Interfacial composition and stability of sodium caseinate emulsions as influenced by calcium ions

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Received 28 July 2000; revised 23 October 2000; accepted 18 December 2000

Abstract

The changes in droplet size distribution, surface protein concentration and composition, microstructure and creaming stability were examined when CaCl$_2$ was incorporated into oil-in-water emulsions (30%, w/w, soya oil) made with 0.5 or 3%, w/w, sodium caseinate. The droplet size distribution of emulsions made with 0.5 or 3% was monomodal until a critical CaCl$_2$ concentration (4 mm in 0.5%, 20 mm in 3% sodium caseinate) after which the distribution shifted towards larger size range. The shape of the size distribution curve was dependent on whether CaCl$_2$ added before or after homogenization. Surface protein concentration increased with CaCl$_2$ addition, which was largely due to enhanced adsorption of the $\alpha$S$_1$ ($\alpha$S$_{1-1}$, $\alpha$S$_{1-2}$) casein at interface of emulsion droplets in all emulsion systems studied. Addition of CaCl$_2$ before or after homogenization caused a decrease in the creaming stability of emulsions made with 0.5% caseinate. In contrast, addition of CaCl$_2$ up to $\leq$10 mM increased the creaming stability of 3% caseinate emulsions, although the stability decreased again above 20 mm CaCl$_2$. The confocal microscopy confirmed that the extent of depletion flocculation in 3.0% caseinate emulsions decreased by addition of CaCl$_2$, but irreversible flocculation occurred when added CaCl$_2$ exceeded 20 mM. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Sodium caseinate; CaCl$_2$; Adsorption; Creaming stability; Flocculation; Emulsions

1. Introduction

Sodium caseinate, which consists of partially aggregated mixture of $\alpha$S$_1$-, $\alpha$S$_2$-, $\beta$- and $\kappa$-caseins, is commonly used as an ingredient in a wide range of formulated food emulsions. Because of the presence of the phosphoseryl residues, the caseins, especially $\alpha$S$_1$- and $\alpha$S$_2$-caseins, have strong tendency to bind calcium ions (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981). The binding of Ca$^{2+}$ to caseins reduces the electrostatic repulsions between the casein molecules which could promote interactions between hydrophobic domains, leading to formation of aggregates (Swaisgood, 1992). This change in the aggregation state of caseins will inevitably influence their adsorption behaviour at the oil/water interface and the stability of resulting emulsions. Previous workers have shown that the adsorption behaviour of caseinate at the oil/water interface and the stability of emulsions formed with caseinate are influenced by the state of aggregation of protein (Mulvihill & Murphy, 1991; Srinivasan, Singh, & Munro, 1996). Srinivasan et al. (1996) reported that addition of CaCl$_2$ to caseinate solutions, prior to emulsion formation, caused increases in the average droplet size and the surface protein concentration. Mulvihill and Murphy (1991) reported that the emulsions formed with highly-aggregated calcium caseinate had higher protein loads at the interface of emulsion droplets than those made with sodium caseinate. The binding of Ca$^{2+}$ to the casein-coated oil droplets results in aggregation of emulsion droplets (Dickinson, Hunt, & Horne, 1992; Agboola & Dalgleish, 1995). For example, Agboola and Dalgleish (1995) reported that the average droplet size of the sodium caseinate emulsion increased with increase in Ca$^{2+}$ concentration added to the emulsion after homogenization.

Relatively little information is available on the details of influence of Ca$^{2+}$ on the adsorption behaviour of caseinate at the oil/water interfaces (Agboola & Dalgleish, 1995; Dickinson & Davies, 1999). No previous studies have been reported on the effects of Ca$^{2+}$ on the composition of the adsorbed protein layers in sodium caseinate emulsions. Hence, the first objective of this work was to explore the effects of addition of CaCl$_2$ to the caseinate emulsions, before or after homogenization, on the surface protein coverage and composition.

Dickinson and Golding (1997) demonstrated that emulsions made with $>2$% sodium caseinate were more unstable towards creaming than emulsions made with lower caseinate concentrations. This destabilization was
attributed to depletion flocculation caused by the presence of high concentrations of non-adsorbed caseinate (Dickinson & Golding, 1997). Recently, Dickinson and Golding (1998) reported that addition of CaCl$_2$ to 4 or 6 wt% sodium caseinate solutions, prior to emulsion formation, enhanced the creaming stability. They suggested that the addition of Ca$^{2+}$ resulted in association of caseinate sub-micelles into larger aggregates which, owing to their size, were incapable of inducing depletion flocculation. The second objective of our work was to extend these findings and study the effects of addition of CaCl$_2$ into the caseinate solutions prior to making the emulsions or to emulsions after they were made on the stability of emulsions. In addition, it is known that ionic strength influences the binding of Ca$^{2+}$ to caseins (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981), which could subsequently influence the emulsion formation and stability of emulsion. Therefore, the effects of NaCl (200 mm) addition to the emulsions containing various levels of Ca$^{2+}$ were also studied. As the emulsions made with 0.5 and 3 wt% sodium caseinate have been shown to exhibit markedly different interfacial composition (Srinivasan et al., 1996) and stability properties (Dickinson & Golding, 1997), these two concentrations of sodium caseinate were chosen for studying the effects of calcium ions.

2. Materials and methods

2.1. Materials

Sodium caseinate (ALANATE 180) was obtained from the New Zealand Dairy Board, Wellington, New Zealand. This product contained ~96% dry matter of which about 94% was protein, 1.38% Na, and 0.06% Ca. 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

Protein solutions were prepared by adding the sodium caseinate powder to Milli-Q water (water purified by treatment with a Milli-Q apparatus, Millipore Corp. Bedford, MA), and then stirring for 60 min at room temperature to ensure complete dispersion. In some cases, different concentrations of CaCl$_2$ were added to the protein solutions and pH adjusted to 7.0 using 1 M NaOH or 1 M HCl. Appropriate quantities of soya oil were then mixed with the protein solution to give 30% oil in the final emulsion. The mixture was heated to 55°C and then homogenized in a two-stage valve homogeniser (Rannie a/s, Roholmsvej 8, DK 2620 Albertslund, Denmark) at 207 bar for the first stage and 34 bar for the second stage. In some cases, different amounts of CaCl$_2$ were added to the emulsions after they were made in the absence of CaCl$_2$. The emulsions were stored at 20°C. At least two separate emulsions were prepared for each treatment.

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 droplet size distribution using 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

Surface protein concentration was determined by the method described by Srinivasan et al. (1996). The composition of the adsorbed protein at the surface of the emulsion droplets was determined using SDS-PAGE adapted from Hunt and Dalgleish (1994). The method and the sample treatment are described in detail elsewhere (Ye & Singh, 2000). The sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Singh and Creamer (1991). The percentage composition of each sample was determined by scanning the areas for $\alpha_s$-casein, $\beta$-casein and $\kappa$-casein expressing the individual protein peaks as a fraction of the sum total on a laser densitometer (LKB Ultroscan XL, LKB Produkter AB, Bromma, Sweden).

Analysis of six separate emulsions, made with 3.0% sodium caseinate and 30% soya oil, showed that the variations were $\pm 0.05 \mu$m for $d_{43}$, ~4% for surface protein concentration, ~4% for $\alpha_s$-casein, ~4% for $\beta$-casein and ~5% for $\kappa$-casein.

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:

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\text{Stability rating} \% = \frac{\text{fat in the lower phase} \%}{\text{fat in the original emulsion} \%} \times 100
\]

Analysis of six separate emulsions, made with 3.0% sodium caseinate and 30% soya oil, showed that the variations were ~5% for the stability rating.
2.6. Confocal laser microscopy

A Leica (Heidelberg, Germany) confocal scanning laser microscope (CSLM) with a 100 mm oil immersion objective lens and an Ar/Kr laser with an excitation line of 488 nm (in such a way that only the fluorescent wavelength band can reach the detector system) was used to determine the microstructure of emulsions. Emulsions were made as described above and about 3 ml of sample was taken in a test tube and Nile Blue (fluorescent dye) was mixed through and then placed on a microscope slide. The slide was then covered with a coverslip and observed under the microscope.

3. Results

3.1. Droplet size distributions and average droplet diameters

Figs. 1 and 2 show the droplet size distributions of emulsions made with 0.5% and 3.0% sodium caseinate, in which CaCl₂ was added before or after homogenization. When CaCl₂ was added before emulsification, the size distribution of droplets in emulsions made with both 0.5 and 3.0% caseinate was monomodal until a critical CaCl₂ concentration (>1 mM in 0.5%, >15 mM in 3.0%) after which the distribution shifted towards larger particle sizes (Figs. 1(a) and 2(a)).

In comparison with the above changes, the droplet size distribution of emulsions in which CaCl₂ was added after homogenization showed a general reduction in the proportion of emulsion droplets in the original size range and an increase in proportion of large particles (Figs. 1(b) and 2(b)). This gave a bimodal size distribution as concentration of CaCl₂ was increased. However, the shape of the size distribution of small particles hardly changed although some very large particles were formed from aggregation of small ones. The difference in droplet sizes in emulsions made with 0.5 or 3.0% caseinate was essentially the difference in the critical concentration of CaCl₂ required to change the particle size distribution (2 mM for 0.5%, 10 mM for 3.0%).

When CaCl₂ was added to 0.5% sodium caseinate, prior to emulsion formation, the average droplet diameter ($d_{43}$) increased gradually as the concentration of CaCl₂ increased from 0 to 3 mM (Table 1). However, at higher levels of CaCl₂ addition, a very large increase $d_{43}$ of emulsion droplets occurred. Addition of 200 mM NaCl to these emulsions had no significant effect on the $d_{43}$ of emulsions.
containing up to 3 mM CaCl$_2$. In contrast, the $d_{43}$ of emulsions containing 4 or 5 mM CaCl$_2$ decreased markedly upon addition of NaCl. Dispersion of emulsions in SDS solution caused a marked decrease in $d_{43}$ of emulsions containing 4 or 5 mM CaCl$_2$ but did not change the $d_{43}$ values of emulsions containing ≤3 mM CaCl$_2$. After SDS treatment, the $d_{43}$ values did not fully return to those of emulsions without CaCl$_2$ (Table 1), indicating that some larger droplets were formed probably by recoalescence during homogenization.

When CaCl$_2$ was added to emulsions after they were made, the $d_{43}$ did not change with increase in CaCl$_2$ up to 2 mM, but increased considerably from 1.0 to 6.7 $\mu$m with further increase in CaCl$_2$ (from 3 to 5 mM). The $d_{43}$ reduced to ~1.4 $\mu$m when 200 mM NaCl was added to emulsions (Table 1). After SDS treatment, the $d_{43}$ values fully returned to that of emulsion made without added CaCl$_2$, in contrast to emulsions where CaCl$_2$ was added before homogenization. It was also observed that $d_{43}$ values of emulsions where CaCl$_2$ was added before homogenization did not change with time by dilution and stirring in the MasterSizer, whereas these values decreased with time for emulsions in which CaCl$_2$ was added after homogenization. The $d_{43}$ decreased from 4.6 to 1.6 $\mu$m for emulsion containing 4 mM CaCl$_2$ or from 6.6 to 2.4 $\mu$m for emulsion with 5 mM CaCl$_2$ added, when the emulsions were stirred for 5 min in the MasterSizer.

In emulsions made with 3.0% sodium caseinate, the $d_{43}$ remained unaffected with increase in CaCl$_2$ concentrations up to 15 mM, but increased markedly (from 0.81 to 30.8 $\mu$m) with further increase in CaCl$_2$ to 20 mM. Addition of 200 mM NaCl to emulsions did not significantly affect the $d_{43}$ values, except in emulsions containing 18 or 20 mM CaCl$_2$ where a small decrease in $d_{43}$ values was observed (Table 2). However, SDS treatment caused a marked decrease in the $d_{43}$ values of emulsions containing 18 or 20 mM CaCl$_2$.

When CaCl$_2$ was added to emulsions made in absence of CaCl$_2$, the $d_{43}$ values increased from 0.81 to 13.23 $\mu$m with increase in CaCl$_2$ from 0 to 25 mM. The $d_{43}$ decreased to 0.81 $\mu$m when 200 mM NaCl was added to emulsions, except that emulsions containing 20 mM CaCl$_2$ still had a higher $d_{43}$ value (5.41 $\mu$m) compared with the control (no CaCl$_2$ or NaCl) (Table 2). SDS treatment also caused a decrease in $d_{43}$ values of emulsions containing >20 mM CaCl$_2$.

3.2. Surface protein concentration and composition

As the concentration of CaCl$_2$ added to 0.5% caseinate solution, prior to emulsification, increased from 0 to 5 mM, the surface protein concentration of emulsions increased gradually from 0.68 to 1.25 mg/m$^2$ (Fig. 3(a)). When 200 mM NaCl was added to the emulsions, the surface protein concentration increased for emulsions containing below 2 mM CaCl$_2$ but decreased for emulsions containing >2 mM CaCl$_2$ (Fig. 3(a)). Interestingly, when CaCl$_2$ was added to the emulsions after they were made, the changes in surface protein concentration were similar to those in emulsions made in the presence of CaCl$_2$ (Fig. 3(b)).

In emulsions made with 3.0% sodium caseinate in the presence of CaCl$_2$, the surface protein concentration increased markedly from 1.6 to 6.3 mg/m$^2$ as the concentration of CaCl$_2$ increased from 0 to 20 mM (Fig. 4(a)). When 200 mM NaCl was added, the surface protein concentration increased slightly for emulsions containing <10 mM CaCl$_2$, but decreased for emulsions containing >10 mM CaCl$_2$ compared with the emulsions made in the absence of NaCl at a given CaCl$_2$ concentration (Fig. 4(a)). Generally,
similar results were obtained when CaCl₂ was added to the emulsions after they were made (Fig. 4(b)).

In emulsions made with 0.5% caseinate in the absence of CaCl₂, the proportions of adsorbed caseins were: α₁-casein (α₁ + α₂-caseins) ~35%, β-casein ~49% and κ-casein ~18% as compared with the proportions in the original caseinate solution (α₁-casein ~45%, β-casein ~41% and κ-casein ~14%) (Fig. 5). In agreement with our previous studies (Srinivasan et al., 1996, 1999), this suggests that β-casein was adsorbed in preference to α₁-casein under these conditions. As the concentration of CaCl₂ was increased from 0 to 3 mM, the relative proportion of α₁-casein increased from ~35 to ~55% with corresponding decreases in the proportions of adsorbed β-casein from ~50 to ~30% (Fig. 5(a)). The proportions of adsorbed κ-casein did not change with CaCl₂ concentration. Addition of 200 mM NaCl to these emulsions had no effect on the proportions of adsorbed caseins, except in the case of emulsions containing 0 or 5 mM CaCl₂ (Fig. 5(a)).

In the case of emulsions in which CaCl₂ was added after they were made, the proportion of α₁-casein increased slightly with a corresponding decrease in the proportion of β-casein at 1 mM CaCl₂ addition (Fig. 5(b)). There were no further changes beyond 2 mM CaCl₂ addition. Addition of 200 mM NaCl to these emulsions did not affect the interfacial composition (Fig. 5(b)).

The interfacial composition (α₁-casein ~49%, β-casein ~23% and κ-casein ~27%) (Fig. 6) of emulsions made with 3.0% sodium caseinate, in the absence of CaCl₂ was different from that of emulsions made with 0.5% sodium caseinate. It appeared that α₁-casein (α₁ + α₂-caseins) was adsorbed in preference to β-casein under these conditions. Low concentrations of CaCl₂ (<10 mM) added prior to emulsion formation caused an increase in the proportion of α₁-casein and a decrease in the proportions of β- and κ-caseins (Fig. 6(a)). Further increase in CaCl₂ concentration resulted in a decrease in the proportion of α₁-casein with corresponding increases in β- and κ-caseins. Addition of 200 mM NaCl to emulsions did not significantly affect the interfacial composition of these emulsions (Fig. 6(a)). Generally similar results of interfacial compositions were found when CaCl₂ was added to emulsions after they were made (Fig. 6(b)).

### 3.3. Stability of 0.5% caseinate emulsions

The effect of addition of CaCl₂ before or after emulsion formation on creaming stability of emulsions made with 0.5% sodium caseinate is shown in Fig. 7. In the case of CaCl₂ added prior to emulsion formation, increase in CaCl₂ from 0 to 5 mM caused a major decrease in the creaming stability of emulsions (from ~70 to ~2%) (Fig. 7(a)). The addition of CaCl₂ to emulsions after homogenisation also resulted in a decrease in creaming stability, but the decrease (~70–~40%) was less marked than in the case of addition of CaCl₂ prior to emulsion formation (Fig. 7(b)). Addition of 200 mM NaCl to both emulsions had no considerable effect on creaming stability (Fig. 7). It appears that the creaming stability strongly follows the particle size (i.e. the oil surface area) of emulsions under these conditions.

These emulsions were examined using confocal laser scanning microscopy to observe the state of droplet aggregation and have a visual perspective of the aggregation. In emulsions containing no or low concentrations of CaCl₂, emulsion droplets appeared to be homogeneous with no sign of flocculation (Fig. 8(a) and (b)). Emulsions containing 5 mM CaCl₂ showed large number of small particles aggregated together.
and separated from the aqueous phase (Fig. 8(c)). Emulsions in which 3 mM CaCl$_2$ was added after emulsion formation (Fig. 9), showed some aggregated droplet clusters along with some small droplets (Fig. 9(b)), in accordance with the changes in particle size distributions of these emulsions (Fig. 1). As the CaCl$_2$ concentration was further increased, more clusters (about 10–15 $\mu$m) were formed from droplet aggregation (Fig. 9(c)). The appearance of these aggregates was different from that in emulsions in which CaCl$_2$ was added before homogenization, in which much larger aggregates involving large numbers of droplets were formed (Fig. 8(c)).

3.4. Stability of 3.0% caseinate emulsions

The creaming stability of emulsions made with 3.0% caseinate, in the absence of CaCl$_2$, was considerably low due to the depletion flocculation (Dickinson & Golding, 1997). The addition of CaCl$_2$ both prior to and after emulsion formation dramatically influenced the stability rating of these emulsions (Fig. 10). When CaCl$_2$ was added to the caseinate solution prior to emulsion formation, the stability rating increased markedly from ~7 to ~73% as the CaCl$_2$ concentration was increased from 0 to 12 mM. Between 12 to 18 mM CaCl$_2$ the stability rating remained constant at ~75%, but it decreased abruptly to ~10% as the CaCl$_2$ was further increased to 20 mM (Fig. 11(a)).

Compared to the above results, the creaming stability of emulsions made with 3.0% caseinate was more sensitive to the addition of CaCl$_2$ to emulsions after they were made. The stability increased to ~70% at ~10 mM CaCl$_2$ (Fig. 11(b)), and did not decrease at high CaCl$_2$ concentrations, although the average droplet size of emulsions increased further (Table 2).

Addition of 200 mM NaCl to emulsions improved markedly the stability rating (to ~70%) of emulsions made with 3.0% caseinate in the absence of CaCl$_2$ and of those containing lower CaCl$_2$ concentrations (<12 mM) regardless of the method of CaCl$_2$ addition.

Confocal micrographs of emulsions made with 3.0% sodium caseinate showed that the droplets were joined together to form a network structure (Fig. 11(a)). Addition of 5 mM CaCl$_2$ to protein solution before emulsion formation showed smaller aggregates and more individual droplets (Fig. 11(b)) as compared with significant flocculated structure in the emulsion made in the absence of CaCl$_2$ (Fig. 11(a)). Addition of 12 mM CaCl$_2$ resulted in fine and homogeneous state of emulsion droplets (Fig. 11(c)). These changes in flocculation...
were obviously responsible for the changes observed in creaming stability of emulsions (Fig. 11).

Fig. 12 demonstrates that the changes in microstructures of emulsions made with 3.0% caseinate when CaCl₂ was added after homogenization. Addition of 5 mM CaCl₂ also showed some decrease in the extent of flocculation (more individual droplets) (Fig. 12(b)) while addition of 10 mM CaCl₂ virtually eliminated the flocculation of emulsion droplets (Fig. 12(c)). By contrast, large aggregates of emulsion droplets were formed at 25 mM CaCl₂ (Fig. 12(d)).

4. Discussion

It is well known that, when Ca²⁺ is added to caseinate solution, Ca²⁺ binding with phosphoserine of caseins reduces electrostatic repulsion between the molecules (Baumy & Brule, 1986), resulting in the formation of aggregates in solution (Swaisgood, 1992). The turbidity of caseinate solution increased markedly with increase in Ca²⁺ concentration at the molar ratios of Ca²⁺/caseinate \( > \sim 3.2 \) (Tables 1 and 2) indicating aggregation of caseinate molecules. This aggregation would decrease the number of casein molecules available for adsorption, and may restrict the protein molecules undergoing spreading and rearrangement effectively. As a result, caseinate may not able to cover immediately all the new surface area of oil droplet created during homogenization and achieve a state of lowest free energy. Consequently, coalescence of the smaller droplets during homogenisation may occur, resulting in an increase in droplet size of emulsions (Figs. 1(a) and 2(a)) upon addition of low concentrations of CaCl₂.

A large increase in the droplet size at higher ratios of Ca²⁺/protein (Tables 1 and 2) could be attributed due mainly to protein bridging flocculation between droplets. High levels of casein aggregation, combined with decreased flexibility of casein molecules, probably lead to insufficient amount of protein being available to cover the total surface of oil droplets. Therefore, the aggregated particles of caseins adsorbed at the oil droplet surface extend away from the interface and the outside part of protein particle adsorbs to another oil droplet. This flocculation was largely irreversible upon dilution with water and stirring in the MasterSizer, but the droplets could be dissociated by dispersion in SDS solution. However, at high levels of calcium addition some
Ca$^{2+}$ bridging between casein-coated droplets probably also occurs.

The flocculation by calcium bridges between protein molecules adsorbed on different emulsion droplets may be responsible for increased droplet sizes in emulsions in which Ca$^{2+}$ was added after homogenization (Figs. 1(b) and 2(b)). The flocculation caused by calcium bridging was reversible upon dilution and stirring. Dickinson et al. (1992) reported that the particle size distribution of β-casein-stabilized emulsion, in which calcium was added after homogenization, was less sensitive to flocculation than that of emulsions made in the presence of calcium. They inferred that emulsions stabilized by proteins with a high affinity for calcium are more prone to flocculation by calcium bridges between protein molecules adsorbed on different emulsion droplets when calcium is added after homogenization, and that the flocculation is reversible upon dilution.

The extent of decrease in the average particle size upon addition of NaCl to the flocculated emulsions can distinguish between different aggregation mechanisms. Most of the aggregated particles in emulsions were dissociated by NaCl addition in the case when CaCl$_2$ was added after homogenisation, whereas dissociation occurred to a limited extent in emulsions in which

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**Fig. 7.** Changes in stability rating (%) as a function of CaCl$_2$ concentration in emulsions (30% soya oil) stabilized by 0.5% sodium caseinate. CaCl$_2$ was added: (a) before, or (b) after emulsion formation (●), and 200 mM NaCl was added to emulsions at each CaCl$_2$ concentration (▼).

**Fig. 8.** Confocal micrographs of 0.5% sodium caseinate-stabilized oil-in-water emulsions: (a) 0 mM, (b) 3 mM, or (c) 5 mM CaCl$_2$ was added to caseinate solution prior to emulsion formation.
CaCl₂ was added before homogenization (Tables 1 and 2). This may be because Na⁺ competed directly with specific sites for Ca²⁺ binding between the droplets in the former case, but only provided little more protein to the interface from the aqueous phase, due to dissociation of aggregated caseins, in the latter case. Turbidity of sodium caseinate solution containing high added CaCl₂ decreased with NaCl addition confirming dissociation of casein aggregates. The strength of Ca²⁺ binding to both αs₁-casein and β-casein is inversely related to the ionic strength as dictated by the molarity of NaCl (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981).

In agreement with previous studies (Srinivasan et al., 1996; Dickinson & Davies, 1999), addition of CaCl₂ prior to emulsification increased the surface protein coverage; this could be attributed to aggregation of caseins, due to binding of Ca²⁺, and subsequent adsorption of aggregated caseins at the droplet surface. In addition, Ca²⁺ binding reduces charge repulsion between casein molecules and hence could increase protein packing at the interface.

A marked increase in surface protein coverage with CaCl₂ addition to the emulsions (Figs. 3(b) and 4(b)) indicates that CaCl₂ was added before homogenization (Tables 1 and 2). This may be because Na⁺ competed directly with specific sites for Ca²⁺ binding between the droplets in the former case, but only provided little more protein to the interface from the aqueous phase, due to dissociation of aggregated caseins, in the latter case. Turbidity of sodium caseinate solution containing high added CaCl₂ decreased with NaCl addition confirming dissociation of casein aggregates. The strength of Ca²⁺ binding to both αs₁-casein and β-casein is inversely related to the ionic strength as dictated by the molarity of NaCl (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981).

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some Ca$^{2+}$ might be involved in binding with caseins in the
aqueous phase and forming calcium bridges between case-
ins in aqueous phase and that adsorbed at the emulsion
droplet surface. In addition, the binding of Ca$^{2+}$ to the case-
ins adsorbed at the droplet surface may cause more compact
packing of adsorbed caseins (Horne & Leaver, 1995;
Dickinson, 1998), which could allow more protein to be
adsorbed.

The increase in surface protein coverage observed
upon addition of NaCl to the emulsions made in the
absence of Ca$^{2+}$ (Figs. 3 and 4) is in agreement with
the results of Srinivasan, Singh, and Munro (2000).
Increasing the ionic strength may reduce the electrostatic repulsions 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 molecules at the interface is also facilitated at higher ionic strength (Tornberg, 1978). A slight decrease in surface protein concentration of emulsions containing high concentrations of Ca\(^{2+}\) upon NaCl addition (Figs. 3 and 4) could be attributed to competition by the Na\(^+\) for the Ca\(^{2+}\) binding sites of caseins, thereby reducing the amounts of casein aggregates adsorbed at the interface.

The present results clearly showed that the addition of Ca\(^{2+}\) before and after homogenization enhanced the adsorption of $\alpha_s$-casein ($\alpha_s$-$\alpha_\text{1} + \alpha_s$-$\alpha_\text{2}$-caseins) at the droplet surface (Figs. 5 and 6). This may be because the binding

Fig. 12. Confocal micrographs of 3.0% sodium caseinate-stabilized oil-in-water emulsions: (a) 0 mM, (b) 5 mM, (c) 10 mM, or (d) 25 mM CaCl\(_2\) was added to emulsion after emulsion formation.
capacity of $\alpha_{s1}$- and $\alpha_{s2}$-caseins to Ca$^{2+}$ is higher than $\beta$-casein (Dalgleish & Parker, 1980; Parker & Dalgleish, 1981; Pappas & Rothwell, 1991) and the extent of aggregation of $\alpha_{s1}$-casein increases with Ca$^{2+}$ binding. Increase in adsorbed $\alpha_{s}$-casein when Ca$^{2+}$ was added was probably due to adsorption of these $\alpha_{s}$-casein-rich aggregates at the droplet surface. When the concentration of added Ca$^{2+}$ was high, more $\beta$-casein adsorbed from the aqueous phase. The proportion of $\kappa$-casein at the surface decreased with the addition of Ca$^{2+}$ (Figs. 5 and 6); this may be because Ca$^{2+}$ binding ability of $\kappa$-casein is very limited.

The addition of NaCl (raising the ionic strength of environment) to emulsions slightly increased the adsorption of $\alpha_{s}$-casein ($\alpha_{s1} + \alpha_{s2}$-caseins) (Figs. 5 and 6), confirming the results of Srinivasan et al. (2000).

The present results demonstrate that the creaming stability of emulsions made with 0.5% sodium caseinate decreased with increase in the concentration of Ca$^{2+}$ (Fig. 7). This decrease appears to be related to an increase in the droplet sizes, as predicted by the Stoke’s law (Walstra, 1987).

The instability (Fig. 7) of the emulsions made with 3% sodium caseinate, in the absence of Ca$^{2+}$ and NaCl, could be attributed to depletion flocculation (Dickinson & Golding, 1997). Dickinson and Golding (1997) reported that the extent of depletion flocculation is mainly dependent on the size and concentration of unbound or unadsorbed protein; the presence of caseinate sub-micelles (15–20 nm) above certain critical concentration in aqueous phase is responsible for the depletion flocculation.

Binding of Ca$^{2+}$ to caseins increases the size of casein particles in the aqueous phase of emulsion as well as the protein concentration in the aqueous phase decreases, due to increase casein adsorption. Our results show that 3.0% caseinate emulsions containing 12 mM (added before emulsification) or 10 mM Ca$^{2+}$ (added after emulsification), which had $\sim$1.7% caseinate in the aqueous phase, showed high creaming stability (Fig. 11) and no flocculation (Figs. 11(c) and 12(c)). However, at this aqueous phase concentration, a strong flocculation was observed in emulsions made with sodium caseinate ($\sim$2.5%) in the absence of Ca$^{2+}$. This suggests that the depletion flocculation was prevented largely by increase in the size of casein particles in the aqueous phase. These results essentially confirm the findings of Dickinson and Golding (1998).

It was also found that irreversible flocculation of oil droplets occurred when the concentration of added CaCl$_2$ exceeded 20 mM. In the case of emulsions where Ca$^{2+}$ was added before emulsification, this flocculation was probably due largely to protein bridging whereas in emulsions where CaCl$_2$ was added after emulsification, Ca$^{2+}$ bridging between droplets was responsible.

Acknowledgements

The authors wish to thank the New Zealand Dairy Research Institute for providing the facilities for particle size measurements.

References


