ELSEVIER

Food Chemistry 69 (2000) 237–244

Food Chemistry

www.elsevier.com/locate/foodchem

Influence of NaCl addition on the properties of emulsions formed with commercial calcium caseinate

Aiqian Ye, Magesh Srinivasan[1], Harjinder Singh*

Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand

Received 17 July 1999; received in revised form 16 September 1999; accepted 16 September 1999

Abstract

Oil-in-water emulsions containing 30% soya oil, calcium caseinate and various NaCl concentrations were prepared in a two-stage homogeniser. The average volume-surface diameter (d_{32}) of emulsion droplets decreased with increasing NaCl from 0 to 20 mM, but remained constant beyond 20 mM. The surface protein concentration (mg/m^2) decreased with NaCl addition up to 50 mM, but increased with increasing NaCl concentration above 50 mM. α_s-(α_{s1}-+α_{s2}-)Casein adsorbed at the droplet surface in preference to β-casein in emulsions made both with and without NaCl. In emulsions made with $<2\%$ calcium caseinate, the droplet size distributions were broad and bimodal, but they became narrow when 50 or 150 mM NaCl was added prior to emulsion formation. The changes in creaming stability were consistent with the droplet sizes in emulsions. It appears that the aggregated protein in calcium caseinate dispersion was dissociated by NaCl, which consequently improved its emulsifying properties.

Keywords: Adsorption; Calcium caseinate; Emulsion; NaCl

1. Introduction

Large amounts of caseinates, especially sodium caseinate and calcium caseinate, are used as ingredients by the food industry. The properties of caseinates are influenced by the method of manufacture, cation type, and the properties of the food system in which they are incorporated (Morr, 1982). Caseinates provide fat emulsification in coffee creamers, whipped toppings, soups and meat emulsions and contribute to viscosity in various food products. Caseinate is a composite of four different proteins (α_{s1}-, α_{s2}-, β-, κ-caseins, in weight proportions of approximately 4:1:4:1) which are coprecipitated at pH 4.6. Various caseins show different adsorption behaviour at oil–water interfaces because of their different chemical or physico-chemical properties. β-Casein adsorbs most rapidly at oil-water interfaces and is most effective in decreasing the interfacial tension (Dickinson, 1989). In oil-in-water emulsions made with sodium caseinate, all of the casein types are rapidly adsorbed at the droplet surface and decrease the interfacial tension (Hunt & Dalgleish, 1994; Robson & Dalgleish, 1987; Srinivasan, Singh & Munro, 1996). This provides stability to the resultant emulsion with respect to coalescence and flocculation.

Most of the previous reports on the emulsifying properties and adsorption behaviour of caseinates at the oil/water interface have focused on sodium caseinate (Fang & Dalgleish, 1993; Galazka & Dickinson, 1995; Hunt & Dalgleish, 1994, 1996; Robson & Dalgleish, 1987; Srinivasan et al., 1996; Tornberg, 1978). Little information is available on the adsorption behaviour of calcium caseinate in oil-in-water emulsions (Mulvihill & Murphy, 1991). The effects of NaCl on the adsorption behaviour and stability of emulsions formed with calcium caseinate have not been reported.

Calcium caseinate is a highly aggregated caseinate as indicated by its turbidity and high levels of sedimentable protein (Lee, Anema, Schrader & Buchheim, 1996; Srinivasan, Singh & Munro, 1999). The emulsifying capacity and adsorption behaviour of proteins at the oil/water interface are affected significantly by the state of aggregation of protein (Mulvihill & Murphy, 1991; Oortwijn & Walstra, 1979; Singh, Fox & Cuddigan, 1993). Mulvihill and Murphy reported that the aggregated caseins/caseinates were less surface-active than the dispersed caseinates, and that the aggregated caseins/caseinates have higher surface protein loads in emulsions.

* Corresponding author. Tel.: +64-6-350-4401; fax: +64-6-350-5655.

[1] Current address: Dairy Product Technology Centre, California Polytechnic State University, San Luis ObisPo, CA 93407, USA.

E-mail address: h.singh@massey.ac.nz (H. Singh).

0308-8146/00/$ - see front matter
PII: S0308-8146(99)00253-8

The state of aggregation of protein in solution is dependent on pH, ionic strength and temperature (Swaisgood, 1992). Therefore, it would be expected that the aggregation state of protein in calcium caseinate solution would be influenced by addition of NaCl, which in turn would affect its adsorption behaviour in emulsions. In this report, the effects of addition of NaCl to calcium caseinate dispersions, on the formation and stability of oil-in-water emulsions, have been investigated. The relationship between the aggregation state of protein in calcium caseinate dispersions and the properties of emulsions is discussed.

2. Materials and methods

2.1. Materials

Calcium caseinate (Alanate 380) was obtained from the New Zealand Dairy Board, Wellington, New Zealand, it contained ~96% dry matter of which ~94% was proteins and 1.58% calcium and 0.07% sodium. Soya oil was purchased from Davis Trading Company, Palmerston North, New Zealand. All of the chemicals used were of analytical grade obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

2.2. Emulsion preparation

Emulsions were prepared using calcium caseinate dispersion and 30% soya oil. Calcium caseinate dispersions were prepared by adding calcium caseinate powder to Milli-Q water (water purified by reverse osmosis followed by treatment with a Milli-Q apparatus, Millipore Corp. Bedford, MA), and then stirring for 30 min at 50°C in a water bath to ensure complete dispersion. Different amounts of NaCl were added to this dispersion, and pH adjusted to 7.0 using 1 M NaOH or 1 M HCl. Appropriate quantities of soya oil (to give 30% oil in the final emulsion) were added to the caseinate dispersions, the mixture heated to 55°C and then homogenized in a two-stage valve homogenizer (Rannie a/s, Roholmsvej 8, DK 2620 Albertslund, Denmark) at 207 bar for the first stage and 34 bar for the second stage. Emulsions were prepared at least in triplicate.

2.3. Determination of average particle size and specific surface area

A Malvern MasterSizer MSE (Malvern Instruments Ltd, Worcestershire, UK) was used to determine the volume-surface average diameter (d_{32}) and specific surface area (area per unit mass). The parameters that were used to analyse the particle size distribution were defined by the presentation code 2NAD. Relative refractive index (N) was 1.095, i.e. the ratio of refractive index of emulsion particle (1.456) and that of the dispersion medium (1.33). The absorbance value of emulsion particle was 0.001.

2.4. Determination of surface protein concentration and composition

Emulsions were centrifuged at 45,000 *g* for 40 min at 20°C in a temperature-controlled centrifuge (Sorvall RC5C, DuPont Co., Wilmington, DE). The subnatants were carefully removed using a syringe. The cream layer was dispersed in deionized water and recentrifuged at 45,000 *g* for 40 min. The subnatant was filtered sequentially through 0.45 and 0.22 μm filters (Millipore Corp, Bedferd, MA). The filtrates were analysed separately for total protein using the Kjeldahl method (1026, Distilling Unit and 1007 Digestor Blorck, Tecator AB, Hoganas, Sweden). The sediment was removed and total protein was determined using the Kjeldahl method. The surface protein concentration (mg/m^2) was calculated from the surface area of the oil droplets, determined by MasterSizer, and the difference in the amount of protein used to prepare emulsion and that measured in the subnatants and sediment after centrifugation.

$$\text{Adsorbed protein (g)} = \text{total protein (g) taken for making an emulsion} - [\text{protein (g) present in the subnatant} + \text{protein (g) present in the sediment}]$$

$$\text{Surface protein concentration (mg/m}^2) = \frac{\text{total (mg) protein absorbed}}{\text{total fat surface area}}$$

The composition of the adsorbed protein at the surface of the emulsion droplets was determined using SDS-PAGE, as described by Srinivasan et al. (1996). A certain amount of cream was spread onto a filter paper and a known amount of dried cream was mixed with SDS buffer (0.5 M Tris, 2% SDS, 0.05% mercaptoethanol, pH 6.8). A portion (5 μl) of this dispersion was applied to the SDS gels previously prepared on a Miniprotein II system (Bio-Rad Laboratories, Richmond, CA). After destaining, the gels were scanned on a laser densitometer (LKB Ultroscan XL, LKB Produkter AB, Bromma, Sweden). The percentage composition of each sample was determined by scanning the areas for α_s-(α_{s1}-+α_{s2}-), β- and κ-caseins and expressing the individual casein peaks as a fraction of the sum total.

2.5. Creaming stability

Immediately after preparation, the emulsions (30 g) were transferred into the centrifuge tubes and maintained

at 20°C for 24 h. The samples were then centrifuged at 185 *g* for 15 min; a sample (5 g) from the lower phase was carefully removed using a syringe and analysed for fat content by the Mojonnier method. The stability rating was calculated as follows:

$$\text{Stability rating}\,(\%) = \frac{\text{fat in the lower phase}\,(\%)}{\text{fat in the original emulsion}\,(\%)} \times 100$$

2.6. Turbidity measurements

The turbidity of calcium caseinate dispersions was measured at 900 nm using an UV-160A Spectrophotometer (UV-visible recording spectrophotometer, Shimadzu Corp) and 2 mm quartz sample cell.

2.7. Sedimentation of calcium caseinate dispersions

The calcium caseinate dispersions (30 g) with added NaCl and adjusted to pH 7.0 were centrifuged at 10,000 *g* for 40 min at 20°C. Total protein in the supernatant was determined using the Kjeldahl method.

Analysis of 6 separate emulsions, made with 2.5% calcium caseinate and 30% soya oil, showed that the variations were ±0.02 μm for d_{32}, ~4% for surface protein concentration, ~6% for stability rating, ~4% for α_s-casein, ~5% for β-casein and ~7% for κ-casein.

3. Results

3.1. Addition of NaCl to 2.5% calcium caseinate dispersions

Various quantities of NaCl were added to 2.5% calcium caseinate dispersion prior to making emulsions. The changes in turbidity and protein sedimention (10,000 *g* for 40 min) are shown in Fig. 1. Turbidity (absorbance at 900 nm) of calcium caseinate dispersion decreased markedly with increase in NaCl concentration from 0 to 50 mM, but no further change in turbidity occurred beyond 50 mM. The changes in the amount of sedimentable protein showed a similar trend. These results indicate that the concentration of the large particles in calcium caseinate dispersion decreased with increasing NaCl concentration. Lee et al. (1996) also reported that the turbidity of calcium caseinate suspensions in water was reduced when NaCl was added to the system.

3.2. Effect of NaCl concentration on emulsions made with 2.5% calcium caseinate

The d_{32} of emulsion droplets decreased from 0.73 to 0.60 μm as the concentration of NaCl was increased from 0 to 20 mM, but the d_{32} remained constant at ~0.60 μm with further increase in NaCl concentration (Fig. 2). The particle size distributions of these emulsions are shown in Fig. 3. The distribution of particles shifted towards smaller particle sizes as the concentration of NaCl was increased from 0 to 20 mM. There was no further change in the size distribution profile as the NaCl concentration was increased beyond 20 mM.

Surface protein concentration (mg/m^2) of the emulsion droplets decreased from ~2.5 to ~2.1 mg/m^2 with increase in the concentration of NaCl from 0 to 50 mM (Fig. 2). This was followed by a gradual increase in

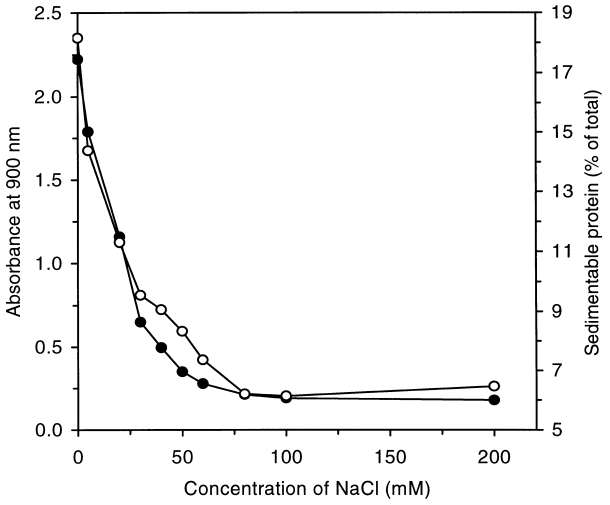

Fig. 1. Effect of addition of NaCl on the turbidity (absorbance at 900 nm) (●) and protein sedimentation (○) (10,000 *g* for 40 min) in 2.5% calcium caseinate dispersion.

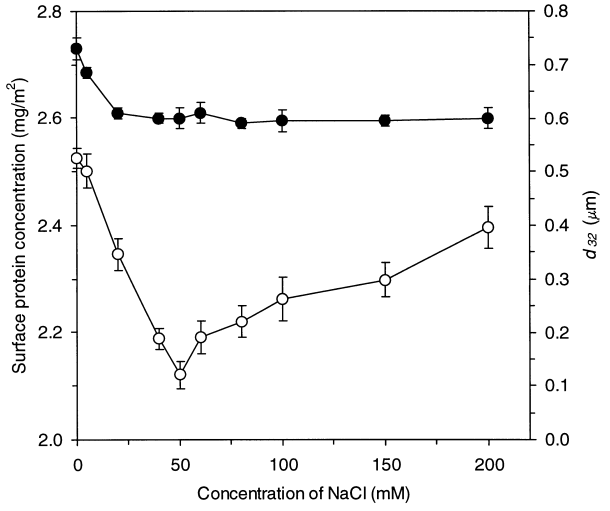

Fig. 2. Changes in the volume-surface average droplet diameter (d_{32}) (●) and surface protein concentration (mg/m^2) (○) of emulsions (30% soya oil, 2.5% calcium caseinate) as a function of NaCl concentration. NaCl was added to the calcium caseinate dispersion prior to emulsion formation. The data presented are average of four measurements and standard deviations are also shown.

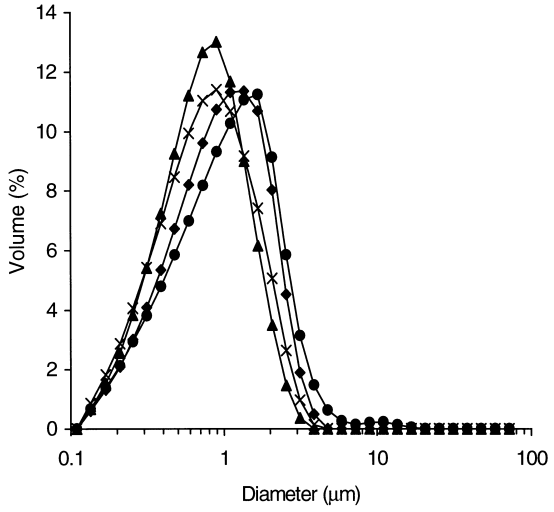

Fig. 3. Changes in particle size distribution of emulsions formed with 2.5% calcium caseinate, and containing (●) 0 mM, (□) 5 mM, (▲) 40 mM or (X) 100 mM NaCl. NaCl was added to the calcium caseinate dispersion prior to emulsion formation.

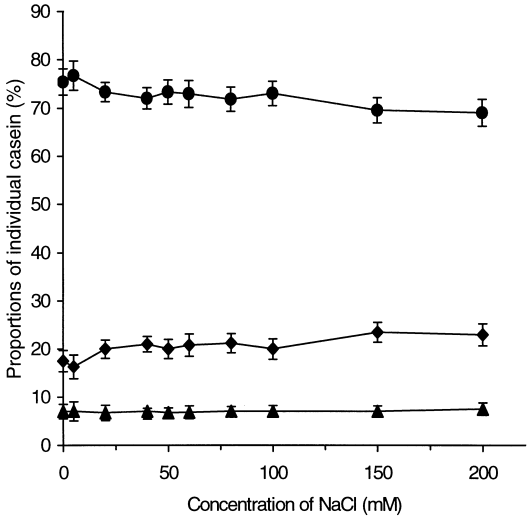

Fig. 4. Effect of addition of NaCl, prior to emulsion formation, on the proportions of (●) α_s-(α_{s1}-+α_{s2}-) casein, (□) β-casein and (▲) κ-casein at the droplet surface (cream phase) in emulsions. The data presented are average of four measurements and standard deviations are also shown.

surface protein concentration (from ~2.1 to ~2.4 mg/m^2) as the concentration of NaCl was increased from 50 to 200 mM (Fig. 2). The higher surface protein concentration (~2.5 mg/m^2) of emulsions made with 2.5% calcium caseinate (pH 7.0, 30% soya oil) compared with that of emulsions made with sodium caseinate under the same conditions (about 1.3 mg/m^2; Srinivasan et al., 1996) was probably due to aggregation of caseins in calcium caseinate dispersion. Mulvihill and Murphy (1991) also observed that emulsions formed with high-calcium caseinate had higher surface protein load than those formed with the less aggregated sodium and ammonium caseinates.

Fig. 4 shows the proportions of individual caseins in the adsorbed layer of the emulsions droplets (cream phase). The proportions of adsorbed α_s-(α_{s1}-+α_{s2})-casein decreased slightly (from ~75 to ~68%), with corresponding increases in the proportions of β-casein, as the concentrations of NaCl increased from 0 to 200 mM. The proportions of adsorbed κ-casein remained constant at about 7.0%. The proportions of individual caseins in the original calcium caseinate dispersion were: 46.5% α_s-casein, 42.4% β-casein and 11.1% κ-casein. This suggests that the α_s-casein (α_{s1}-+α_{s2}-) was adsorbed at the droplet surface in preference to β-casein, and there was a slight decrease in this preferential adsorption of α_s-casein with the addition of NaCl prior to emulsion formation.

The creaming stability of emulsions (expressed as stability rating) made with 2.5% calcium caseinate in the presence of different amounts of NaCl is shown in Fig. 5. The stability rating increased from ~52 to ~66% with increase in the concentration of NaCl from 0 to 50 mM, but no significant further increase was observed beyond 50 mM NaCl addition. This trend was similar to that observed for the changes in d_{32} of emulsions (Fig. 2) with the concentration of added NaCl.

The above results clearly showed that the emulsifying properties of calcium caseinate were sensitive to the presence of NaCl during emulsification. However, addition of NaCl (50–200 mM) to emulsions after they were formed had no significant effects on d_{32} values, surface protein concentration and composition, and stability rating of emulsions.

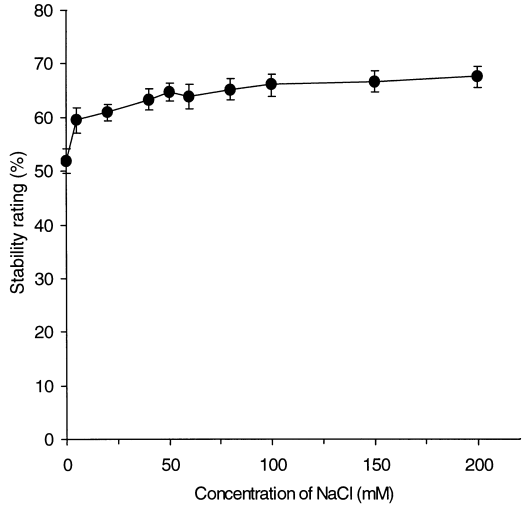

Fig. 5. Changes in the stability rating of emulsions made with 30% soya oil and 2.5% calcium caseinate at pH 7.0 as a function of NaCl concentration. NaCl was added to calcium caseinate solution prior to emulsion formation. The data presented are average of four measurements and standard deviations are also shown.

Further studies on the behaviour of emulsions made with different calcium caseinate concentrations, as influenced by NaCl added prior to emulsion formation, were carried out, in order to obtain an understanding of the properties of emulsions containing sufficient or insufficient protein when NaCl was added.

3.3. Effect of NaCl on emulsions made with various concentrations of calcium caseinate

Fig. 6 shows the d_{32} of emulsion droplets made with various concentrations of calcium caseinate (0.5–5.0%) in the absence or presence of NaCl (50–150 mM). The d_{32} of emulsions made without NaCl decreased with increasing concentration of calcium caseinate, the decrease being greater when protein concentration was increased from 0.5 to 2.0%. Addition of NaCl at 50 or 150 mM caused a decrease in the d_{32} values, particularly in emulsions made with low caseinate concentrations. There was no significant difference between the d_{32} values for emulsions containing 50 and 150 mM NaCl.

The droplet size distributions of emulsions made with 0.5 and 1.0% calcium caseinate in the absence of NaCl were broad with bimodal characteristics (Fig. 7). However, the droplet size distribution became monomodal when the concentration of protein was increased beyond 2.0% (Fig. 7). When the above emulsions were diluted with 0.02% EDTA and 2% SDS solution, the size distribution of droplets became narrower; the effect was more pronounced at lower caseinate concentrations (results not shown). As the dilution of emulsion with EDTA and SDS displaces the protein from the surface and dissociates any large protein aggregates, these results indicate that, at a low concentration of calcium caseinate, bridging flocculation may occur between emulsion droplets. When 150 mM NaCl was added to calcium caseinate dispersions prior to emulsion formation, the monomodal distributions were observed in all emulsions, indicating that there was no flocculation in emulsions made with calcium caseinate in the presence of 150 mM NaCl (Fig. 7B).

The surface protein concentrations in emulsions made with various concentrations of calcium caseinate (0.5–5.0%), in the absence or presence of NaCl (50 or 150 mM), are shown in Fig. 8. The surface protein concentration increased almost linearly from 1.1 to 4.4 mg/m^2 with increase in the concentration of calcium caseinate in emulsions made in the absence of NaCl. In

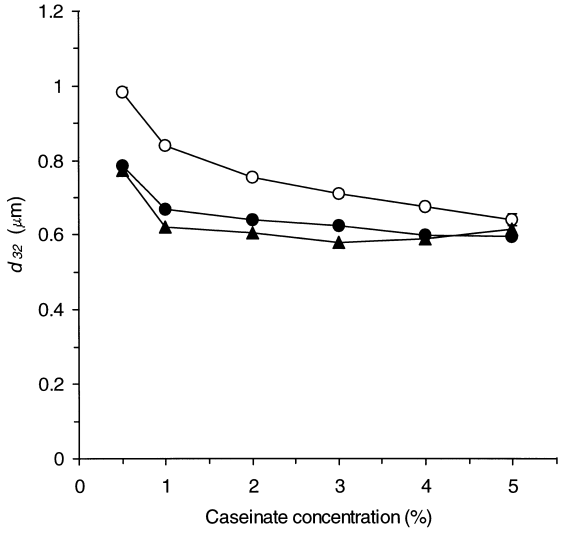

Fig. 6. Changes in volume-surface average diameter (d_{32}) of emulsions (30% soya oil, pH 7.0) as a function of concentration of calcium caseinate. Emulsions made in the absence of NaCl (○), or in the presence of 50 mM (●) or 150 mM (▲) NaCl. NaCl was added to the calcium caseinate solution prior to emulsion formation. Each data point is an average of three determinations on separate emulsions.

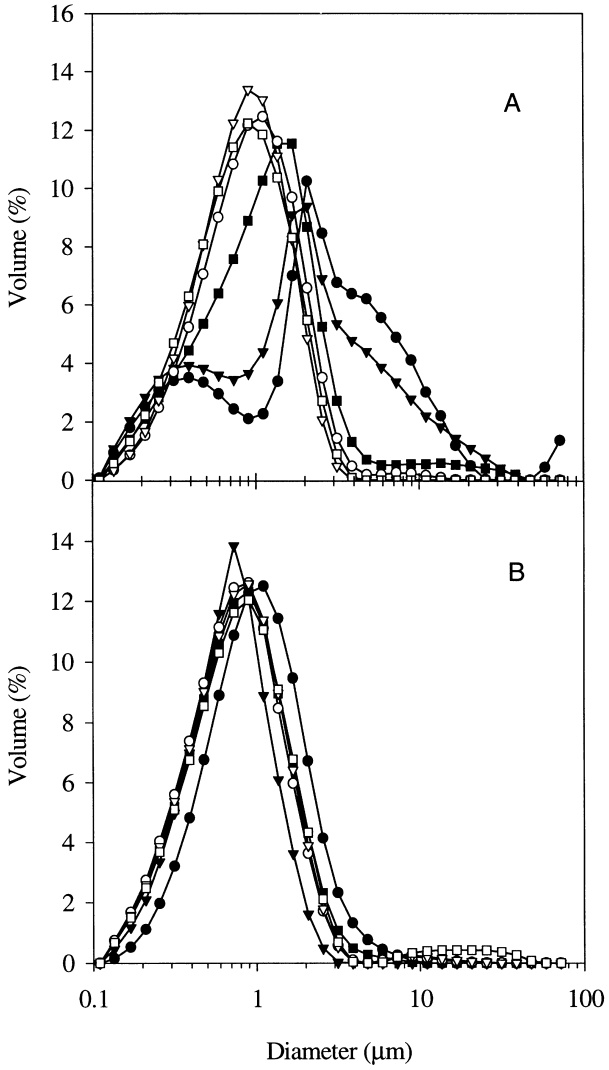

Fig. 7. Particle size distributions in emulsions (30% soya oil, pH 7.0) made with (●) 0.5%, (▲) 1.0%, (■) 2.0%, (○) 3.0%, (△) 4.0% or (□) 5.0% calcium caseinate, in the absence (A) or presence of 150 mM NaCl (B). NaCl was added to the calcium caseinate solution prior to emulsion formation.

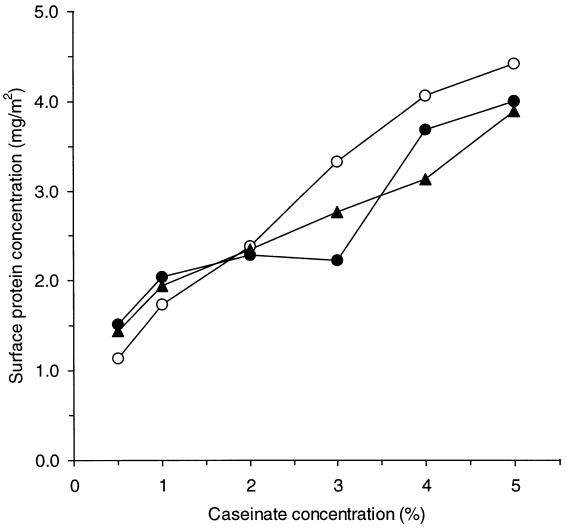

Fig. 8. Changes in surface protein concentration (mg/m^2) of emulsions (30% soya oil, pH 7.0) as a function of calcium caseinate concentration. Emulsions made in the absence of NaCl (○), or in the presence of 50 mM (●) or 150 mM (▲) NaCl. NaCl was added to the calcium caseinate solution prior to emulsion formation. Each data point is an average of three determinations on separate emulsions.

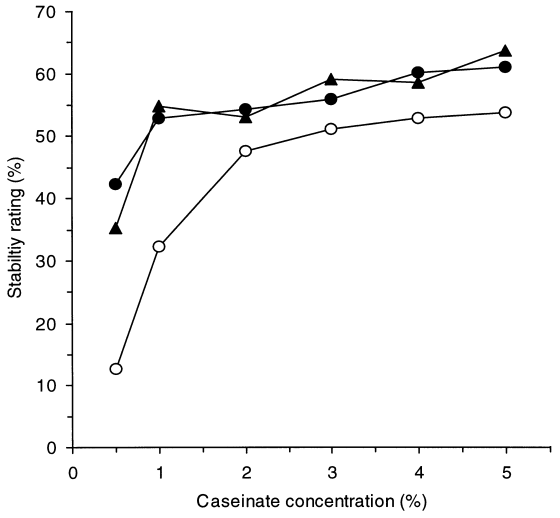

Fig. 9. Changes in stability rating of emulsions (30% soya oil, pH 7.0) as a function of calcium caseinate concentration. Emulsions made in the absence of NaCl (○), or in the presence of 50 mM (●) or 150 mM (▲) NaCl. NaCl was added to the calcium caseinate solution prior to emulsion formation. Each data point is an average of three determinations on separate emulsions.

contrast, the surface protein concentrations in sodium caseinate-stabilized emulsions have been shown to reach a plateau value, corresponding to a monolayer adsorption (Hunt & Dalgleish, 1994; Srinivasan et al., 1996).

The surface protein concentrations in emulsions made with 0.5 and 1.0% protein with added NaCl (50 or 150 mM) were slightly higher than the emulsions made in the absence of NaCl; there was no difference between 50 and 150 mM NaCl. The surface protein concentration in emulsions made with 2.0% protein was unaffected by the addition of 50 or 150 mM NaCl. In contrast, the surface protein concentrations in emulsions made with $>2\%$ caseinate decreased by the addition of NaCl.

In emulsions made in the absence of NaCl, the creaming stability increased markedly with an increase in calcium caseinate concentration from 0.5 to 3.0%, but no further changes occurred beyond 3.0% (Fig. 9). The presence of 50 or 150 mM NaCl enhanced the stability of all emulsions, especially those made with 0.5 and 1.0% caseinate. The presence of NaCl (50 or 150 mM) caused the emulsion, made with 1.0% caseinate, to have stability rating (55%) that was close to that of emulsions made with high caseinate concentrations. There was no significant difference in the creaming stability of emulsions containing 50 and 150 mM NaCl, except in the case of emulsions made with 0.5% caseinate (Fig. 9).

4. Discussion

The present results clearly showed that the caseins were aggregated to a large extent in calcium caseinate dispersions, in agreement with the results of Mulvihill and Murphy (1991) and Srinivasan et al. (1999). Addition of up to 50 mM NaCl, to 2.5% calcium caseinate, resulted in a marked reduction in the extent of casein aggregation (Fig. 1). As the solubility of α_{s1}-,α_{s2}- and β-caseins is strongly affected by the binding of calcium ions (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981), it is likely that the binding of Ca^{2+} to the caseins was reduced with the addition of NaCl. Binding of Ca^{2+} to the caseins at neutral pH reduces their negative charge, which diminishes the electrostatic repulsions between the molecules. In this way, calcium binding promotes hydrophobic interactions which can lead to increasing association and ultimately to precipitation (Swaisgood, 1992). The strength of binding of Ca^{2+} for α_{s1}- and β-caseins decreases by increasing ionic strength (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981). In addition, the decrease in aggregation could also be attributed to the competition by Na^+ for specific sites on the phosphoserine groups of caseins, thereby resulting in the formation of sodium caseinate (Gaucheron, Legraet, Boyaval & Piot, 1997). Gaucheron et al. (1997) reported that addition of NaCl in the range 0–0.12 M to the caseinate, containing 1.5 mM Ca^{2+}, caused an increase in the concentration of free Ca^{2+}, suggesting that Na^+ can be exchanged with bound cations in the caseinate.

It was found that the surface protein concentration in emulsions decreased with NaCl addition (from 0 to 50 mM) prior to emulsification (Fig. 2). This may be due to dissociation of calcium caseinate aggregates into smaller casein complexes or monomers and their subsequent adsorption at the droplet surface. The average particle

size (d_{32}) results (Fig. 2), which showed a decrease with increasing NaCl concentration up to 20 mM, appear to support this view. The emulsions stabilized by highly aggregated proteins generally have lower fat surface areas in the same power input range (Mulvihill & Murphy, 1991). Previous works (Agboola & Dalgleish, 1995; Dickinson, Hunt & Horne, 1992; Srinivasan et al., 1996) have shown that addition of calcium to sodium caseinate increased the particle size of oil-in-water emulsions because of Ca^{2+} induced aggregation of caseins.

The effect of the dissociation of aggregated caseins, in calcium caseinate dispersion, on the droplet size was more apparent in emulsions made with a relatively low concentration of caseinate. For example, the broad particle size distribution of emulsions, made with 1.0% caseinate, indicated that insufficient quantities of protein were available to form a stable emulsion; insufficient surfactant concentration causes droplets to coalesce or flocculate to reduce interfacial area during homogenisation. However, the size distributions of droplets of these emulsions became narrow when NaCl (50 or 150 mM) was added prior to emulsion formation. It is suggested that the casein complexes and monomers formed as a result of dissociation were more flexible and were able to spread efficiently around the surface of oil droplets to fully cover the interface, hence preventing flocculation/coalescence during homogenisation.

It is not immediately apparent why addition of $\geqslant 50$ mM NaCl caused a steady increase in surface protein concentration (Fig. 2). It is possible that the dissociated caseins aggregated again at high ionic strength, but this aggregation was not related to Ca^{2+}. Most of the aggregates, related to binding of Ca^{2+} to caseins, were probably dissociated at $\sim$50 mM NaCl, but self-association of caseins may have occurred at higher concentrations of NaCl. In aqueous solutions, the caseins have a strong tendency to associate due to their high hydrophobicity and peculiar charge distribution. The self-association properties of α_{s1}- and α_{s2}- caseins are very dependent on the ionic strength due to their amphipathic and highly charged structures (Swaisgood, 1992). α_{s1}-Casein exhibits progressive consecutive self-association to dimers, tetramers, hexamers, etc. with the degree of association being strongly dependent upon the pH and ionic strength of the solution. Unlike α_s-caseins, the association of β-casein involves the hydrophobic domain to form the core of the polymer micelle, and the interaction is predominantly hydrophobic (Swaisgood, 1992). Therefore, it is likely that aggregates of α_s-casein were formed in the caseinate dispersion under the high ionic strength conditions (i.e. >50 mM NaCl) and that the adsorption of these aggregates resulted in an increase in surface protein concentrations.

Alternatively, increasing the ionic strength may reduce the electrostatic repulsion between the adsorbed film and arriving molecules, thereby increasing the rate of adsorption and consequently increasing the proportions of protein particles irreversibly adsorbed at the interface. More compact packing of protein molecules at the interface is also facilitated at higher ionic strength (Tornberg, 1978). This would also cause an increase in surface protein concentration with increase in NaCl concentration.

The interfacial composition of sodium caseinate-stabilized emulsions has been reported by several workers (Dickinson, 1989; Euston, Singh, Munro & Dalgleish, 1995; Hunt & Dalgleish, 1994, 1996; Robson & Dalgleish, 1987; Srinivasan et al., 1996). β-Casein is adsorbed in preference to α_{s1}-casein in emulsions stabilized by a mixture of these caseins because it is most surface-active and hydrophobic (Dickinson, 1989). However, Robson and Dalgleish demonstrated no preferential adsorption of β-casein in sodium caseinate-stabilized emulsions immediately after homogenization, although on aging, β-casein displaced some of the adsorbed α_{s1}-casein. Hunt and Dalgleish (1994) found no preference for β-casein or α_{s1}-casein in sodium caseinate-stabilized emulsions. Srinivasan et al. (1996) reported that the preferential adsorption of β-casein in sodium caseinate was dependent on the concentration of protein used in making emulsions. At low protein concentration, β-casein was adsorbed in preference to α_s-casein, whereas a larger amount of α_s-casein than β-casein was present at the interface at high protein concentrations. These results suggest that the preferential adsorption of β-casein was easily diminished, possibly because caseins in sodium caseinate are aggregated to some extent. It is possible that β-casein in calcium caseinate dispersion existed in an aggregated state (probably related to Ca^{2+}-induced aggregation), which resulted in a loss of its flexibility and surface-activity. α_{s1}-Casein adsorbed in preference to β-casein because the large casein aggregates, which contain relatively high proportions of α_s-casein (Srinivasan et al., 1999), were adsorbed in preference at the oil-water interface during homogenisation (Walstra & Oortwijn, 1982).

As the extent of aggregation, due to binding of Ca^{2+} to caseins, decreased with increase in the concentration of NaCl, β-casein would be expected to exhibit some increase in surface activity. However, the results shown in Fig. 4 indicate only a small increase in β-casein adsorption. It is likely that β-casein adsorption was inhibited at high ionic strengths. Hunt and Dalgleish (1996) reported that in emulsions made with 2.0 % sodium caseinate, 20% oil at pH 7.0, the proportion of α_{s1}-casein increased markedly with a corresponding decrease in the proportion of β-casein when increasing the concentration of KCl above 25 mM; the percentages of κ- and α_{s2}-caseins in the adsorbed protein remained constant as the concentration of KCl was increased up to 200 mmol/dm^3. They suggested that aggregation of

β-casein, as the ionic strength was increased, caused it to become less surface active, and α_{s1}-casein was less affected by the presence of KCl.

The creaming stability appeared to be determined by the size and the extent of flocculation of droplets in emulsion in the present study. Emulsions made with low calcium caseinate concentrations (< 2%) showed protein bridging flocculation, which resulted in very low creaming stability. Emulsions made with sufficient protein (> 2%) (Fig. 6) or in the presence of NaCl (Fig. 2) showed no bridging flocculation and the droplet size decreased slightly with increase in caseinate concentration. Consequently the creaming stability of these emulsions improved (Figs. 5 and 9). The trends in surface protein concentration did not appear to relate to that in creaming stability.

References

Agboola, S. O., & Dalgleish, D. G. (1995). Calcium-induced destabilization of oil-in-water emulsions stabilized by caseinate or by β-lactoglobulin. *Journal of Food Science, 60*, 399–403.

Dalgleish, D. G., & Parker, T. G. (1980). Binding of calcium ions to bovine α_{s1}-casein and precipitability of the protein-calcium ion complexes. *Journal of Dairy Research, 47*, 113–122.

Dickinson, E. (1989). Surface and emulsifying properties of caseins. *Journal of Dairy Research, 56*, 471–477.

Dickinson, E., Hunt, J. A., & Horne, D. S. (1992). Calcium induced flocculation of emulsions containing adsorbed β-casein or phosvitin. *Food Hydrocolloids, 6*, 359–370.

Euston, S. E., Singh, H., Munro, P. A., & Dalgleish, D. G. (1995). Competitive adsorption between sodium caseinate and oil-soluble and water-soluble surfactants in oil-in-water emulsions. *Journal of Food Science, 60*, 1124–1131.

Fang, Y., & Dalgleish, D. G. (1993). Dimensions of the adsorbed layers in oil-in-water emulsions stabilized by caseins. *Journal of Colloid and Interface Science, 156*, 329–334.

Galazka, V. B., & Dickinson, E. (1995). Surface properties of protein layers adsorbed from mixtures of gelatin with various caseins. *Journal of Texture Studies, 26*, 401–409.

Gaucheron, F., Legraet, Y., Boyaval, E., & Piot, M. (1997). Binding of cations to casein molecules: importance of physico-chemical conditions. *Milchwissenschaft, 52*, 322–327.

Hunt, J. A., & Dalgleish, D. G. (1994). Adsorption behaviour of whey protein isolate and caseinate in soya oil-water emulsions. *Food Hydrocolloids, 8*, 175–187.

Hunt, J. A., & Dalgleish, D. G. (1996). The effect of the presence of KCl on the adsorption behaviour of whey protein and caseinate in oil-in-water emulsions. *Food Hydrocolloids, 10*, 159–165.

Lee, S. K., Anema, S. G., Schrader, K., & Buchheim, W. (1996). Effect of high hydrostatic pressure on Ca-caseinate systems. *Milchwissenschaft, 51*, 17–21.

Morr, C. V. (1982). Functional properties of milk proteins, and their use as food ingredients, In P. F. Fox, *Developments in dairy chemistry* (Vol. 1, pp. 370–399). London: Elsevier Applied Science Publishers.

Mulvihill, D. M., & Murphy, P. C. (1991). Surface active and emulsifying properties of caseins/caseinates as influenced by state of aggregation. *International Dairy Journal, 1*, 13–37.

Oortwijn, H., & Walstra, P. (1979). The membrane of recombined fat globules. 2. Composition. *Netherlands Milk and Dairy Journal, 33*, 134–154.

Parker, T. G., & Dalgleish, D. G. (1981). Binding of calcium ions to bovine β-casein. *Journal of Dairy Research, 48*, 71–76.

Robson, E. W., & Dalgleish, D. G. (1987). Interfacial composition of sodium caseinate emulsions. *Journal of Food Science, 52*, 1694–1698.

Singh, H., Fox, P. F., & Cuddigan, M. (1993). Emulsifying properties of protein fractions prepared from heated milk. *Food Chemistry, 47*, 1–6.

Srinivasan, M., Singh, H., & Munro, P. A. (1996). Sodium caseinate-stabilized emulsions: factors affecting coverage and composition of surface proteins. *Journal of Agricultural and Food Chemistry, 44*, 3807–3811.

Srinivasan, M., Singh, H. & Munro, P. A. (1999). Adsorption behaviour of sodium and calcium caseinates in oil-in-water emulsions. *International Dairy Journal*, *9*, 337–341.

Swaisgood, H. E. (1992). Chemistry of the caseins. In P. F. Fox. *Advanced dairy chemistry* (Vol. 1, pp. 63–110). London: Elsevier Applied Science Publishers.

Tornberg, E. (1978). Functional characterization of protein-stabilized emulsion: emulsifying behaviour of proteins in a valve homogenizer. *Journal of Science of Food Agriculture, 29*, 867–879.

Walstra, P., & Oortwijn, H. (1982). The membranes of recombined fat globules. 3. Mode of formation. *Netherlands Milk and Dairy Journal, 36*, 103–107.