybean oil and (●) 10% (w/w) soybean oil. Error bars represent standard deviation. Average of at least three measurements. Analysis was carried out by integrated light scattering (Mastersizer X).

and the MFGM isolate may be attributed to the difference in protein composition between the two isolates and the structural organization of the membrane material.

A complete coverage of the interface took place only at high concentrations of MFGM isolate (i.e. >5% w/v in 10% oil-in-water emulsions), because of the large aggregates of membrane material present in the MFGM isolate. In the emulsions prepared with buttermilk isolate, a large amount of casein micelles was present,

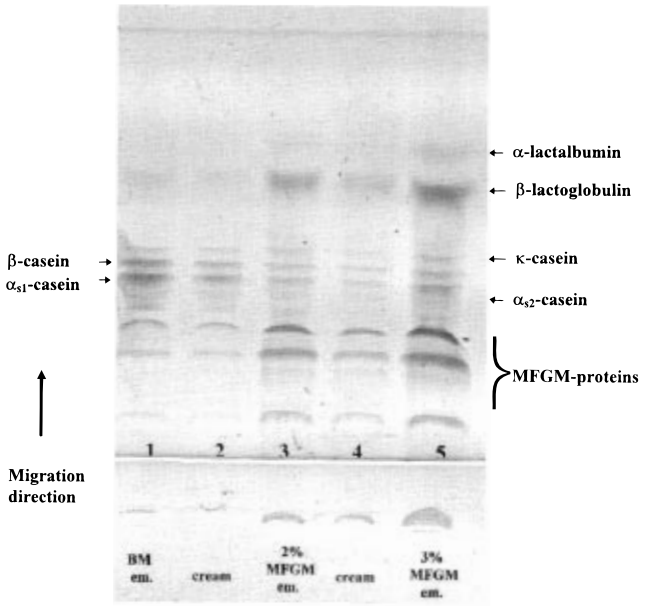

Figure 6. Typical SDS−PAGE gel of proteins obtained from samples of 10% oil-in-water emulsions and resuspended cream (oil droplets resuspended in the same volume of the original emulsion): (lane 1) 1% (w/v) buttermilk isolate emulsion; (lane 2) oil phase from emulsion in lane 1; (lane 3) 2% (w/v) MFGM isolate emulsion; (lane 4) oil phase from emulsion in lane 3; (lane 5) 3% MFGM isolate emulsion. Analysis was performed on 20% homogeneous gel, in reducing conditions.

and the required surface coverage could be achieved with a lower protein concentration than that of the emulsions prepared with MFGM isolate. Figure 6 illustrates the protein composition, as determined by SDS−PAGE, of whole emulsions and the oil droplets separated by centrifugation. No preferential adsorption of MFGM proteins compared to caseins or whey proteins seemed to occur at the interface, and the amounts of the different proteins adsorbed depended on their concentrations in the original emulsion. In emulsions made with buttermilk isolate, most of the proteins adsorbed at the interface were of skim milk origin: caseins and whey proteins (Figure 6). In contrast, in the MFGM isolate emulsions very few caseins were present at the oil/water interface (since the fraction contained little casein). In addition, a large amount of β-lactoglobulin was present in the MFGM isolate emulsion and its oil phase. A more detailed analysis of the three main MFGM protein bands present in the MFGM isolate emulsions and oil phases suggested that no preferential adsorption occurred among MFGM proteins.

We have noted that both of the isolates contain quantities of phospholipid, which is generally known to be highly surface active and to at least enhance the capacity of proteins to form emulsions (Fang and Dalgleish, 1993b), although the mechanism of the action is not well understood, because their interaction with the interface in the presence of proteins may be small (Fang and Dalgleish, 1993b; Courthaudon et al., 1991). However, it is evident that in the isolates described here, the phospholipids seem not to exert an enhancing effect on the emulsifying capacity of the proteins. This suggests that heat treatment on the MFGM material in the buttermilk affects not only the proteins but also the functional properties of the phospholipid part of the membrane isolates.

The poor emulsifying properties of MFGM proteins obtained from industrial buttermilk were confirmed by addition of small quantities of sodium caseinate to

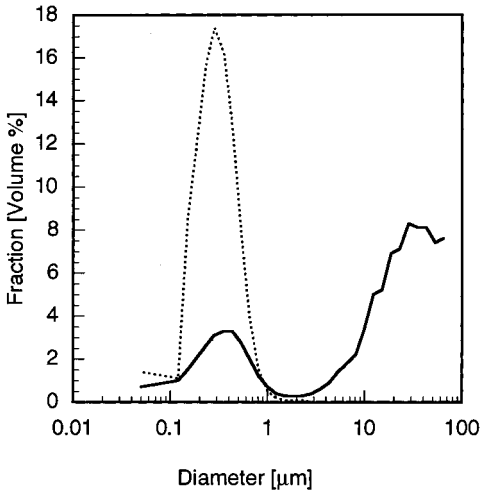

Figure 7. Changes in particle size distribution of 10% oil-in-water emulsion prepared with 2.5% MFGM isolate (solid line) and with 2.5% MFGM isolate and 0.5% caseinate (broken line).

emulsions prepared with MFGM isolate. When 2.5% (w/v) MFGM isolate was used, the 10% oil-in-water emulsions showed a bimodal size distribution (Figure 7). The same emulsion prepared with the addition of 0.5% (w/v) caseinate was characterized by a monomodal droplet size distribution. The instability of emulsions prepared with low concentrations of MFGM isolate was caused by incomplete coverage of the oil/water interface by the large clumps of membrane material, which formed bridges between the emulsion droplets (Corredig and Dalgleish, unpublished results). We have shown above that if enough MFGM isolate is used, stable emulsions can be made, so that the initial instability was not caused by a tendency of the MFGM itself to aggregate. The bridging was prevented by the addition of casein, which allows more complete coverage of the interfaces of the droplets, so that a sharp monomodal distribution of droplet size could be obtained (Figure 7).

CONCLUSIONS

Although the role of MFGM proteins in the stability of the native milk fat globule is recognized (Keenan and Dylewski, 1995), the membrane material released in solution after some butter-making processes is characterized by poor emulsifying properties. The process of heat treatment, churning, and the release of membrane fragments to the water phase causes a high degree of aggregation and changes in the functional properties of membrane proteins and phospholipids. The microfluidization process did not allow the MFGM proteins present in this membrane aggregate to be released into solution and migrate to the interface to act as emulsifiers. It is possible that the rigidity of the MFGM in buttermilk was the result of heat treatment of cream at source. It is known that interactions occur during heat treatment between skim milk proteins and MFGM proteins (Dalgleish and Banks, 1991), and the large amounts of β-lactoglobulin in the MFGM isolate from industrial buttermilk could be used as further evidence of this. The present study demonstrates that industrial buttermilk may contain MFGM proteins with poor functional properties, presumably because of the extensive or complete heat denaturation of the membrane proteins. The complex structure of the membranes in the MFGM isolates did not allow a complete coverage of the oil/water interface, resulting in an unstable emulsion at low MFGM isolate concentration. These

results are in strong disagreement with the data on MFGM proteins and reconstituted butteroil emulsions (Kanno et al., 1991; Oehlmann et al., 1994). However, previous authors used raw cream buttermilk as the MFGM protein source. The industrial origin of buttermilk used in this research made the results presented therein difficult to compare with the previous literature.

It is reported that the fat globule membrane present in buttermilk may enhance the emulsification properties of cheese (Mistry et al., 1996). The large amount of casein micelles present in buttermilk may, however, overcome any effect deriving from the other component present in a smaller amount.

ACKNOWLEDGMENT

We thank Alexandra Smith of the Department of Food Science of the University of Guelph and Andrew Moore of the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA) for help with the transmission electron microscopy.

LITERATURE CITED

Basch, J. J.; Greenberg, R; Farrell, H. M. Identification of the milk fat globule membrane proteins. II. Isolation of major proteins from electrophoretic gels and comparison of their amino acid compositions. *Biochim. Biophys. Acta* **1985**, *12*, 127−135.

Corredig, M.; Dalgleish, D. G. Effect of different heat treatments on the strong binding interactions between whey proteins and milk fat globules in whole milk. *J. Dairy Res.* **1996**, *63*, 441−449.

Courthaudon, J-L.; Dickinson, E.; Christie, W. W. Competitive adsorption of lecithin and β-casein in oil-in-water emulsions. *J. Agric. Food Chem.* **1991**, *39*, 1365−1368.

Dalgleish, D. G.; Banks, J. M. The formation of complexes between serum proteins and fat globules during heating of whole milk. *Milchwissenschaft* **1991**, *46*, 75−78.

Fang, Y.; Dalgleish, D. G. Dimensions of the adsorbed layers in oil-in-water emulsions stabilized by caseins. *J. Colloid Interface Sci.* **1993a**, *156*, 329−334.

Fang, Y.; Dalgleish, D. G. Casein adsorption on the surfaces of oil-in-water emulsions modified by lecithin. *Colloids Surf. B* **1993b**, *1*, 357−364.

Greenwalt, D. E.; Mather, I. H. Characterization of an apically derived epithelial membrane glycoprotein from bovine milk, which is expressed in capillary endothelia in diverse tissues. *J. Cell Biol.* **1985**, *100*, 397−408.

Holt, C.; Davies, D. T.; Law, A. J. R. Effects of colloidal calcium phosphate content and free calcium ion concentration in the milk serum on the dissociation of bovine casein micelles. *J. Dairy Res.* **1986**, *53*, 557−572.

Hunt, J. A.; Dalgleish, D. G. Adsorption behavior of whey protein isolate and caseinate in soya oil-in-water emulsions. *Food Hydrocolloids* **1994**, *8*, 175−187.

Kalab, M. Possibilities of an electron-microscopic detection of buttermilk made from sweet cream in adulterated skim milk. *Scanning Electron Microsc.* **1980**, *3*, 645−651.

Kanno, C. Emulsifying properties of bovine milk fat globule membrane in milk fat emulsion: conditions for the reconstitution of milk fat globules. *J. Food Sci.* **1989**, *54*, 1534−1539.

Kanno, C. Secretory membranes of the lactating mammary gland. *Protoplasma* **1990**, *159*, 184−208.

Kanno, C.; Kim, D. H. A simple procedure for the preparation of bovine milk fat globule membrane and a comparison of its composition, enzymatic activities and electrophoretic properties with those prepared by other methods. *Biol. Chem.* **1990**, *54*, 2845−2854.

Kanno, C.; Shimomura, Y.; Takano, E. Physicochemical properties of milk fat emulsions stabilized with bovine milk fat globule membrane. *J. Food Sci.* **1991**, *56*, 1219−1223.

Keenan, T. W.; Dylewski, D. O. Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. In *Advanced Dairy Chemistry. 2. Lipid*; Fox, P. F., Ed.; Chapman & Hall: London, U.K., 1995; pp 89−130.

Keenan, T. W.; Freudenstein, C.; Franke, W. W. Membranes of mammary gland. XIII. A lipoprotein complex derived from bovine milk fat globule membrane with some preparative characteristics resembling those of actin. *Cytobiologie* **1977**, *24*, 259−278.

Keenan, T. W.; Dylewski, D. O.; Woodford, T. A.; Ford, R. H. Origin of milk fat globules and the nature of the milk fat globule membrane. In *Developments in Dairy Chemistry—2. Lipids*; Fox, P. F., Ed.; Applied Science Publishers: London, U.K., 1983; pp 83−118.

Kim, D. H.; Kanno, C.; Mizokami, Y. Purification and characterization of major glycoproteins, PAS-6 and PAS-7, from bovine milk fat globule membrane. *Biochim. Biophys. Acta* **1992**, *1122*, 203−211.

Kim, H.-H. Y.; Jimenez-Florés, R. Heat induced interactions between the proteins of milk fat globule membrane and skim milk. *J. Dairy Sci.* **1995**, *78*, 24−35.

Luft, J. H. Improvements in epoxyresin embedding methods. *J. Biophys. Biochem. Cytol.* **1961**, *9*, 409−414.

Malin, E. L.; Basch, J. J.; Shieh, J. J.; Sullivan, B. C.; Holsinger, V. H. Detection of adulteration of buttermilk powder by gel electrophoresis. *J. Dairy Sci.* **1994**, *77*, 2199−2206.

Malmsten, M.; Bergenståhl, B.; Nyberg, L.; Odham, G. Sphingomyelin from milk- Characterization of liquid crystalline, liposome and emulsion properties. *J. Am. Oil Chem. Soc.* **1994**, *71*, 1012−1026

McPherson, A. V.; Kitchen, B. J. The bovine fat globule membrane—its formation, composition, structure and behavior in milk and dairy products. *J. Dairy Res.* **1983**, *50*, 107−133.

Mistry, V. V.; Metzger, L. E.; Maubois, J. L. Use of ultrafiltered sweet buttermilk in the manufacture of reduced fat cheddar cheese. *J. Dairy Sci.* **1996**, *79*, 1137−1145.

Oehlmann, S. M.; Duncan, S. E.; Keenan, T. W. Butteroil emulsification with milk-derived membrane and protein fractions. *J. Food Sci.* **1994**, *59*, 53−56, 66.

Ramachandra Rao, H. G.; Lewis, M. J.; Grandison, A. S. Effect of pH on flux during ultrafiltration of sweet whey and buttermilk. *J. Dairy Res.* **1995**, *65*, 441−449.

Shantha, N. C. Thin-layer chromatography-flame-ionization detection Iatroscan system. *J. Chromatogr.* **1992**, *624*, 21−35.

Tvrzická, E.; Votruba, M. Thin-layer chromatography with flame ionization detection. In *Lipid Chromatographic Analysis*; Shibamoto, T., Ed.; Dekker: New York, 1994; pp 51−73.

Received for review June 20, 1997. Revised manuscript received September 11, 1997. Accepted September 12, 1997.[⊗] We acknowledge financial support from the Ontario Dairy Council and OMAFRA. We are also indebted to Gay Lea Foods, Guelph, Ontario, for providing buttermilk samples.

JF970531F

[⊗] Abstract published in *Advance ACS Abstracts*, November 1, 1997.