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Changes in the surface protein of the fat globules during homogenization and heat treatment of concentrated milk

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The changes in milk fat globules and fat globule surface proteins of both low-preheated and high-preheated concentrated milks, which were homogenized at low or high pressure, were examined. The average fat globule size decreased with increasing homogenization pressure. The total surface protein (mg m^{-2}) of concentrated milk increased after homogenization, the extent of the increase being dependent on the temperature and the pressure of homogenization, as well as on the preheat treatment. The concentrates obtained from high-preheated milks had higher surface protein concentration than the concentrates obtained from low-preheated milks after homogenization. Concentrated milks heat treated at 79 °C either before or after homogenization had greater amounts of fat globule surface protein than concentrated milks heat treated at 50 or 65 °C. This was attributed to the association of whey protein with the native MFGM (milk fat globule membrane) proteins and the adsorbed skim milk proteins. Also, at the same homogenization temperature and pressure, the amount of whey protein on the fat globule surface of the concentrated milk that was heated after homogenization was greater than that of the concentrated milk that was heated before homogenization. The amounts of the major native MFGM proteins did not change during homogenization, indicating that the skim milk proteins did not displace the native MFGM proteins but adsorbed on to the newly formed surface.

Keywords: Concentrated milks, homogenization, milk fat globules, milk fat globule surface protein, milk fat globule membrane (MFGM), caseins, whey proteins.

The composition of the fat globule surface layer makes a significant contribution to the physical properties of many dairy products (Anderson et al. 1977; McCrae & Muir, 1991; McKenna et al. 1999). The composition of this surface layer differs largely because of different processing treatments. An increase in the homogenization pressure increases the protein load on the fat globule surface but the protein load decreases as the temperature of homogenization increases (Walstra, 1995; Cano-Ruiz & Richter, 1997). However, the protein load is higher when heated milk (>80 °C) is homogenized (Walstra, 1995), because casein micelles may aggregate into large particles during heating (Dalgleish et al. 1987; Mohammad & Fox, 1987; Singh & Creamer, 1991).

Most of the previous work on the influence of homogenization conditions on fat globules has been carried out on whole milk. No information on highly concentrated milks (>45%) is available. Homogenization of concentrated milk either before or after heating is carried out in the manufacture of whole milk powder to reduce the amount of free fat in the powder and to improve its reconstitution properties (Oldfield & Singh, 2005). The changes in the fat globule surface proteins, as a result of the differences in the conditions of homogenization, have been suggested to influence the reconstitution properties of whole milk powder in water (Mol, 1975; McKenna et al. 1999).

Our previous work has shown that changes in the fat globules and the fat globule surface proteins occur during the preheat treatment and evaporation (Ye et al. 2004a, b). Preheat treatment by direct steam injection (DSI) caused a decrease in the fat globule size and an increase in the surface protein concentration to ~1·8 mg m^{-2} because of adsorption of casein micelles at the fat globule surface and association of whey proteins with the milk fat globule membrane (MFGM). In subsequent evaporation, the size of the milk fat globules decreased whereas the amount of total surface proteins on the fat globules increased as the milk passed through each effect of the evaporator. The major original MFGM proteins, xanthine oxidase, butyrophilin,

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PAS 6 and PAS 7, did not change during evaporation; however, PAS 6 and PAS 7 decreased during preheating. The objective of this work was to examine the effects of homogenization conditions (temperature, pressure and placement of the homogenization step, i.e. before or after heat treatment) on the milk fat globules and the surface proteins of both low-preheated and high-preheated concentrated milks in the manufacture of whole milk powder.

Materials and methods

Materials

Bulked whole milk, obtained from the Fonterra Co-operative Group, New Zealand, was standardized to 34 g fat, 32 g protein, 48 g lactose, 119 g total solids (TS) and 85 g solids-non-fat l^{-1} in the pilot plant at Fonterra, Palmerston North, New Zealand. The milk was pasteurized at 72 °C for 15 s before further processing.

All the chemicals used were of analytical grade and were obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St. Louis, MO, USA) unless specified otherwise.

Preheating and concentration of milk

Samples of bulked whole milk and standardized milk were collected for analysis. Preheating was carried out using a two-stage DSI process to bring the milk to the desired temperature (70 °C for low preheating and 95 °C for high preheating). The milk was then passed through holding tubes (residence time 20 s) to the vacuum vessel, where it was flash cooled to approximately 70 °C prior to entry into the first stage of the evaporator.

For each process run, about 1750 l standardized milk was passed through a three-effect, pilot-scale, falling film evaporator (Wiegand, Karlsruhe, Germany) with a nominal evaporative capacity of 1600 l/h. The temperature of the milk in the first, second and third effects was approximately 70, 63 and 51 °C respectively. The milk was evaporated to approximately 50% TS.

Homogenization and heat treatment of the concentrates

The concentrated milk samples obtained after evaporation were homogenized in a single-stage homogenizer using a pressure of either 4 or 7 MPa at 50, 65 or 79 °C. In some experiments, the samples, homogenized at 50 °C, were heated to 65 or 79 °C in a heat exchanger (no holding time) and then cooled immediately to about 30 °C in a water bath before further analysis.

Determination of average fat globule size and specific surface area of fat globules in the concentrated milk

A Malvern MasterSizer MSE (Malvern Instruments Ltd, Worcestershire, England) was used to determine the fat globule size distribution, as described by Ye et al. (2002). The concentrated milks were dispersed in 20 g SDS/l and 50 mM-EDTA solution (pH 6·8) to dissociate the casein micelles and any aggregation of fat globules.

Isolation and analysis of milk fat globule surface material

Milk fat globule surface material was isolated from standardized milk and diluted concentrated milk by centrifugation at 15 000 ***g*** for 20 min at 20 °C, as described by Ye et al. (2002). The total protein content and the fat content of the cream samples were determined using the Kjeldahl method (AOAC, 1974) and the Mojonnier method (IDF, 1987) respectively.

The individual proteins in the washed cream were determined by polyacrylamide gel electrophoresis (PAGE), as described by Ye et al. (2002). The proteins of the MFGM were identified by comparison with results reported previously (Keenan & Dylewski, 1995; Mather, 2000).

Transmission electron microscopy

The method used was that described by McKenna et al. (1999). The sample was mixed with warm melted (35–40 °C) 3% low-temperature gelling agarose in a 1 : 1 ratio and was further treated and analysed as described by McKenna et al. (1999).

Statistical analysis

All experiments were carried out at least twice. The results were analysed statistically using the Minitab 12 for Windows package (Minitab Inc., State College, PA, USA). Analysis of variance (ANOVA) was used to determine if the means of responses were significant ($P<0·05$).

Results

Heating the concentrate before homogenization

The average fat globule size (d_{43}) of concentrated milk decreased from ~1·80 μm to ~1·3 and ~1·1 μm after homogenization at 4 and 7 MPa respectively (Table 1). Homogenization of the concentrates caused an increase in the surface protein concentration from ~4 to ~6 mg m^{-2} at 4 MPa and to ~7 mg m^{-2} at 7 MPa in the low-preheated concentrates (Table 1). In the high-preheated concentrates, the surface protein concentration increased from ~6 to ~7 mg m^{-2} at 4 MPa and to ~8 mg m^{-2} at 7 MPa depending on the temperature of homogenization. The surface protein concentrations were higher when the concentrate was homogenized at 65 or 79 °C than when the concentrate was homogenized at 50 °C at both 4 and 7 MPa, but there was no significant difference between 65 and 79 °C. The surface protein concentrations of the high-preheated concentrates after homogenization were

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Table 1. Total surface protein concentration and composition of the milk fat globules in homogenized concentrates (TS ~ 49%) that were heated at different temperatures before homogenization

				Composition (%)			
Concentrate	Temperature (°C)	d_{43} (μm)	Surface protein coverage (mg m^{-2})	Caseins	β-Lactoglobulin	α-Lactalbumin	Others (including native MFGM proteins)
Low-preheated (70 °C) concentrate (non-homogenized)							
	50	1·84[a#§]	4·00[a]	78·39[a]	3·27[a]	0·56[a]	17·77[a]
	65	1·84[a]	3·91[a]	77·11[a]	3·95[a]	0·68[a]	18·26[a]
	79	1·84[a]	5·50[b]	78·47[a]	4·33[a]	0·68[a]	16·52[a]
Homogenized at 4 MPa							
	50	1·31[b]	4·96[c]	81·51[b]	5·18[b]	1·18[b]	12·13[b]
	65	1·39[b]	5·71[b]	84·97[b]	3·21[a]	0·57[a]	11·24[b]
	79	1·28[b]	5·97[b]	83·56[b]	4·20[a]	1·47[b]	10·77[b]
Homogenized at 7 MPa							
	50	1·07[c]	5·53[b]	84·91[b]	3·30[a]	0·77[a]	11·01[b]
	65	1·16[c]	6·99[d]	86·54[b]	2·39[a]	0·68[a]	10·38[b]
	79	1·09[c]	6·60[d]	84·99[b]	3·86[a]	0·57[a]	10·57[b]
High-preheated (95 °C, 20 s) concentrate (non-homogenized)							
	50	1·77[a]	5·90[b]	67·69[c]	15·39[c]	2·55[c]	14·37[c]
	65	1·77[a]	6·08[b]	66·53[c]	14·92[c]	3·11[c]	15·54[c]
	79	1·77[a]	7·20[d]	65·01[c]	16·40[c]	5·22[d]	13·55[c]
Homogenized at 4 MPa							
	50	1·34[b]	6·70[d]	67·32[c]	19·59[d]	2·97[c]	10·12[b]
	65	1·30[b]	7·01[d]	69·99[c]	17·31[d]	3·29[c]	9·42[d]
	79	1·31[b]	7·10[d]	65·08[c]	17·18[d]	7·82[e]	9·91[d]
Homogenized at 7 MPa							
	50	1·12[c]	6·89[d]	68·24[c]	18·68[d]	3·19[c]	9·89[d]
	65	1·13[c]	7·98[e]	72·20[c]	16·14[c]	2·40[c]	9·27[d]
	79	1·07[c]	7·82[e]	68·79[c]	15·60[c]	6·28[e]	9·32[d]

Different superscripts within a column indicate significant differences ($P < 0·05$)
§ Data are means calculated from two samples that were produced from the raw milk

1–2 mg m^{-2} higher than those of the low-preheated concentrates in most cases (Table 1).

The protein composition of the fat globule surface, obtained from quantitative analysis of the SDS-PAGE patterns, showed no significant differences in the surface protein composition of low-preheated concentrates before and after homogenization at a given temperature, except for a decrease in the native MFGM proteins (Table 1). Changes in the homogenization pressure (4 or 7 MPa) and temperature (50, 65 or 79 °C) did not significantly affect the relative proportions of the surface proteins. When the low-preheated concentrates were homogenized, caseins constituted approximately 85% of the total surface protein and <5% of the surface protein was whey proteins (Table 1). However, when the high-preheated concentrates were homogenized, the proportion of whey proteins at the fat globule surface increased to >20% (Table 1). Higher proportions of α-lactalbumin (α-la) (~7·0%) were found at the surface of high-preheated concentrates that were homogenized at 79 °C than at the surface of high-preheated concentrates that were homogenized at 50 and 65 °C (Table 1).

Heating the concentrate after homogenization

No significant change in the surface protein concentration was observed when both low-preheated and high-preheated concentrates were heated to 65 °C after homogenization (Table 2). However, heating at 79 °C after homogenization caused an increase (about 1 mg m^{-2}) in the surface protein concentration for both low-preheated concentrates and high-preheated concentrates.

Comparison of the surface protein concentrations in the concentrate that was heated at 79 °C before (Table 1) or after (Table 2) homogenization at 7 MPa showed that the surface protein concentrations were significantly higher in the concentrates that were heated after homogenization. This result indicated the association of whey proteins with casein micelles that were already adsorbed at the surface. Sharma & Dalgleish (1993) also reported that the amounts of whey proteins present in the fat globule membrane were greater in milk that had been homogenized and then heated than in milk that had been heated and then homogenized.

The proportions of β-lactoglobulin (β-lg) and α-la in the low-preheated concentrates heated at 79 °C after

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Table 2. Total surface protein concentration and composition of the milk fat globules in homogenized concentrates (TS ~ 49%) that were heated at different temperatures after homogenization

	Temperature (°C)	Surface protein coverage (mg m^{-2})	Composition (%) Caseins	β-Lactoglobulin	α-Lactalbumin	Others (including native MFGM proteins)
Low-preheated (70 °C) concentrate						
Homogenized at 4 MPa	50	4·96[a#§]	81·51[a]	5·18[a]	1·18[a]	12·13[a]
	65	4·77[a]	83·08[a]	4·57[a]	0·914[a]	11·42[a]
	79	5·76[b]	81·51[a]	5·37[a]	1·19[a]	11·93[a]
Homogenized at 7 MPa	50	5·53[b]	84·91[a]	3·30[b]	0·77[a]	11·01[a]
	65	6·09[c]	85·03[a]	2·95[b]	0·68[a]	11·33[a]
	79	7·81[d]	77·18[b]	9·24[c]	2·45[b]	11·14[a]
High-preheated (95 °C, 20 s) concentrate						
Homogenized at 4 MPa	50	6·70[e]	67·32[c]	19·59[d]	2·97[b]	10·12[b]
	65	6·96[e]	69·83[c]	18·02[d]	2·56[b]	9·60[b]
	79	7·68[d]	70·36[c]	14·98[d]	4·96[c]	9·70[b]
Homogenized at 7 MPa	50	6·89[e]	68·24[c]	18·68[d]	3·19[b]	9·89[b]
	65	6·98[e]	69·42[c]	17·30[d]	3·24[b]	10·05[b]
	79	8·15[f]	66·91[c]	17·02[d]	6·55[d]	9·51[b]

Different superscripts within a column indicate significant differences ($P < 0·05$)
§ Data are means calculated from two samples that were produced from the raw milk

homogenization (Table 2) were higher than those in the low-preheated concentrates heated at 79 °C before homogenization (Table 1). For example, the proportion of β-lg in the low-preheated concentrates heated at 79 °C after homogenization at 7 MPa was ~9·0% (Table 2) compared with ~4% in the low-preheated concentrates heated at 79 °C before homogenization at 7 MPa (Table 1). However, the proportions of β-lg and α-la in the high-preheated concentrates heated after homogenization (Table 2) were similar to those in the high-preheated concentrates heated before homogenization (Table 1).

Cream washed with a dissociating buffer

When the isolated MFGM material (cream) was washed in urea and ETDA buffer, the casein micelles adsorbed at the fat globule surface were dissociated and washed away. The protein molecules adsorbed directly at the interface of fat globules and the protein molecules bound to the interfacial protein layer via covalent bonds remained on the surface of the fat globules (Ye et al. 2004a). The SDS-PAGE patterns of the washed cream samples are shown in Fig. 1. Among the caseins, κ-casein was present in high concentrations in the SDS-PAGE patterns of homogenized concentrates. This indicated that only κ-casein from the casein micelle directly adsorbed on to the fat globules, and that other casein molecules (α_{s1}-, α_{s2}-caseins and β-casein) were not directly adsorbed. The intensity of κ-casein increased in the concentrates homogenized at 7 MPa (Fig. 1). In addition, homogenization at 7 MPa at 79 °C further enhanced the intensities of the κ-casein, β-lg and α-la bands (Fig. 1B).

Changes in the homogenization pressure and temperature did not affect the intensity of the bands of the major native MFGM proteins, including xanthine oxidase, butyrophilin, PAS 6 and PAS 7, in the low-preheated concentrates (Fig. 1A). However, the PAS 6 and PAS 7 bands became very faint in the high-preheated concentrates (Fig. 1B). Furthermore, the SDS-PAGE patterns showed a protein band with a molecular weight (Mr) of ~75 kDa and a faint band with an Mr of ~58 kDa located between butyrophilin (Mr 66 kDa) and PAS 6 (Mr 50 kDa) in both low-preheated and high-preheated homogenized concentrates (Fig. 1). The intensity of these two bands in the homogenized concentrates was greater than that in the concentrate before homogenization and it increased with homogenization pressure from 4 to 7 MPa. The intensities of these bands were fairly similar in the low-preheated and high-preheated concentrates (Fig. 1). This suggested that these proteins were directly adsorbed at the fat globule surface during homogenization.

Electron microscopy

Transmission electron micrographs of both low-preheated and high-preheated concentrates homogenized at 7 MPa showed that casein-micelle-like particles were adsorbed at the fat globule surface (Fig. 2), although not all of the fat globule surface was covered by casein. In the concentrates homogenized at 79 °C or heated to 79 °C after homogenization, many adsorbed micelles were joined with the adjacent micelles in the serum to form chains. In the high-preheated homogenized concentrates, it was observed that the thread-like material between the casein micelles was

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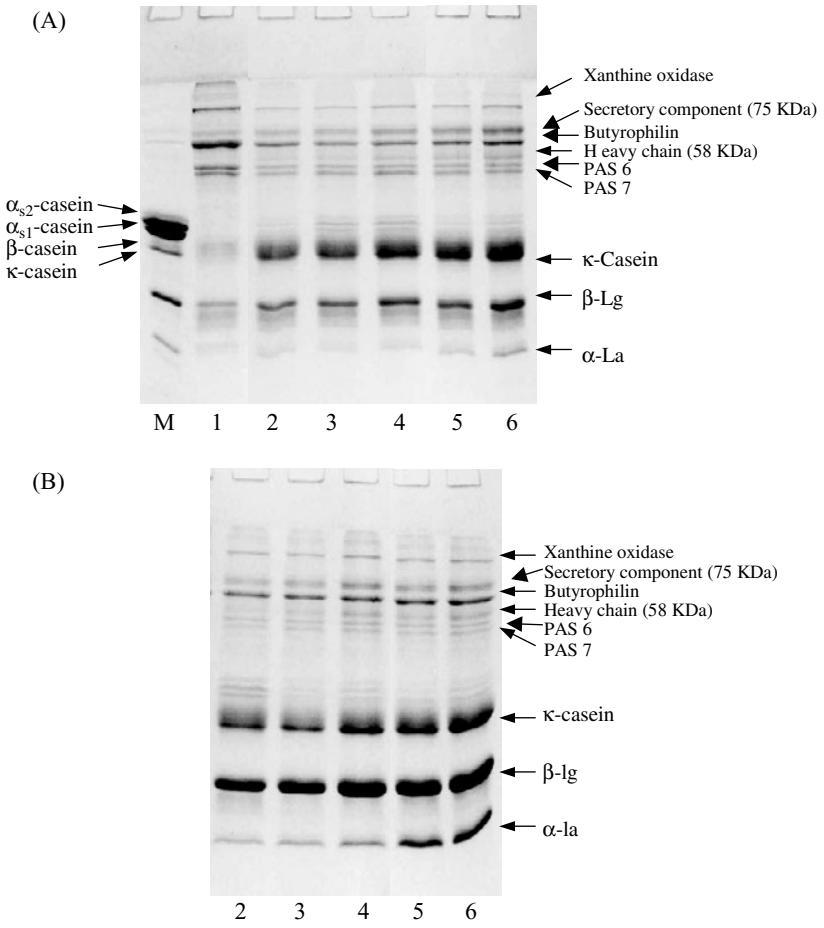

Fig. 1. SDS-PAGE patterns (15% acrylamide gel), under reducing conditions, of fat globule surface material isolated from low-preheated (70 °C) (A) and high-preheated (95 °C, 20 s) (B) concentrates before or after homogenization at different pressures and temperatures. Lane 1, standard milk; lane 2, concentrate; lane 3, concentrate homogenized at 4 MPa and 50 °C; lane 4, concentrate homogenized at 7 MPa and 50 °C; lane 5, concentrate homogenized at 4 MPa and 79 °C; lane 6, concentrate homogenized at 7 MPa and 79 °C; M, whole milk.

involved in the formation of these micelle chains (Figs. 2C, 2D), indicating that the non-micellar material between the casein micelles may be involved in the formation of these casein micelle chains. Hair-like structures have been observed on the surface of casein micelles in heated milks (Davies et al. 1978; Mohammed & Fox, 1987) and have been described as κ-casein/β-lg complexes (Singh & Fox, 1987).

In the low-preheated homogenized concentrates, the amount of thread-like material between the casein micelles was much less although chains of casein micelles were still observed, particularly at the fat globule surface; these chains appeared to be the micelles in contact with each other (Figs. 2A, 2B). McKenna et al. (1999) also reported that the micelles adsorbed at the fat globule surface aggregated with adjacent micelles, and became more electron dense, suggesting that there was some change in the structure of the micelles as they adsorbed to the fat globule surface.

Discussion

Any heat treatments during processing, including preheating of the raw milk, and heating before and after homogenization, result in the association of whey proteins or whey protein/casein micelle complexes with the fat globule surface proteins and subsequently lead to an increase in the total fat globule surface protein concentration (Dalgleish & Banks, 1991; Houlihan et al. 1992; Ye et al. 2004a). During high-temperature preheating, serum proteins associate with the native MFGM proteins and the casein micelles via disulfide bonds; these casein micelle/serum protein complexes are subsequently adsorbed on to the surface of the fat globules during homogenization, resulting in greater amounts of surface serum proteins and greater total surface protein concentrations (Tables 1 and 2). During preheating, only about 1·2 mg whey protein/g fat associates with the native MFGM (Ye et al. 2004b). This amount of whey protein is relatively small compared with

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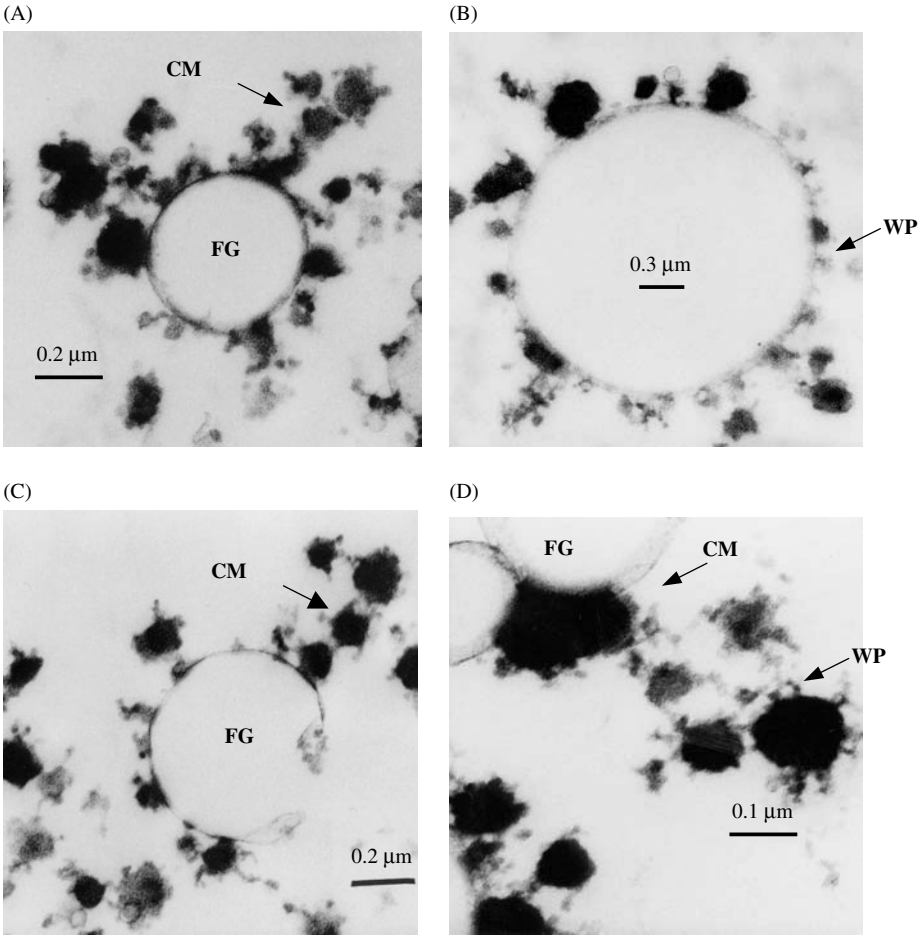

Fig. 2. Transmission electron micrographs of low-preheated (70 °C) (A, B) and high-preheated (95 °C, 20 s) (C, D) concentrates homogenized at 7 MPa and 79 °C (A, C), and concentrates homogenized at 7 MPa and 50 °C and then heated to 79 °C (B, D). FG: fat globules, CM: casein micelles, WP: whey protein. Magnification: × 48 600.

the total surface protein (~60 mg g^{-1} fat or 6–8 mg m^{-2}) after homogenization, in which whey protein constitutes about 12 mg g^{-1} fat. This effect arises mainly because of the adsorption of the casein micelle/whey protein complexes formed during preheating, including casein micelle aggregate chains linked by whey proteins (Fig. 2).

A higher surface protein concentration in the concentrate homogenized at high temperature than in the concentrate homogenized at low temperature was observed in the present study. This result is different from the observations on normal milk, in which the protein load decreases upon homogenization at high temperature (Oortwijn & Walstra, 1979) because the adsorbed casein micelles spread over the fat–water interface and the spreading rate appears to increase with temperature (Walstra, 1995). However, during homogenization of the concentrate, a high temperature may cause more adsorbed casein micelles to aggregate with adjacent micelles in the serum (Fig. 2). When the temperature is higher than the temperature of whey protein denaturation, the associations of whey proteins with the surface also result in an increase in the surface protein.

The results (Fig. 1) for the protein composition of the surface material, washed in the dissociation buffer, also showed an increase in the amount of surface β-lg and α-la after homogenization, indicating that the directly adsorbed κ-casein was likely to be present as κ-casein/serum protein complexes. This suggested that the association of β-lg and α-la with κ-casein induced by high-temperature preheating did not influence the adsorption of κ-casein on to the fat globule surface during homogenization.

Further increases in the total surface protein concentration and the surface serum proteins (Table 2) were observed when the heat treatment was carried out after homogenization, indicating that further interaction between the adsorbed micelles (low preheated) or adsorbed micelle/whey protein complexes (high preheated) and whey proteins or micelle/whey protein complexes could take place. During the heat treatment of homogenized concentrates, β-lg bound to casein micelles may have further associated with the β-lg that had already bound at the fat globule surface as a result of heating and adsorption. This also caused an increase in the amount of casein at the fat

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globule surface. Meanwhile, α-la was associated with the fat globule surface together with the casein micelles that had already associated with β-lg during preheating (95 °C).

The amounts of β-lg and α-la associated with adsorbed κ-casein, induced by heating of the concentrates after homogenization, were higher than those of the concentrates heated before homogenization (Tables 1 and 2). This result suggests that the association of whey proteins with adsorbed casein micelles via disulphide bonds during heat treatment after homogenization could not be inhibited by the adsorption of casein micelles during homogenization. This result is in agreement with observations in normal milk (without concentration) by Sharma & Dalgleish (1993), who showed that casein micelles can associate with more serum proteins after adsorption at the fat globule surface. Sharma & Dalgleish (1994) suggested that more binding sites for the serum proteins become available as the casein micelles are spread over the fat surface rather than being in their native configuration.

In the present work, the SDS-PAGE analysis of the surface protein material washed with urea and EDTA showed the proteins adsorbed directly at the surface. Only κ-casein directly adsorbed at the surface when casein micelles were adsorbed at the fat globule surface (Fig. 1). This phenomenon did not change when casein micelles spread over the surface at higher homogenization pressure. Homogenization at higher pressure and temperature led to more κ-casein directly adsorbing at the surface (Fig. 1). This may be attributed to more casein micelles adsorbed at the surface and broadly spreading over the surface of the fat globule. This implies that the casein micelles are not disintegrated during homogenization and consequently are adsorbed on to the fat interface as ''intact'' micelles. However, results reported by previous workers (Anderson et al. 1977; Darling & Butcher, 1978; Sharma et al. 1996) show that a number of casein monomers or sub-units of micelles are also present at the fat interface of homogenized cream or recombined milk.

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References

Anderson M, Cheeseman GC & Wiles R 1977 Extending shelf life of UHT creams. *Journal of the Society of Dairy Technology* **30** 229–232

AOAC 1974 *Official Methods of Analysis of the Association of Official Analytical Chemists*, 12th Edition. AOAC: Washington DC

Cano-Ruiz ME & Richter RL 1997 Effect of homogenization pressure on the milk fat globule membrane proteins. *Journal of Dairy Science* **80** 2732–2739

Dalgleish DG & Banks JM 1991 The formation of complexes between serum proteins and fat globules during heating of whole milk. *Milchwissenschaft* **46** 75–78

Dalgleish DG, Pouliot Y & Paquin P 1987 Studies on the heat stability of milk. II. Association and dissociation of particles and the effects of added urea. *Journal of Dairy Research* **54** 39–49

Darling DF & Butcher DW 1978 Milk fat globule membrane in homogenized cream. *Journal of Dairy Research* **45** 197–208

Davies FL, Shankar PA, Brooker BE & Hobbs DG 1978 A heat-induced change in the ultrastructure of milk and its effect on gel formation in yoghurt. *Journal of Dairy Research* **45** 53–58

Houlihan AV, Goddard PA, Nottingham SM, Kitchen BJ & Masters CJ 1992 Interactions between the bovine milk fat globule membrane and skim milk components on heating whole milk. *Journal of Dairy Research* **59** 187–195

IDF 1987 Cream. Determination of fat content (Rose Gottlieb gravimetric method). IDF Standard 16C. International Dairy Federation: Brussels

Keenan TW & Dylewski DP 1995 Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. In *Advanced Dairy Chemistry*. Volume 2: *Lipids*, pp. 89–130 (Ed. PF Fox). Chapman & Hall: London

Mather IH 2000 A review and proposed nomenclature for major proteins of the milk-fat globule membrane. *Journal of Dairy Science* **83** 203–247

McCrae CH & Muir DD 1991 Effect of surface protein concentration on the heat stability of systems containing homogenized fat globules from recombined milk. *International Dairy Journal* **1** 89–100

McKenna AB, Lloyd RJ, Munro PA & Singh H 1999 Microstructure of whole milk powder and of insoluble detected by powder functional testing. *Scanning* **21** 305–315

Mohammad KS & Fox PF 1987 Heat-induced microstructural changes in casein micelles before and after heat coagulation. *New Zealand Journal of Dairy Science and Technology* **22** 191–203

Mol JJ 1975 The milk fat globule and the solubility of whole milk powder. *Netherlands Milk and Dairy journal* **29** 221–224

Oldfield D & Singh H 2005 Functional properties of milk powders. In *Encapsulated and Powdered Foods*, pp. 366–383 (Ed. C Onwulata). Taylor & Francis: Boca Raton, FL

Oortwijn H & Walstra P 1979 The membranes of recombined fat globules. II. Composition. *Netherlands Milk and Dairy Journal* **33** 134–154

Sharma R, Singh H & Taylor MW 1996 Recombined milk: factors affecting the protein coverage and composition of fat globule surface layers. *Australian Journal of Dairy Technology* **51** 12–16

Sharma SK & Dalgleish DG 1993 Interactions between milk serum proteins and synthetic fat globule membrane during heating of homogenized whole milk. *Journal of Agricultural and Food Chemistry* **41** 1407–1412

Sharma SK & Dalgleish DG 1994 Effect of heat treatments on the incorporation of milk serum proteins into the fat globule membrane of homogenized milk. *Journal of Dairy Research* **61** 375–384

Singh H & Creamer LK 1991 Denaturation, aggregation and heat stability of milk protein during the manufacture of skim milk powder. *Journal of Dairy Research* **58** 269–283

Singh H & Fox PF 1987 Heat stability of milk: influence of colloidal and soluble salts and protein modification on the pH-dependent dissociation of micellar κ-casein. *Journal of Dairy Research* **54** 523–534

Walstra P 1995 Physical chemistry of milk fat globules. In *Advanced Dairy Chemistry*. Volume 2: *Lipids*, pp. 131–171 (Ed. PF Fox). Chapman & Hall: London

Ye A, Singh H, Taylor MW & Anema S 2002 Characterization of protein components of natural and heat-treated milk fat globule membranes. *International Dairy Journal* **12** 393–402

Ye A, Singh H, Taylor MW & Anema S 2004a Interactions of fat globule surface proteins during concentration of whole milk in a pilot-scale multiple-effect evaporator. *Journal of Dairy Research* **71** 471–479

Ye A, Singh H, Oldfield DJ & Anema S 2004b Kinetics of heat-induced association of b-lactoglobulin and a-lactalbumin with milk fat globule membrane in whole milk. *International Dairy Journal* **14** 389–398