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Influence of Emulsifying Component Composition on Creams Formulated with Fractionated Milkfat

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Dairy systems formulated with fractionated milkfat and milk-derived components have compositional differences that may affect functionality and nutritional aspects as compared to natural dairy products. The composition of 20% milkfat creams formulated with emulsifying components (skim milk, sweet buttermilk, and butter-derived aqueous phase) and low- or medium-melt fractionated butteroil was compared with natural cream. Cream separation temperatures (49 and 55 °C) and processing conditions (commercial and pilot plant) in obtaining emulsifying components were examined for effect on content of surface active agents. Individual fatty acids, lipids, cholesterol, phospholipids, protein levels, and types varied with components. Separation temperature influenced the cholesterol level in the aqueous phase. A commercially produced aqueous phase contained less total lipid, protein, cholesterol, and phospholipid than aqueous phase obtained in the pilot plant. Milkfat globule membrane concentration of emulsifying components affected phospholipid and cholesterol content of formulated creams. Butteroil type affected cholesterol levels and cream formulations.

KEYWORDS: Cream formulation; skim milk; sweet buttermilk; butter-derived aqueous phase; fractionated butteroil; separation; composition

INTRODUCTION

The dairy industry recently has made technological advances in modifying the chemical and physical attributes of milkfat, leading to replacement of traditional milkfat in various dairy and food systems. Modified milkfat differs nutritionally and functionally from natural milkfat but requires emulsification in many food systems. The challenges of emulsification can be overcome by combining altered milkfat with surface active agents into formulated dairy products such as creams. The final dairy product should have chemical and physical attributes comparable to natural dairy products.

Milk-derived components such as skim milk, sweet buttermilk, butter-derived aqueous phase, whey proteins, casein dispersions, and purified milkfat globule membrane (MFGM) suspensions have proven to successfully emulsify butteroil (*1*−*8*). These components contain differing types and amounts of surface active agents that function to stabilize the milkfat in the milk plasma.

The presence of flexible, unstructured casein proteins and structured globular whey proteins makes skim milk a good emulsifying agent. Whey and casein proteins are the primary proteins associated with sweet buttermilk and butter-derived aqueous phase, but buttermilk and aqueous phase are also abundant in phospholipids from MFGM fractions (*9*−*11*). Casein proteins have open structures, high levels of apolar amino acid residues, and uneven amino acid distribution that allow adsorbance at oil−water interfaces. The lack of a tertiary structure makes individual casein proteins more heat stable and better at lowering surface tension, thus giving them the ability to stabilize more surface area than whey proteins (*12*, *13*).

Processing conditions for attaining milk-derived components may influence their composition and emulsifying properties when formulated into cream. The compact, organized structure of whey proteins influences their behavior, most importantly functionality, during heat processing. Thermal unfolding of whey proteins, particularly β-lactoglobulin, improves emulsifying characteristics; however, prolonged denaturation decreases activity at the interface (*14*).

Thermal treatment of raw cream causes rearrangement of structural components on the MFGM that results in a change in its surface properties (*15*). Heating whole milk (80 °C) or cream (65 °C) strongly affects the functional properties of the membrane fraction and will cause whey proteins, particularly β-lactoglobulin, to associate with the MFGM, thereby increasing the protein content and proportionally decreasing the lipid in

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Table 1. Fatty Acid Analysis (wt %) of Low-Melt and Medium-Melt Fractionated Butteroils

fatty acid (wt %) butteroil fraction	butryic 4:0	caproic 6:0	caprylic 8:0	capric 10:0	lauric 12:0	myristic 14:0	palmitic 16:0	palmitoleic 16:1	stearic 18:0	oleic 18:1	linoleic 18:2
low-melt	4.6	2.3	2.2	3.8	3.7	9.4	19.6	1.8	8.6	33.1	2.1
medium-melt	6.3	2.7	1.4	3.9	3.1	8.5	29.3	3.7	9.6	23.5	3.0

the membrane (*16*, *17*). Conversely, mild heat treatment at 45 °C for 10 min has resulted in the loss of approximately 50% of the total protein from the MFGM of heated washed cream. Melting of the lipid phase at >40 °C may result in rearrangement of the fat globule surface and loss of some MFGM protein components (*18*). Other researchers (*19*) have found no significant difference due to heat treatment (75 °C for 16 s or 140 °C for 4 s) in protein composition of MFGM isolated from whipped cream.

It has been demonstrated that 30% milkfat creams formulated from skim milk or sweet buttermilk and butter-derived aqueous phase have a protein and phospholipid composition comparable to the emulsifying agent(s) used in each formulation. Electron microscopy, however, revealed that emulsions were water-in-oil as opposed to desired oil-in-water emulsions (*4*). Fat globules occurring in creams processed by Elling et al. (*6*) had surface proteins and MFGM fragments sufficiently adsorbed, resulting in oil-in-water emulsions. Unlike Oehlmann et al. (*4*) and Kanno et al. (*2*), Elling et al. (*6*) did not use MFGM suspensions as an emulsifying agent. Elling and co-workers (*6*) determined that reformulated creams (20% milkfat) consisting of buttermilk or buttermilk/aqueous phase exhibited protein and phospholipid compositions similar to natural homogenized cream. A MFGM composition of creams consisting of sweet buttermilk alone and sweet buttermilk and butter-derived aqueous phase was comparable to natural homogenized cream (*6*).

The objectives of this research were (i) to evaluate the influence of separation temperature (49 and 55 °C) on the content of surface active agents in milk-derived components (skim milk, sweet buttermilk, and butter-derived aqueous phase); (ii) to examine differences in composition of milk-derived components (sweet buttermilk and butter-derived aqueous phase) obtained from pilot plant operations as compared to a commercial operation; and (iii) to compare the composition of natural creams to 20% milkfat creams formulated with modified butteroils (with different melting ranges) and milk-derived components.

MATERIALS AND METHODS

Separation into Cream and Skim Milk. Raw milk was obtained from the Virginia Tech dairy farm, prewarmed to 49 or 55 °C, and separated into 30−35% milkfat cream and skim milk with a pilot plant separator (Elecrem separator, model 1G, 6400 rpm, Bonanza Industries, Inc., Calgary, Alberta). Creams, obtained at 49 and 55 °C separation, were standardized to 30−33% milkfat using skim milk obtained from the appropriate separation. Each cream was vat pasteurized at 68.3 °C for 30 min, cooled to 13 °C in an ice bath, and refrigerated (3.3 °C).

Preparation of Buttermilk and Butter-Derived Aqueous Phase. Components (sweet buttermilk and butter-derived aqueous phase) were obtained as described by Elling et al. (*6*). Creams (30−33% milkfat from separations at 49 or 55 °C) were tempered to 13−14 °C. Sweet buttermilk and butter were obtained from cream by mechanical churning (Gem Dandy Standard Electric Churn, Bonanza Industries, Inc.). Butter granules were separated from sweet buttermilk and pressed to remove excess moisture.

The butter-derived aqueous phase was produced in the pilot plant for comparison to commercially produced aqueous phase. The pilot plant produced aqueous phase was collected by melting butter obtained from churning. Melted butter (55−60 °C) was cooled (3 °C) without agitation for approximately 30 min to enhance separation, and the aqueous phase was collected and refrigerated overnight. Solidified lipid was removed from aqueous phase by filtering through cheesecloth. The pilot plant processed aqueous phase was vat pasteurized at 62.8 °C for 30 min, cooled, and stored at 3.3 °C.

Commercially produced (Grasslands Dairy Products, Inc., Greenwood, WI) sweet buttermilk and butter-derived aqueous phase were obtained for comparison to the sweet buttermilk and butter-derived aqueous phase that were processed in the pilot plant of Virginia Tech's Department of Food Science and Technology. At the commercial processing facility, cream (38.5% milkfat) was preheated to 85 °C and subsequently pasteurized at 87.2−87.8 °C. Following pasteurization, the cream was cooled to 6.1 °C by a plate heat exchanger. The cream then went through a two stage commercial separation process and was tempered at 10.6 °C for 8−10 h, and the buttermilk was obtained by churning. Serum (aqueous phase) was recovered following separation.

All components (skim milk, 49 and 55 °C separations; sweet buttermilk, 49 and 55 °C separations; and commercially obtained butter-derived aqueous phase) were pasteurized at the pilot plant in a tubular heat exchanger (Microthermics UHT/HTST Lab 25-HV, Microthermics, Inc., Raleigh, NC) at 71.7 °C for 15 s, cooled to 21.5 °C, packaged, and subsequently stored at 3.3 °C for further analyses.

Characterization of Low-Melt and Medium-Melt Fractionated Butteroils. Low-melt and medium-melt fractionated butteroils were obtained from anhydrous milkfat utilizing the Tirtiaux fractionation procedure at the Wisconsin Center for Dairy Research (University of Wisconsin, Madison). Low-melt fraction butteroil had a dropping point of 18 °C whereas medium-melt fraction butteroil had a dropping point of 26 °C (*20*). Percent solid fat was zero at approximately 25 and 30 °C for low-melt and medium-melt fractions, respectively. Both butteroils had butterlike flavors and medium yellow coloration (*20*). Fatty acid profiles for low-melt and medium-melt fractionated butteroils are provided in **Table 1** (*20*).

Cream Formulation. Creams (20% milkfat) were formulated from low-melt or medium-melt fractionated butteroils and pasteurized components: skim milk (49 or 55 °C separation) or buttermilk (49 or 55 °C separation) and commercial butter-derived aqueous phase. The pilot plant-produced aqueous phase was utilized only for compositional comparison to the commercially produced aqueous phase. Butteroils were melted at 45−50 °C prior to reformulation. Natural creams were prepared by separation of raw milk at 49 or 55 °C into 30−33% cream and skim milk. Skim milk from the appropriate separation was used to standardize each natural cream to 20% milkfat. Creams were preheated to 55 °C and homogenized in a two stage homogenizer (APV Gaulin, Inc., model 15MR, Everett, MA) at 13.6/3.4 Mpa (first/second stages), cooled to 29.4 °C, and pasteurized at 77.8 °C for 15 s in a tubular heat exchanger (Microthermics UHT/HTST Lab 25-HV, Microthermics, Inc.). **Table 2** describes cream formulations.

Fat, Protein, Cholesterol, and Phospholipid Determination of Components and Creams. Fat, protein, phospholipid, and cholesterol analyses were performed for pilot plant and commercially processed components (skim milk, buttermilk, and butter-derived aqueous phase) and creams. The DC Bio Rad assay (Bio Rad Laboratories, Hercules, CA) was employed to determine protein content. Total lipid content of components was determined according to Bligh and Dyer (*21*). The Babcock procedure for cream (*22*) was used to measure fat content of reformulated and natural creams. Analysis of phospholipid content required lipid extraction as outlined by Folch et al. (*23*). Phospholipids in the lipid extract were separated with a silicic acid column (*24*), and a quantitative analysis of the amount of phosphorus in the phospholipid extract was made using the spectrophotometric method described by Rouser and co-workers (*25*). The value obtained from the analysis was multiplied by a factor of 25 to convert from phosphorus to phospho-

Table 2. Description of 20% Milkfat Natural and Formulated Creams

formulated cream content	separation temp (°C)
80% skim milk + 20% low-melt butteroil	49
80% skim milk + 20% low-melt butteroil	55
80% skim milk + 20% medium-melt butteroil	55
70% buttermilk + 20% low-melt butteroil + 10% aqueous phase[a]	49
70% buttermilk + 20% low-melt butteroil + 10% aqueous phase[a]	55
70% buttermilk + 20% medium-melt butteroil + 10% aqueous phase[a]	55
natural cream (20% milkfat)	49
natural cream (20% milkfat)	55

[a] Commercial supplier.

lipid content (*26*). Cholesterol content was determined according to Stadtman (*27*).

Free Fatty Acid Profiles of Components and Creams. Free fatty acid profiles were determined by gas chromatography according to methods described by Supelco, Inc. (*28*). Fatty acid determinations were performed in triplicate and reported as an average on one replication. An internal standard (heptadecanoic acid, 17:0) was added (150 μL) to 10 mL of fresh sample before lipid extraction. Lipids were extracted by adding 10 mL of sample, 10 mL of ethanol, 3 mL of 28% ammonium hydroxide, 25 mL of petroleum ether, and 25 mL of diethyl ether to a separatory funnel. The mixture was shaken for approximately 5 min and allowed to sit undisturbed for 20 min. The bottom phase was drained, and the ether phase was dried under nitrogen. Approximately 3 mL of 0.5 N NaOH in methanol was added to the sample and heated on a steam bath for 15 min. Five milliliters of water was added, and the pH was adjusted to 2.0 with 2 N HCl. Free fatty acids were extracted with 5 mL of petroleum ether and 5 mL of diethyl ether.

Extract volumes of 0.2−0.3 μL were directly injected into an HP5890A gas chromatography system (Hewlett-Packard Co., Avondale, PA) fitted with a Nukol (Supelco, Inc., Bellefonte, PA) fused silica capillary column (15 m, 0.53 i.d.) and a flame ionization detector. The carrier gas (helium) was used at a flow rate of 20 mL/min. The initial oven temperature of 110 °C was held for 2 min followed by a ramp of 8 °C/min to a final temperature of 200 °C. Fatty acid standards (Supelco, Inc.) were diluted in diethyl ether to provide the standard solution. Quantification of free fatty acids was determined as described by Woo and Lindsay (*29*).

Characterization of Proteins by Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) separation of proteins in components and MFGM material from creams was conducted according to Laemmli (*30*) on one replication. Components were mixed 1:1 with 3× solubilization buffer, heated in a boiling water bath for approximately 5 min, and centrifuged for 2 min. Aliquots (5 μL) of supernatant from each component were separated by SDS−PAGE, using 8−20% Tris-glycine precast gradient gels of 1.5 mm thickness (Novex, San Diego, CA). Purified MFGM protein was used as a reference. Protein bands were fixed and stained with Coomassie blue in methanol, acetic acid, and water. Gels were destained first in 40% ethanol, 7% acetic acid in water, and second in 10% acetic acid in water and photographed.

Analysis of Cream Milkfat Surface Material. The amount of phospholipid and protein adsorbed on the surface of the milkfat globule was analyzed as described by Elling et al. (*6*). Creams were separated into a lipid rich cream plug and a serum phase by centrifuging (60 min, 2 °C, 175 000*g*) in a Beckman L2-65B Ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). The cream plug contained the components of the MFGM material and fat globule associated protein. Membrane material was released from the milkfat by two cycles of slow freezing and thawing and collected by centrifugation. The pellet was lyophilized in a freeze-drier (Freezemobile 12 SL, Virtis Co., Inc., Gardiner, NY) with drying chamber (10-MR-SM Vacuum Stoppering and Manifold Drying Chamber, Virtis Co., Inc.). Protein was determined with the DC Bio Rad Protein Assay, and SDS−PAGE (*30*) was used to determine the types of protein surrounding milkfat globules.

Statistical Analyses. The study was replicated three times. Analyses were performed on duplicate samples. An augmented randomized block design was employed for data analysis for fat, protein, phospholipid, cholesterol, and amount of milkfat globule surface material. Contrasts were used to compare effects of separation temperature and pilot plant vs commercially processed components. Statistical analyses were conducted with SAS (Cary, NC). A p value of 0.01 was used to minimize type 1 error in determination of significant differences.

RESULTS AND DISCUSSION

Component Composition and Potential Emulsifying Characteristics. Knowledge of component composition and nature of surface material is valuable in developing an understanding of the emulsion characteristics and functionality of formulated cream. Compositional differences of skim milk, sweet buttermilk, and butter-derived aqueous phase obtained in the pilot plant by separation of milk at 49 and 55 °C as well as commercially obtained sweet buttermilk and aqueous phase were found (**Table 3**).

Skim milk was significantly lower ($p \leq 0.01$) in fat, cholesterol, and phospholipid than buttermilk or butter-derived aqueous phase but had a higher ($p \leq 0.01$) protein content than butter-derived aqueous phase. Sweet buttermilk and butter-derived aqueous phase were higher in fat because both were derived from churned cream (30% milkfat). The butter-derived aqueous phase was significantly higher ($p \leq 0.01$) in lipid content than sweet buttermilk. The commercially produced butter-derived aqueous phase was lower ($p \leq 0.01$) in fat than aqueous phase produced in the pilot plant, suggesting that separation of milkfat from the butter-derived aqueous phase in the pilot plant was not as efficient as the commercial operation. However, the lipid content did not differ ($p > 0.05$) between sweet buttermilk processed in the pilot plant and commercially produced buttermilk. The separation temperature used in obtaining components did not have a significant ($p > 0.05$) effect on lipid content.

Phospholipid concentration serves as an indication of the presence of MFGM fragments in the components. As a lipid group, phospholipids prove to be excellent emulsifying agents due to their amphiphillic nature, having affinity for both polar and apolar regions (*31*). Pilot plant-produced butter-derived aqueous phase was significantly higher ($p \leq 0.01$) in phospholipid content than skim milk and sweet buttermilk, while sweet buttermilk contained more ($p \leq 0.01$) phospholipid than skim milk (**Table 3**). Some membrane material is associated with skim milk (*12*). However, buttermilk and butter-derived aqueous phase contain not only skim milk proteins (casein and whey proteins) but also a large fraction of MFGM material known to be high in phospholipid and protein. Fragments remain in the churned products after the native MFGM is disrupted by the churning process. Phospholipid content in butter-derived aqueous phase has been found to be over 40 times higher than in skim milk, and sweet buttermilk contains seven times as much phospholipid as skim milk (*6*). There was no difference ($p > 0.01$) in phospholipid content between pilot plant-processed sweet buttermilk and commercially manufactured buttermilk. However, pilot plant-processed butter-derived aqueous phase had a significantly higher ($p \leq 0.01$) phospholipid content than butter-derived aqueous phase produced commercially.

Similar trends were observed for cholesterol content in components. Higher amounts of cholesterol were expected to be associated with sweet buttermilk and butter-derived aqueous phase since cholesterol is concentrated in the MFGM. Aqueous phase obtained from 55 °C separation was significantly higher ($p \leq 0.01$) in cholesterol than aqueous phase from 49 °C separation.

Table 3. Composition[a] and Statistical Contrasts for Compositions of Pilot Plant Produced and Commercially Processed Components

Compositions of Components

component (separation temp; °C)	lipid (%)	protein (mg/g component)	cholesterol (mg/g component)	phospholipid (mg/g component)	% phospholipid in lipid
skim milk (49)	0.205	29.65	0.087	0.155	7.56
skim milk (55)	0.210	30.19	0.079	0.156	7.43
buttermilk (49)	0.682	27.78	0.147	1.013	14.85
buttermilk (55)	0.690	28.93	0.167	1.004	14.55
commercial buttermilk	0.753	24.46	0.175	1.393	18.50
AP[b] (49)	1.464	22.43	0.345	5.015	34.26
AP (55)	1.538	26.12	0.390	4.634	30.13
commercial AP	0.786	11.67	0.135	0.741	9.43
standard error	0.100	1.145	0.007	0.220	3.59

Statistical Control for Compositions of Components

component (separation temp; °C)	lipid (%)	protein (mg/g component)	cholesterol (mg/g component)	phospholipid (mg/g component)
skim milk (49) vs skim milk (55)	0.9703	0.7425	0.3616	0.9980
buttermilk (49) vs buttermilk (55)	0.9524	0.4916	0.0562	0.9793
AP (49) vs AP (55)	0.6121	0.0387	0.0003[c]	0.2429
pilot plant buttermilk vs commercial buttermilk	0.5965	0.0149[c]	0.0453	0.1777
pilot plant AP vs commercial AP	0.0003[c]	0.0001[c]	0.0001[c]	0.0001[c]
skim milk vs buttermilk	0.0004[c]	0.1927	0.0001[c]	0.0019[c]
skim milk vs AP	0.0001[c]	0.0002[c]	0.0001[c]	0.0001[c]
buttermilk vs AP	0.0001[c]	0.0031[c]	0.0001[c]	0.0001[c]

[a] Values are means for three replications. [b] AP = aqueous phase. [c] Statistically significant at $p \leq 0.01$.

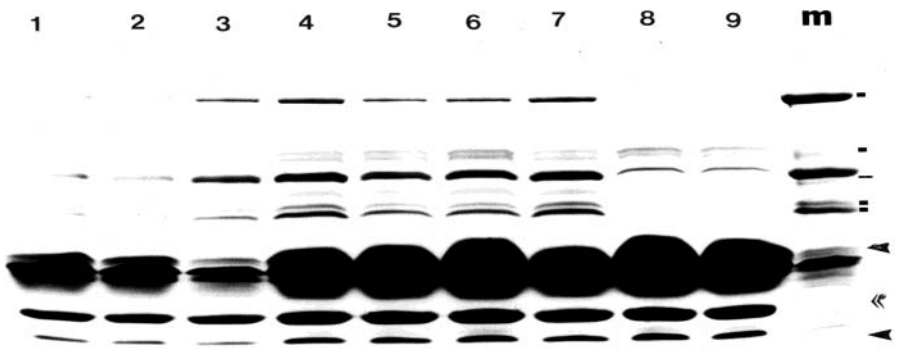

Figure 1. Polypeptide profiles of pilot plant-produced components and commercially obtained components. Lanes: 1, 49 °C butter-derived aqueous phase; 2, 55 °C butter-derived aqueous phase; 3, commercial butter-derived aqueous phase; 4, commercial sweet buttermilk; 5, 49 °C sweet buttermilk; 6, 55 °C sweet buttermilk; 7, unpasteurized commercial sweet buttermilk; 8, 49 °C skim milk; 9, 55 °C skim milk; and m, purified MFGM extract. Dashes from top to bottom: xanthine oxidase, CD36, butyrophilin, and PAS 6/7. Arrowheads from top to bottom: casein and whey proteins, α-lactalbumin and β-lactoglobulin, respectively.

The protein content of buttermilk resembles that of skim milk (approximately 30 g/L of protein) (*32*, *33*). Skim milk proteins (caseins and whey proteins, α-lactalbumin, and β-lactoglobulin) have been found to make up 75% percent of the total protein in buttermilk (*34*). The protein content of pilot plant processed skim milk and buttermilk was significantly higher ($p \leq 0.01$) than the pilot plant-processed butter-derived aqueous phase. In contrast, Elling et al. (*6*) found that butter-derived aqueous phase contained significantly higher amounts of protein in comparison to skim milk and sweet buttermilk. Both sweet buttermilk and butter-derived aqueous phase from commercial processors were lower ($p \leq 0.01$) in protein than pilot plant-processed buttermilk and butter-derived aqueous phase.

Gel electrophoresis indicated that casein proteins were the predominant proteins in components, regardless of separation temperature and source of processing (**Figure 1**). The whey protein bands, α-lactalbumin, and β-lactoglobulin were distinct in all components, with less visible bands in butter-derived aqueous phases. Sweet buttermilk and commercially obtained butter-derived aqueous phase had darker bands of butyrophilin, xanthine oxidase, and PAS 6/7. PAS 6/7 was absent in the skim milk. The band representing CD36 was discernible in all components with the exception of the butter-derived aqueous phases.

Types and amounts of free fatty acids associated with components were determined by gas chromatography (**Table 4**). The free fatty acid profile of the components appeared to be unaffected by separation temperature. Palmitic acid (16:0) was the most abundant fatty acid in all of the components followed by oleic acid (18:1). High levels of palmitic acid and oleic acid are expected since they contribute 20−25 and 30−38% of total fatty acid content, respectively (*35*). Skim milk contained the lowest amounts of all of the fatty acids. Skim milk also was lower in the unsaturated fatty acids, palmitoleic (16:1), oleic (18:1), and linoleic (18:2) acid. The butter-derived aqueous phase contained the largest amounts of the individual fatty acids followed by buttermilk. Commercial buttermilk contained lower levels of free fatty acids than pilot plant-processed sweet buttermilk. In contrast, the commercially

Table 4. Fatty Acid Analysis[a] of Components Used for Cream Formulation

	fatty acid content (μg/mL component)										
component	butyric 4:0	caproic 6:0	caprylic 8:0	capric 10:0	lauric 12:0	myristic 14:0	palmitic 16:0	palmitoleic 16:1	stearic 18:0	oleic 18:1	linoleic 18:2
49 °C skim milk	ND	ND	ND	ND	ND	0.73	1.65	ND	0.47	0.78	ND
55 °C skim milk	ND	ND	ND	ND	ND	0.71	1.51	ND	0.42	0.74	ND
49 °C buttermilk	1.43	3.14	1.87	3.05	2.65	6.04	13.7	1.02	3.31	8.22	1.05
55 °C buttermilk	1.77	3.59	4.60	3.51	2.98	6.62	15.2	1.14	3.91	9.31	1.08
commercial buttermilk	0.62	1.38	0.90	1.95	1.34	3.00	6.80	0.58	1.92	4.44	0.66
49 °C AP	3.20	4.14	2.99	3.79	3.87	12.02	28.49	ND	10.58	13.56	ND
55 °C AP	3.06	3.94	2.59	3.29	3.69	11.35	28.69	ND	11.43	11.29	ND
commercial AP	4.89	6.40	2.95	5.60	6.32	12.48	31.69	2.98	10.53	23.79	3.32

[a] Means are triplicate measurements from replication three; ND, below detectable limits; AP, aqueous phase.

Table 5. Composition[a] and Statistical Contrasts for Compositions of Natural and Formulated Creams[b]

Compositions of Natural and Formulated Creams

formulation (separation temp; °C)	fat (%)[c]	protein (mg/g)	cholesterol (mg/g)	phospholipid (mg/g)	% phospholipid in lipid
20% lmbo + 80% skim milk (49)	20.17	40.12	0.693	0.067	0.033
20% lmbo + 80% skim milk (55)	20.17	40.29	0.679	0.063	0.031
20% mmbo + 80% skim milk (55)	20.08	31.57	0.507	0.066	0.033
20% lmbo + 70% bm + 10% AP (49)	20.67	34.62	0.750	0.539	0.261
20% lmbo + 70% bm + 10% AP (55)	20.75	34.82	0.707	0.515	0.248
20% mmbo + 70% bm + 10% AP (55)	21.25	27.83	0.564	0.522	0.246
natural cream (49)	20.75	27.77	0.610	0.486	0.234
natural cream (55)	21.92	29.88	0.614	0.492	0.225
standard error	0.378	2.564	0.020	0.065	0.039

Statistical Contrasts for Compositions of Natural and Formulated Creams

formulation (separation temp; °C)	fat (%)	protein (mg/g)	cholesterol (mg/g)	phospholipid (mg/g)
skim milk lmbo (49) and skim milk lmbo (55) vs skim milk mmbo (55)	0.8821	0.0156	0.0001[d]	0.9927
Bm/AP lmbo (49) and Bm/AP lmbo (55) vs Bm/AP mmbo (55)	0.3428	0.0457	0.0001[d]	0.9487
skim vs Bm/AP	0.0608	0.0345	0.0103[d]	0.0001[d]
skim vs natural	0.0115	0.0027[d]	0.4348	0.0001[d]
Bm/AP vs natural	0.2980	0.1462	0.0039[d]	0.5461
natural (49) vs natural (55)	0.0884	0.5691	0.9014	0.9452
skim milk lmbo (49) vs skim milk lmbo (55) and skim milk mmbo (55)	0.9409	0.2036	0.0009[d]	0.9731
Bm/AP lmbo (49) vs Bm/AP lmbo (55) and Bm/AP mmbo (55)	0.5553	0.3122	0.0003[d]	0.8016

[a] Values are means for three replications. [b] lmbo, low-melt butteroil; mmbo, medium-melt butteroil; Bm/AP, creams formulated with buttermilk and aqueous phase. [c] Babcock method (*22*). [d] Statistically significant at $p \leq 0.01$.

obtained aqueous phase contained higher amounts of all of the free fatty acids (especially the short chain fatty acids) with the exception of caprylic (8:0) and stearic (18:0) acid.

Characterization of Natural and Formulated Creams. The implications of formulation, separation temperature in obtaining components, and butteroil melting range characteristics were considered in making comparisons of natural and formulated creams. Transmission electron microscopy revealed that all creams were oil-in-water emulsions (data not presented) (*36*). Oehlmann et al. (*4*) produced water-in-oil emulsions in reformulated, 30% milkfat creams. Perhaps processing sequence or formulation balance was improper for stabilization of an oil-in-water emulsion. Elling et al. (*6*) emulsified reduced cholesterol butteroil (20%) with sweet buttermilk (70%) and butter-derived aqueous phase (10%) and produced desired oil-in-water emulsions.

Compositional differences among formulated creams reflected compositional differences in components utilized in the formulation. Cream formulations using skim milk were higher ($p \leq 0.01$) in protein than natural creams (**Table 5**). This observation is in contrast to that of Elling et al. (*6*), who reported no difference in protein content among creams formulated with low cholesterol butteroil and skim milk or buttermilk and aqueous phase. Separation temperature, melting range characteristics of butteroil, and formulation had an influence on the cholesterol content of creams. Formulated creams processed from components obtained by 49 °C separation had higher ($p \leq 0.01$) levels of cholesterol than similar creams manufactured from 55 °C separation components whereas there was no difference in cholesterol in natural creams separated at the two temperatures. Creams containing medium-melt fractionated butteroil were lower ($p \leq 0.01$) in cholesterol than creams comprised of low-melt fraction butteroil (**Table 5**). Creams formulated with sweet buttermilk and butter-derived aqueous phase were higher ($p \leq 0.05$) in cholesterol than skim milk and natural creams. This can be attributed to the fact that 75, 10, and 15% of total cholesterol are associated with core lipid, MFGM, and skim phase membrane material, respectively (*37*). As components, both sweet buttermilk and butter-derived aqueous phase had higher ($p \leq 0.01$) lipid values than skim milk and were more

Table 6. Fatty Acid Analysis[a] of Formulated and Natural Creams

	fatty acid content (μg/mL cream)										
cream formulation[b] (separation temp; °C)	butyric 4:0	caproic 6:0	caprylic 8:0	capric 10:0	lauric 12:0	myristic 14:0	palmitic 16:0	palmitoleic 16:1	stearic 18:0	oleic 18:1	linoleic 18:2
20% lmbo + 80% skim milk (49)	15.71	11.68	26.78	11.68	10.45	15.31	23.29	2.29	7.31	25.91	2.75
20% lmbo + 80% skim milk (55)	13.91	12.25	21.14	13.02	7.95	18.66	35.86	6.32	9.08	30.89	4.00
20% mmbo + 80% skim milk (55)	8.63	10.32	21.42	10.08	7.79	18.82	50.80	2.38	17.61	27.96	3.61
20% lmbo + 70% Bm + 10% AP (49)	4.56	8.96	5.84	11.50	13.88	15.65	34.21	2.87	10.40	34.19	4.29
20% lmbo + 70% Bm + 10% AP (55)	9.45	9.92	9.36	12.31	11.24	15.31	29.28	2.92	8.87	29.84	3.83
20% mmbo + 70% Bm + 10% AP (55)	10.65	11.60	6.24	12.88	13.20	18.48	48.55	2.29	16.08	28.28	3.53
natural cream (49)	8.36	7.21	3.70	8.24	11.26	17.97	44.48	3.75	13.98	30.06	3.08
natural cream (55)	12.25	8.80	4.16	9.65	12.97	20.17	49.12	3.74	15.80	41.70	3.74

[a] Means are triplicate measurements from replication three. [b] lmbo, low-melt butteroil; mmbo, medium-melt butteroil; Bm, buttermilk; AP, aqueous phase.

abundant in fragments of MFGM constituents, indicating that higher amounts of cholesterol may occur in these particular components.

The phospholipid concentration of formulated and natural creams also was influenced by the components used in formulation of the creams. Skim milk formulated creams had significantly lower ($p \leq 0.01$) phospholipid levels than natural creams while creams formulated with sweet buttermilk and butter-derived aqueous phase did not differ in phospholipid content from natural creams (**Table 5**). Elling et al. (*6*) found that higher phospholipid contents of creams processed from sweet buttermilk and butter-derived aqueous phase resulted in better emulsion stability than creams formulated from skim milk.

Free fatty acid analysis of components aided in understanding fatty acid profiles of the creams. However, fatty acids contributed by fractionated butteroils in formulated creams also must be considered. Individual free fatty acid profiles were determined for natural and formulated creams on one replication of the study (**Table 6**). As in component profiles, palmitic acid (16:0) was the most abundant fatty acid in the creams, followed by oleic acid (18:1). Creams formulated with medium-melt fractionated butteroil were most comparable to natural creams in palmitic (16:0) and stearic acid (18:0) levels, the primary saturated fatty acids in milk. The medium-melt butteroil contributed more palmitic (29.3%) and stearic (9.6%) acid to the creams than the low-melt fractionated butteroil (19.6 and 8.6%, respectively) (**Table 1**). Therefore, creams formulated with medium-melt butteroil were higher in these long chain saturated fatty acids.

Characterization of Milkfat Globule Surface Material. Unprocessed cream samples have relatively higher amounts of intact native MFGM components, particularly phospholipids and proteins. These particular components are highly surface active, acting as emulsifying agents at the oil−water interface. Creams that undergo essential processing steps such as homogenization and pasteurization to prolong stability and shelf life contain fragments of the MFGM. Fragmenting of the MFGM results in increased attachment of skim milk proteins to lipid globules. Lipid globules of formulated and natural creams were principally emulsified by casein and whey proteins and some MFGM fragments.

Lipid globules were roughly spherical in shape with membrane fragments and serum proteins present on lipid globule surfaces. A minimum of 135 globules was measured for each cream. Generally, creams processed with low-melt butteroil and skim milk had many small globules that were relatively uniform in size (0.49 ± 0.24 μm), while creams containing medium-melt butteroil, sweet buttermilk, and butter-derived aqueous phase were made up of large diameter fat globules that varied greatly in size (0.87 ± 0.63 μm).

The composition of MFGM isolated from creams was not affected ($p > 0.01$) by melting range characteristics of butteroil or separation temperature used to produce components (**Table 7**). The amount of total protein associating with lipid globules was significantly ($p \leq 0.01$) higher in natural creams and creams formulated with buttermilk and aqueous phase than in creams formulated with skim milk. The staining intensity of the protein bands in the SDS−PAGE confirms this observation (**Figure 2**). Phospholipid content was significantly higher ($p \leq 0.01$) in surface material collected from natural creams and creams formulated with sweet buttermilk and aqueous phase than in MFGM of creams formulated with skim milk. This is a reflection of the higher levels of phospholipid contained in buttermilk and aqueous phase components used in formulating the creams (**Table 3**). The composition of lipid surface material obtained from natural creams was more comparable to formulations consisting of buttermilk and butter-derived aqueous phase than creams formulated with skim milk. These results are in agreement with those of Elling et al. (*6*).

SDS−PAGE was utilized to characterize types of proteins associated with lipid globules occurring in natural and formulated creams. The separation temperature in obtaining components and the melting range profile of butteroil did not result in visible differences in proteins associated with MFGM of the creams (**Figure 2**). Electrophoretic migration of membrane material from all cream formulations was similar to that of purifed MFGM extract. The purified membrane material contained higher amounts of native MFGM proteins and lower levels of the casein and whey proteins. Casein proteins were the most abundant protein in the milkfat surface material of all cream formulations. Corredig and Dalgleish (*11*, *16*) found that casein proteins made up about 50% of total protein in buttermilk isolates. Oortwijn and Walstra (*1*) quantified proteins adsorbed on milkfat droplets in recombined cream consisting of milkfat in skim milk, whey, or casein protein dispersions. In comparisons of the amounts of proteins adsorbed from skim milk and whey, these researchers found casein proteins to be more adsorbed to fat globules. In oil-in-water emulsions made from milkfat plus skim milks and varying in content of fat and protein,

Table 7. Composition[a] of and Statistical Contrasts for Composition of Surface MFGM Isolated from Lipid Globules of Natural and Formulated Creams

Composition of Surface MFGM

formulation[b] (separation temp; °C)	MFGM/ cream (mg/g)	protein/ MFGM (mg/10 mg)	phospholipid/ MFGM (mg/10 mg)
20% lmbo + 80% skim milk (49)	28.48	2.858	0.032
20% lmbo + 80% skim milk (55)	27.15	3.199	0.032
20% mmbo + 80% skim milk (55)	24.49	3.707	0.028
20% lmbo + 70% Bm + 10% AP (49)	20.24	4.672	0.110
20% lmbo + 70% Bm + 10% AP (55)	20.79	5.079	0.109
20% mmbo + 70% Bm + 10% AP (55)	19.16	5.457	0.111
natural cream (49)	17.04	5.504	0.132
natural cream (55)	17.18	5.764	0.132
standard error	4.96	0.429	0.015

Statistical Contrasts for Composition of Surface MFGM

formulation[b] (separation temp; °C)	MFGM/ cream (mg/g)	protein/ MFGM (mg/10 mg)	phospholipid/ MFGM (mg/10 mg)
skim milk lmbo (49) and skim milk lmbo (55) vs skim milk mmbo (55)	0.5930	0.2177	0.8485
Bm/AP lmbo (49) and Bm/AP lmbo (55) vs Bm/AP mmbo (55)	0.8272	0.2869	0.9160
skim milk vs Bm/AP	0.1233	0.0001[c]	0.0001[c]
skim milk vs natural	0.0524	0.0001[c]	0.0001[c]
Bm/AP vs natural	0.5248	0.1714	0.1343
natural cream (49) vs natural cream (55)	0.9850	0.6750	0.9714
skim milk lmbo (49) vs skim milk lmbo (55) and skim milk mmbo (55)	0.6681	0.2765	0.9433
Bm/AP lmbo (49) vs Bm/AP lmbo (55) and Bm/AP mmbo (55)	0.9662	0.2762	0.9765

[a] Values are means for three replications. [b] lmbo, low-melt butteroil; mmbo, medium-melt butteroil; Bm/AP, creams formulated with buttermilk and aqueous phase. [c] Statistically significant at $p \leq 0.01$.

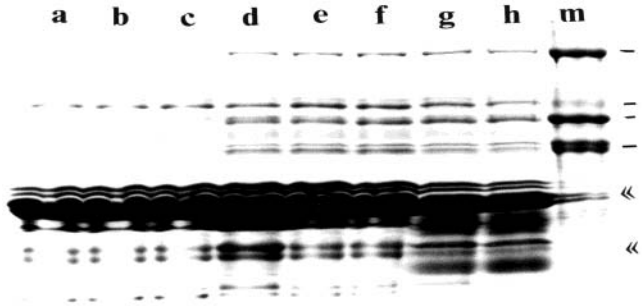

Figure 2. Polypeptide profiles of MFGM material isolated from lipid globules of natural and formulated creams. Lanes: a, 49 °C skim milk, low-melt butteroil; b, 55 °C skim milk, low-melt butteroil; c, 55 °C skim milk, medium-melt butteroil; d, 49 °C sweet buttermilk, commercial butter-derived aqueous phase, low-melt butteroil; e, 55 °C sweet buttermilk, commercial butter-derived aqueous phase, low-melt butteroil; f, 55 °C sweet buttermilk, commercial butter-derived aqueous phase, medium-melt butteroil; g, 49 °C natural cream; h, 55 °C natural cream; and m, purified MFGM extract. Dashes from top to bottom: xanthine oxidase, CD36, butyrophilin, and PAS 6/7. Arrowheads from top to bottom: casein and whey proteins, α-lactalbumin and β-lactoglobulin, respectively.

casein and whey proteins were adsorbed; however, preferential adsorbance of casein over whey proteins occurred (*5*). SDS−PAGE indicated that proteins not adsorbed were predominantly whey proteins (*5*).

The whey proteins β-lactoglobulin and α-lactalbumin were present in MFGM from all formulations and natural creams. On the basis of staining intensity, MFGM from cream containing buttermilk and aqueous phase from the 49 °C separation had the highest level of these proteins, followed by the natural creams. MFGM from skim milk formulations appeared to contain the lowest amounts of these proteins. Corredig and Dalgleish (*11*) performed SDS−PAGE on industrial buttermilk isolates and found β-lactoglobulin but almost no α-lactalbumin. It was hypothesized that heat treatment of the creams prior to buttermaking resulted in formation of complexes linked by disulfide bonds between β-lactoglobulin and MFGM proteins. Corredig and Dalgleish (*16*) found no β-lactoglobulin or α-lactalbumin in buttermilk and MFGM isolates from unpasteurized cream. These proteins remained in the soluble phase after centrifugation of buttermilk. Elling et al. (*6*) reported that casein was the major individual protein attached to lipid globule surfaces of creams; however, whey proteins were not observed due to cream washing. Other researchers reported that heating whole milk caused incorporation of whey proteins into the MFGM, resulting in increased protein content and decreased lipid (*17*). Membrane glycoproteins such as PAS 6 and PAS 7 disappeared or were weakly stained in the gels of heated milk (*17*). In this study, CD36 was present in MFGM of all cream formulations while xanthine oxidase and butyrophilin were most prevalent in MFGM profiles of creams containing buttermilk and aqueous phase, followed by the natural creams. As in the skim component, PAS 6/7 appeared to be absent from MFGM from the skim milk formulations. Butteroil contributed no membrane material to the cream formulations. On the basis of profiles of components (**Figure 1**), buttermilk contributes the majority of these polypeptides so it would be expected that MFGM from creams containing buttermilk would have higher levels of these proteins. Sweet buttermilk and to a lesser extent butter-derived aqueous phase contribute most of the native MFGM proteins that reassociate with lipids after formulation and processing.

CONCLUSION

Compositional differences in milk components influence type and amount of surface active agents available for emulsification of milk fat in milk plasma. Commercially processed products available for large scale processing may not provide the same emulsifying capacity as pilot plant products utilized during product development.

Components used to formulate 20% milkfat creams affected emulsifying characteristics of reformulated creams. The composition of components used in the formulation process served as a predictor of characteristics of formulated creams. Butter-derived aqueous phase and sweet buttermilk were higher in cholesterol and phospholipids than skim milk, resulting in creams formulated with these two components having significantly higher levels of cholesterol and phospholipids.

Melting range characteristics of fractionated butteroils influenced the fatty acid profile of formulated creams. Incorporation of low-melt fractionated butteroils in place of medium-melt fractionated butteroils substantially lowered the amount of long chain saturated fatty acids within treatments and slightly increased unsaturated fatty acids such as oleic acid and linoleic acid. Cholesterol was lower ($p \leq 0.01$) in creams formulated with medium melting range butteroil than similar creams with low-melting range butteroil. Thus, fractionated butteroils changed the compositional profile, and possible nutritional benefits, of formulated creams. Creams with sweet buttermilk and butter-

derived aqueous phase were comparable to natural creams with regard to emulsifying agents and milkfat globule surface material properties.

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