Effect of Homogenization Pressure on the Milk Fat Globule Membrane Proteins

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ABSTRACT

The effect of high pressure homogenization on the milk fat globule membrane proteins was investigated. Milk with 1.5 or 3.0% milk fat was heated in vats at low or high temperature (65°C for 30 min or 85°C for 20 min), homogenized, cooled, and centrifuged to separate the cream and serum phases. The amount of protein load per surface area increased as homogenization pressure increased but decreased with heat treatment. The composition of the proteins forming the milk fat globule membrane in homogenized milk was not affected by homogenization pressure or fat concentration, but significant differences in the composition of the milk fat globule membrane were caused by the heat treatment that was applied before homogenization. The milk fat globule membrane proteins in homogenized milk were composed of native membrane proteins, caseins, α-lactalbumin, and β-lactoglobulin. Caseins represented about 70% of the proteins in the milk fat globule membrane. In milk heated at 85°C for 20 min, the ratios of adsorbed α-lactalbumin and β-lactoglobulin relative to adsorbed caseins were higher than in milk heated to 65°C.
(**Key words**: high pressure homogenization, fat globules, caseins)

Abbreviation key: **MFGM** = milk fat globule membrane, $\mathbf{d_{vs}}$ = mean diameter for volume surface, Γ = mass of protein adsorbed per surface area, **OW** = oil-water.

INTRODUCTION

The native membrane that surrounds milk fat globules consists of a complex mixture of proteins, glycoproteins, enzymes, phospholipids, triglycerides, and other minor components (17). This milk fat globule membrane (**MFGM**) acts as a natural emulsifying agent that prevents flocculation and coalescence of milk fat globules and also protects the fat against enzymatic action (25).

Agitation, cooling, natural creaming, and lipolysis during the processing of milk alter the MFGM, but heat treatment and homogenization produce the greatest changes in the MFGM. Heat treatment of milk causes changes in the MFGM by promoting interactions between plasma proteins and native MFGM components (6, 8, 11, 22). It is not clear whether the interactions result from direct binding between denatured serum proteins and native MFGM proteins through intermolecular disulfide bonds (6, 22) or by displacement of polypeptides in the native MFGM by milk serum proteins (8). Homogenization of milk causes a reduction of fat globule size and a concurrent increase in the milk fat surface area, which alters the original MFGM because the concentration of native MFGM is insufficient to cover the fat surfaces that are formed during homogenization. Adsorption of new material from the milk serum at the oil-water (**OW**) interface occurs to cover this increase in surface area. The new MFGM consists of native MFGM plus adsorbed plasma proteins, with casein as the dominant group, but not at the exclusion of whey proteins (7, 10, 17, 22, 25).

Several studies detail the composition of the MFGM of homogenized and unhomogenized milk (1, 3, 12, 13, 15, 16, 18, 21, 22), but reports of the effect of homogenization pressure on the MFGM material of milk have not been published. Our objective was to evaluate the effect of high homogenization pressure on the composition of MFGM proteins in milk.

MATERIALS AND METHODS

Materials

Fresh raw milk was obtained from the Texas A&M University Dairy Farm (College Station) and was separated using a cream separator (De Laval 104; The Laval Separator Co., Poughkeepsie, NY). Milk fat concentrations of 1.5 and 3.0% were obtained by blending the skim milk and cream.

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Heat Treatment and Homogenization

Milk was heated in vats at low or high temperatures (65°C for 30 min or 85°C for 20 min) in a water bath equipped with an agitator. Immediately after heat treatment, milk was homogenized at the respective heating temperature in a high pressure homogenizer (Mini-Lab type 8.30H; APV Rannie Inc., St. Paul, MN) at 30, 60, and 90 MPa. The samples were cooled to 4°C immediately after homogenization, and 0.02% sodium azide was added to preserve the milk before extraction of the MFGM.

Particle Size Analysis

The mean diameter for volume-surface ($\mathbf{d_{vs}}$) and specific surface area were determined in milk samples after homogenization using a light-scattering apparatus (Coulter LS 130; Coulter Corporation, Miami, FL).

Determination of Protein Load

The concentration of protein in the homogenized milk samples and in the serum phase of the samples after centrifugation [10,500 × *g* for 30 min at 20°C with addition of 28.6 g of sucrose/100 g of milk (24)] was determined using the micro-Kjeldahl method (2). The percentages of milk fat remaining in the serum phase after centrifugation and in the milk samples were determined using the Mojonnier method for ether extraction (4). The mass of adsorbed protein was calculated as the difference between the amount of protein measured in the serum phase and the amount of protein present in the original sample. The protein load (Γ) was calculated as milligrams of protein per square meter of fat surface area, using the formula

$$\Gamma = \frac{(p_o - p_s)\rho_f}{sa(f_o - f_s)}$$

where

Γ = protein load (milligrams of protein per square meter of milk fat),
p_o = protein content of milk (milligrams of protein per gram of milk),
p_s = protein content of the serum (milligrams of protein per gram of milk),
ρ_f = density of milk fat (0.916 gram per milliliter at 20°C),
sa = specific surface area of milk fat (square meters of milk fat per milliliter of milk fat),
f_o = fat fraction of the milk (grams of milk fat per gram of milk), and
f_s = fat fraction of the serum (grams of milk fat per gram of milk).

The specific surface area of the milk fat was calculated from the particle size analysis.

Separation of MFGM Proteins

The MFGM proteins of homogenized milk samples were obtained following the method of Kanno and Kim (9) with modifications. Cream was separated from the homogenized milk by centrifugation at 10,500 × *g* for 30 min and 20°C after addition of 28.6 g of sucrose/100 g of milk to increase the difference in the density between the fat and serum phases. Cream was washed three times with deionized water at 6700 × *g* for 10 min at 20°C. The washed cream was diluted to approximately 30% fat and churned. The buttermilk and butter granules were collected separately, and the butter granules were melted at 40°C and centrifuged (6700 × *g* for 5 min at 20°C) to recover the serum from the butter oil. The buttermilk and butter serum were combined and centrifuged for 15 min at 3000 × *g* at 4°C. The MFGM proteins were concentrated from the aqueous phase by acidification of the serum phase to pH 4.8 with 0.01*N* HCl to precipitate the membrane proteins. The precipitate was allowed to form for 30 min at room temperature (25°C) and then was collected by centrifugation (10,500 × *g* for 30 min at 4°C). The floating cream layer was removed using a Pasteur pipette. Distilled water was added to the precipitate and pH was adjusted to 6.8 using 0.01*N* NaOH.

Quantification of Individual Proteins

The Coomassie Plus Protein Assay Reagent (Pierce Co., Rockford, IL) was used to determine protein concentrations of the MFGM samples prior to electrophoresis. Ten micrograms of MFGM proteins from each sample were loaded on each gel. Analysis by SDS-PAGE under reducing and nonreducing conditions was conducted using the method of Laemmli (14) and a Novex Xcell Mini-Cell and Novex precast Tris-glycine polyacrylamide gels of 18% acrylamide concentration and 8 to 16% gradient acrylamide concentration (Novex Co., San Diego, CA). Sample buffer with 0.5 *M* Tris·HCl, 20% glycerol, 10% SDS, and 0.1% bromophenol blue was added 1:1 (vol/vol) to the MFGM solution to denature the samples for nonreducing conditions. β-Mercaptoethanol (5.0%) was added to the sample buffer to denature the sam-

ples for reducing conditions. Samples were heated in boiling water for 2 min prior to being loaded on gels. The premixed molecular mass marker (Boehringer Manheim Co., Indianapolis, IN) and milk protein standards α-CN, κ-CN, α-LA, and β-LG (Sigma Chemical Co., St. Louis, MO) were applied to each gel. To eliminate variations between gels, 0.5 μg of BSA (Pierce Co., Rockford, IL) was loaded on each gel as an internal standard. A regression equation to determine protein concentration in gels was developed by applying 0.05, 0.10, 0.25, 0.50, and 1.00 μg of BSA in duplicate on the acrylamide gels. Gels were stained with Coomassie blue solution and destained overnight in a mixture of 30% methanol and 10% acetic acid. Images of the gels were obtained using a scanner (HP ScanJet IIcx; Hewlett-Packard Co., Palo Alto, CO). The relative concentration of the polypeptides that were positive by Coomassie blue was calculated from the relative peak areas in the gel images using gel image analysis software (23) and the regression equation obtained from the BSA standards.

Statistical Analysis

All experiments were conducted in duplicate or triplicate using a complete factorial experimental design. The data were analyzed for main effects and for all two-way interactions using the general linear models procedure and the least significant difference means test of SAS (20).

RESULTS AND DISCUSSION

Protein Load

In order to separate the cream from the milk homogenized at high pressures by centrifugation, sucrose (28.6 g of sucrose/100 g of milk) was added to increase the difference in densities between the fat and the serum phases. Even with the addition of sucrose, not all of the milk fat could be separated from the homogenized samples by centrifugation, probably because of the increase in density of the homogenized milk fat globules after homogenization. The fat globules that are formed during homogenization are covered with plasma proteins to coat the increased surface area caused by homogenization (7, 16, 17, 21, 22), and their density could increase to a density near that of the serum phase, especially for the smaller fat globules, making it difficult to separate all of the fat from milk by centrifugation. The fraction of milk fat remaining in the serum phase after centrifugation was taken into account for the calculation of Γ, and, therefore, the Γ obtained represents only the values for the fraction of fat globules that were separated using centrifugation.

TABLE 1. Protein load (Γ) and mean diameter for the volume-surface (d_{vs}) in milk under different experimental conditions.

Variable	d_{vs}	Γ
	(μm)	(mg/m^2)
Pressure		
30 MPa	0.45[a]	6.12[c]
60 MPa	0.25[b]	9.79[b]
90 MPa	0.19[c]	11.88[a]
Temperature		
65°C	0.35[a]	9.69[a]
85°C	0.25[b]	8.84[b]
Fat concentration		
1.5%	0.28[a]	9.24[a]
3%	0.32[a]	9.28[a]

[a,b,c]Means within a column for each variable without a common superscript differ ($P < 0.05$).

Table 1 shows the statistical analysis of Γ and d_{vs} of homogenized milk under different experimental conditions. The Γ increased as homogenization pressure increased but decreased as heat treatment increased. The d_{vs} of homogenized milk decreased as homogenization pressure and heat treatment increased. An inverse relationship existed between Γ and the milk fat globule size. Walstra and Oortwijn (26) used collision theory to predict that larger casein micelles were preferentially adsorbed over small micelles, and this preference was more pronounced for small fat globules, which caused the Γ for the smaller fat globules to be increased. Sharma et al. (21) also reported that Γ increased as fat globule size decreased. Those researchers obtained Γ of 6.56 and 8.14 mg/m^2 for d_{vs} of 0.48 and 0.30 μm, respectively, for milk with 4.0% milk fat homogenized at 17 MPa and 52°C. These values were similar to the Γ of 6.12 mg/m^2 that were obtained in this experiment for milk homogenized at 30 MPa, which had a d_{vs} of 0.45 μm. The Γ on the fat globule surfaces increased significantly at the higher homogenization pressures. The Γ for samples with d_{vs} of 0.25 and 0.19 μm was 9.79 and 11.88 mg/m^2, respectively, for milk homogenized at 60 and 90 MPa (Table 1). Tomas et al. (24) homogenized milk at 35 MPa and 52°C and obtained Γ of 11 mg/m^2 in milk emulsions when the fat to protein mass ratio was <4 and Γ of 6.6 mg/m^2 for the fat to protein mass ratio was 13. Oortwijn and Walstra (18) obtained a Γ of about 9 mg/m^2 with milk emulsions that had been homogenized at 10 MPa and 40°C with fat to protein mass ratios between 4 and 10. These values were higher than the values obtained in this experiment and those reported by Sharma et al. (21).

TABLE 2. Percentage distribution of proteins in the milk fat globule membrane (MFGM) of homogenized milk under different experimental conditions.

Variable	Native membrane	Caseins	β-LG	α-LA
Pressure				
30 MPa	13.14^{ab}	67.57^{a}	6.80^{a}	4.76^{a}
60 MPa	12.61^{b}	69.34^{a}	6.32^{a}	4.76^{a}
90 MPa	13.49^{a}	66.74^{a}	6.87^{a}	4.90^{a}
Temperature				
65°C	12.33^{b}	68.02^{a}	5.10^{b}	4.13^{b}
85°C	13.83^{a}	67.74^{a}	8.22^{a}	5.48^{a}
Fat concentration				
1.5%	14.21^{a}	68.30^{a}	6.11^{a}	4.71^{a}
3.0%	11.95^{b}	67.47^{a}	7.21^{a}	4.90^{a}
Reducing conditions				
No	16.06^{a}	65.20^{b}	4.77^{b}	4.63^{a}
Yes	10.10^{b}	70.57^{a}	8.56^{a}	4.98^{a}

[a,b]Means within a column for each variable without a common superscript differ ($P < 0.05$).

The fat to protein ratios used in this experiment (0.47 and 0.94) did not affect the amount of Γ or d_{vs} (Table 1). Oortwijn and Walstra (18) reported that Γ was little affected by the fat to protein ratio until the amount of protein was insufficient to coat the surface of the lipid properly. The milk fat to protein ratios used in this case were <1, and the amount of protein was apparently sufficient to coat the fat globules properly at all homogenization pressure conditions.

Heat treatment affected Γ (Table 1) and is related to denaturation of proteins and the higher kinetic energy associated with increased temperature.

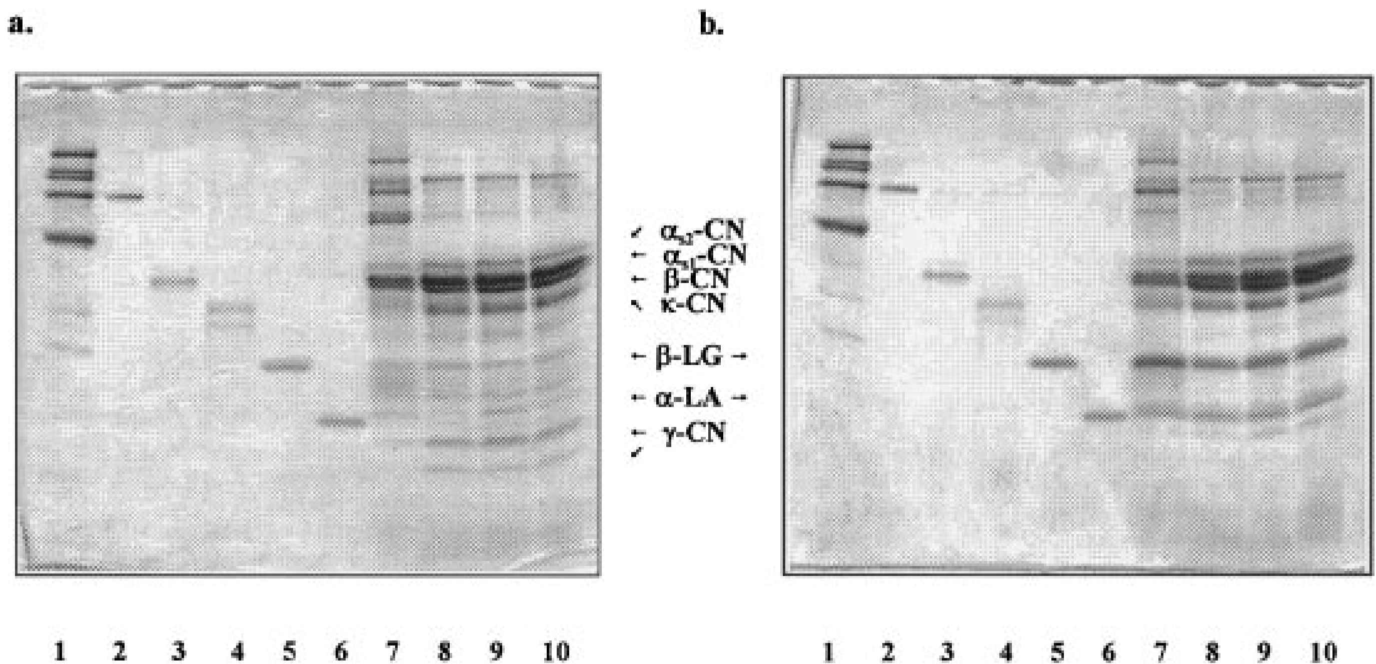

Figure 1. The SDS-PAGE of milk fat globule membrane (MFGM) proteins of milk with 3.0% fat separated using 18% acrylamide gels and reducing conditions. a. Lane 1, molecular mass markers of 200, 116, 97.4, 66.2, and 39.2 kDa (top to bottom); lane 2, BSA standard; lane 3, α_s-CN standard; lane 4, κ-CN standard; lane 5, β-LG standard; lane 6, α-LA standard; and lanes 7 to 10, MFGM proteins of milk with 3.0% fat, heated at 65°C for 30 min and homogenized at 0, 30, 60, and 90 MPa. b. Lane 1, molecular mass markers: 200, 116, 97.4, 66.2, and 39.2 kDa (top to bottom); lane 2, BSA standard; lane 3, α_s-CN standard; lane 4, κ-CN standard; lane 5, β-LG standard; lane 6, α-LA standard; and lanes 7 to 10, MFGM proteins of milk with 3.0% fat, heated at 85°C for 20 min, and homogenized at 0, 30, 60, and 90 MPa.

Caseins apparently are adsorbed at the OW interface as casein micelles in homogenized milk (5, 10, 21, 25). High heat treatments can cause whey proteins to associate with casein micelles via the formation of disulfide groups between denatured whey proteins and the caseins, especially κ-CN (22, 25), which would increase the total amount of protein absorbed if casein molecules were absorbed as intact casein micelles. However, Γ decreased as the heat treatment of the milk increased (Table 1). High heat treatment could cause direct adsorption of denatured β-LG and α-LA (19) that do not interact with the κ-CN during homogenization at the OW interface, limiting the surface available for casein micelles to be adsorbed and therefore decreasing the total amount of protein load. Also, the disintegration of casein micelles into submicelles and α-CN and β-CN monomers by high homogenization pressures might possibly have decreased the total amount of protein that was adsorbed at the fat globule surfaces. Sharma et al. (21), using electron microscopy, found that the milk fat globule surfaces were covered with intact casein micelles and that partial spreading of casein micelles occurred. Those researchers also observed smaller particles, which probably resulted from disintegration of casein micelles, and a thin layer of protein, which was probably whey proteins. Also, the higher homogenization temperature and the resultant higher kinetic energy may have caused more spreading of the casein micelles over the OW interface (25).

Composition of the MFGM Proteins

Differences in the composition of the MFGM proteins were not visible among homogenization pressures of 30, 60, or 90 MPa within heat treatments or in milks with different concentrations of milk fat. However, differences in the concentration of whey proteins were observed between heat treatments of 65 and 85°C (Figure 1) and between reducing and nonreducing conditions of electrophoresis. Bands for proteins with low molecular mass in the region of 20 to 11 kDa were observed in the gels of the MFGM proteins of milk samples heated at 65°C under nonreducing and reducing conditions, but these bands were not present in the gels of milk samples heated at 85°C. The mobility of these bands approximately corresponds to the γ fraction of caseins (molecular masses of 20.5, 11.8, and 11.6 kDa), which might be a result of plasmin activity on β-CN. This proteolytic enzyme is associated with casein micelles and could have survived the heat treatment at 65°C, but not at 85°C (25), and be associated with the casein micelles on the milk fat globule surface. This would explain the presence of the γ-CN in the gels of MFGM proteins of milk with lower heat treatment, but not in the gels of MFGM proteins of milk with the higher heat treatment. In gels of MFGM proteins of milk heated at 85°C and under reducing electrophoresis conditions (Figure 1b), the density of the bands of α-LA and β-LG increased compared with the density of the bands of these proteins in milk heated at 65°C (Figure 1a).

The percentage distribution of the proteins forming the MFGM in milk after homogenization is shown in Table 2. These values represent the composition of the membrane proteins of the fraction of the fat globules that were separated under the experimental conditions. There were no significant differences in the relative amounts of different plasma proteins adsorbed in the OW interface because of homogenization pressure or milk fat concentration. However, significant differences were found that were due to heat treatment and reducing conditions during electrophoresis.

Caseins were adsorbed preferentially over whey proteins at the OW interface and represented about 70% of the proteins in the MFGM after homogenization (Table 2). Several authors (18, 21, 22, 25) have reported preferential adsorption of caseins over whey proteins at the OW interface in homogenized milk. Sharma et al. (21) reported that caseins constituted approximately 90% of the MFGM proteins in recombined milk. In recombined milk, milk fat is emulsified into skim milk, and the MFGM material is formed only by serum proteins. In contrast, in this experiment, cream was combined with skim milk, and milk fat globules retained some of the native membrane proteins. Approximately 10% of the proteins in the MFGM formed during homogenization were proteins from the native membrane (Table 2). Keenan et al. (10) found that about 10% of the surface of homogenized fat globules in milk was covered by their natural membranes. Differences in the concentration of caseins were only observed after their concentrations were determined using reducing and nonreducing electrophoresis (Table 2). This result indicated that some caseins interacted with original membrane proteins, whey proteins, or both. These interactions were probably disulfide linkages because the addition of β-mercaptoethanol caused the increase in the percentage of caseins in the membrane. Keenan et al. (10) found that casein micelles are associated with membrane-like material at the interface between fat globules and the serum phase.

Homogenization pressure did not affect the percentage of the proteins from the native membrane

TABLE 3. Percentage distribution of individual casein proteins in the milk fat globule membrane (MFGM) of homogenized milk under different experimental conditions.

Variable	α_{s2}-CN	α_{s1}-CN	β-CN	κ-CN
Pressure				
30 MPa	7.98^{a}	26.30^{a}	23.36^{a}	9.93^{a}
60 MPa	8.49^{a}	27.50^{a}	24.40^{a}	8.96^{a}
90 MPa	7.18^{a}	26.80^{a}	24.44^{a}	8.31^{a}
Temperature				
65°C	7.50^{a}	27.46^{a}	23.29^{a}	9.78^{a}
85°C	8.27^{a}	26.28^{a}	24.84^{a}	8.35^{a}
Fat concentration				
1.5%	8.08^{a}	26.48^{a}	24.30^{a}	9.43^{a}
3.0%	7.68^{a}	27.25^{a}	23.84^{a}	8.70^{a}
Reducing conditions				
No	6.83^{b}	26.84^{a}	24.58^{a}	6.94^{b}
Yes	8.94^{a}	26.89^{a}	23.55^{a}	11.19^{a}

^{a,b}Means within a column for each variable without a common superscript differ (P < 0.05).

(molecular masses between 50 and 150 kDa) that was associated with the milk fat globules after homogenization, but differences in the distribution of these proteins were found that were due to heat treatment of the milk, milk fat concentration, and electrophoresis procedure (Table 2). The effects of temperature and the changes observed under different electrophoresis conditions on the distribution of native membrane proteins can be explained by interactions of the native membrane proteins with plasma proteins. More of the high molecular mass bands (50 to 150 kDa) were found at 85 than at 65°C and when the protein distribution was determined using nonreducing conditions. When β-mercaptoethanol was added prior to electrophoresis, the percentage of these proteins decreased, which indicated that these proteins interacted with plasma proteins and caused an apparent increase in the percentage of these proteins. Plasma proteins can form complexes with native membrane proteins by formation of disulfide linkages and appear to increase the relative amount of native membrane proteins with high molecular masses in the MFGM formed after homogenization. Houlihan et al. (8) found that it was possible for heat-denatured serum proteins to interact with native membrane proteins.

The percentages of α-LA and β-LG in the MFGM were affected by temperature, as was observed using the gels obtained under nonreducing and reducing conditions of electrophoresis (Table 2). However, homogenization pressure or milk fat concentration did not affect the relative concentration of these proteins in the MFGM of homogenized milk. Heat treatment of the milk to 85°C caused an increase in α-LA and β-LG as part of the MFGM after homogenization compared with the percentage of these proteins in milk heated at 65°C (Table 2). When the concentrations of α-LA and β-LG were determined using reducing conditions (Table 2), the amount of β-LG forming part of the MFGM was higher than under nonreducing electrophoresis conditions (Figure 2), indicating that an interaction occurred between this protein and the caseins and native proteins from the original MFGM. The amount of α-LA that was associated with the membrane did not change whether analyzed by reducing or by nonreducing electrophoresis conditions. However, an interaction between temperature and electrophoresis conditions was found for α-LA. The percentage of α-LA decreased as the temperature increased from 65 to 85°C when the proteins were de-

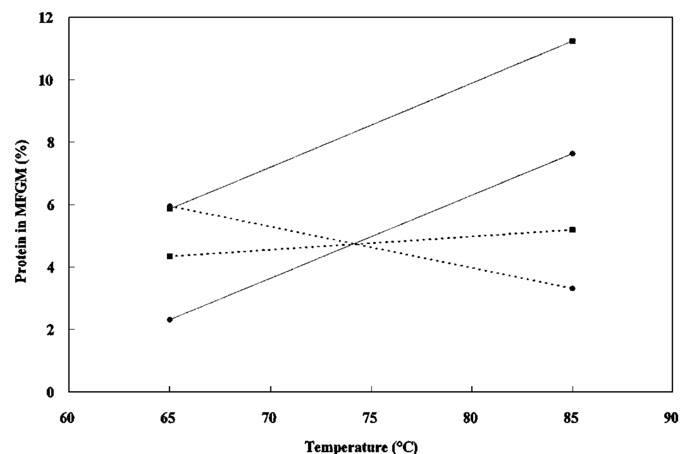

Figure 2. Interaction between temperature and reducing and nonreducing electrophoretic conditions on the percentage of β-LG and α-LA in the milk fat globule membrane (MFGM) of homogenized milk; β-LG, reducing (—■—); β-LG, nonreducing (---■---); α-LA, reducing (—●—); and α-LA, nonreducing (---●---).

TABLE 4. Comparison of ratios of plasma proteins in unhomogenized milk serum to the ratios of plasma proteins in the milk fat globule membrane in milk homogenized at 65 and 85°C.

Ratios	Unhomogenized milk serum[1]	Homogenized milk[2]	
		65°C	85°C
α_{s1}-CN/β-CN	1.08	1.23	1.05
α_{s2}-CN/β-CN	0.28	0.35	0.41
α_{s1}-CN/α_{s2}-CN	3.85	3.55	2.54
κ-CN/β-CN	0.35	0.53	0.42
α-LA/β-LG	0.38	0.39	0.68
Caseins/WP[3]	5.81	9.09	3.54

[1]Walstra and Jenness (26).

[2]From this work under reducing conditions during electrophoresis.

[3]Whey protein (α-LA and β-LG).

termined using nonreducing electrophoresis conditions (Figure 2). When α-LA was determined using reducing electrophoresis conditions, its percentage on the MFGM proteins increased as the heat treatment increased to 85°C (Figure 2).

The statistical analysis of the percentage distribution of the individual caseins forming the MFGM in homogenized milk is shown in Table 3. The percentages of α_{s1}-CN and β-CN did not change whether the composition of the membrane was determined using reducing or nonreducing electrophoresis conditions, but the percentages of α_{s2}-CN and κ-CN increased when they were determined using reducing conditions. Sharma et al. (21) used dissociating agents and found that the dissociation of κ-CN from the fat globule surface increased from 25 to 50% with the addition of β-mercaptoethanol, and the dissociation of β-LG slightly increased under these conditions.

The distribution of caseins and whey proteins in the MFGM of homogenized milk was compared with the distribution of these proteins in the serum phase of unhomogenized milk; the percentage of individual proteins in the membrane was determined using the sum of the plasma proteins in the MFGM during reducing conditions as 100%. Ratios of plasma proteins in the MFGM of homogenized milk at 65 or 85°C that were calculated from the electrophoresis data under reducing conditions and the ratios of these proteins in unhomogenized milk are presented in Table 4. The ratio of total casein to α-LA and β-LG increased from 5.81 in unhomogenized milk to 9.09 in milk that had been homogenized at 65°C, indicating preferential adsorption of caseins over whey proteins after homogenization at 65°C. Sharma et al. (21) found a casein to whey protein ratio of 8.83 in milk homogenized at 17 MPa and 52°C. The ratio of casein to whey protein decreased to 3.54 when the heat treatment was at 85°C. The ratio of α-LA to β-LG in the MFGM of milk homogenized after heated treatment at 65°C was the same as in unhomogenized milk, but the ratio of these two proteins increased to 0.68 in the MFGM of milk heated at 85°C and then homogenized, indicating preferential adsorption of α-LA over β-LG in the OW interface. Sharma and Dalgleish (22) reported a ratio of α-LA to β-LG of 0.60 in the MFGM of milk that was heated to 70 to 75°C after homogenization.

CONCLUSIONS

The SDS-PAGE analysis of the proteins in the MFGM of homogenized milk suggested two mechanisms for the adsorption of plasma proteins at the fat globule surface that were dependent on the heat treatment of the milk prior to homogenization. Heat treatment at 65°C did not significantly denature whey proteins, and caseins were adsorbed preferentially over whey proteins at the OW interface. Heat treatment of milk at 85°C denatured α-LA and β-LG to the extent that these proteins could form disulfide linkages with other proteins. The β-LG mainly was attached to κ-CN in the casein micelles or to other unidentified protein, and these complexes were adsorbed at the OW interface during homogenization. Because the Γ decreased when the heat treatment of the milk increased from 65 to 85°C, even when β-LG was attached to the casein micelles, the binding site between the fat globule surface and the plasma proteins possibly was through the β-LG and κ-CN complex; spreading of this complex was an additional possibility. Also, the decrease in Γ as heating temperature increased could have been the result of spreading of the plasma proteins at the OW interface because of the higher kinetic energy of the system. At 85°C, the adsorption of α-LA and β-LG directly at the fat surface would limit the available surface for adsorption of caseins, which would reduce the ratio of caseins to whey proteins in the MFGM of homogenized milk.

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