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Influence of retorting (121 °C for 15 min), before or after emulsification, on the properties of calcium caseinate oil-in-water emulsions

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Abstract

Oil-in-water emulsions, containing 30% soya oil and various concentrations of calcium caseinate, were prepared in a two-stage valve homogenizer. The emulsions were sealed in glass bottles and then heated at 121 °C for 15 min in an autoclave. In some experiments, the caseinate solutions were heated at 121 °C for 15 min in an autoclave, and then mixed with soya oil (to give 30% oil in the final emulsion), followed by homogenization. Heat treatment (121 °C for 15 min) of calcium caseinate emulsions or heat treatment of calcium caseinate solutions prior to emulsion formation, at all caseinate concentrations, resulted in an increase in surface coverage and altered the proportions of individual caseins at the droplet surface. Heat treatment of calcium caseinate solutions at 121 °C for 15 min resulted in the formation of several new peptides, due to protein degradation, and polymerization of casein molecules, as revealed by SDS-PAGE. Both the polymerized caseinate material and degradation products were adsorbed onto the droplet surface efficiently during emulsification; the degradation products were more readily adsorbed than the parent protein. Creaming stability of calcium caseinate emulsions, after storage at 20 °C for 24 h, increased with an increase in caseinate concentration. Creaming stability of these emulsions improved further when the emulsions were retorted or when emulsions were made using retorted calcium caseinate solutions.

Keywords: Heating; Calcium caseinate; Emulsion; Adsorption, Stability

1. Introduction

Caseinates are manufactured by precipitating the casein from milk by lowering the pH to 4.6, either by the addition of dilute mineral acid or by converting some of the lactose into lactic acid through the action of added culture. The casein curd is then washed with water, dissolved in the appropriate alkali to increase the pH to about 7.0, and the dispersion is then spray-dried. When NaOH is used to adjust the pH, the product formed is called sodium caseinate, and when dispersed in water it contains individual casein molecules and some small casein aggregates (Lucey, Srinivasan, Singh, & Munro, 2000). When calcium hydroxide is used to adjust the pH, calcium caseinate is obtained. Because of the high sensitivity of caseins to calcium ions (Swaisgood, 1992), this product contains casein aggregates of varying sizes (Moughal, Munro, & Singh, 2000; Srinivasan, Singh, & Munro, 1999; Ye, Srinivasan, & Singh, 2000). As the state of aggregation of proteins influences their emulsifying properties, there are considerable differences between the emulsifying abilities of sodium and calcium caseinates.

Many food products, based on emulsions, are subjected to heat treatment prior to distribution. The heat treatment is applied generally to extend the microbiological shelf-life of the product. Heat-induced changes in proteins of skim milk systems (Singh & Creamer, 1992) and sodium caseinate solutions (Guo, Fox, Flynn, & Mohammad, 1989) have been studied extensively and models have been advanced describing the coagulation of proteins in these systems. However, the heat-induced

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changes in calcium caseinate solutions have not been reported.

Heat stability of oil-in-water emulsions containing milk proteins has also been studied by Hunt and Dalgleish (1995). Emulsions formed with sodium caseinate (2 wt.% protein, 20% soya oil) were stable to heating at 90 °C for 30 min or 121 °C for 15 min, but the emulsions produced with whey proteins formed gels under these conditions. No information has been published on heat-induced changes in emulsions formed with calcium caseinate. In this paper, the effects of a typical retort heat treatment (121 °C for 15 min) of calcium caseinate emulsions, on particle size, surface protein coverage and creaming stability were investigated at different caseinate concentrations. The effects of heating calcium caseinate solutions prior to emulsion formation were also explored.

2. Materials and methods

2.1. Materials

Calcium caseinate (ALANATE 380) was obtained from the New Zealand Dairy Board, Wellington, New Zealand; typical values for protein, calcium and sodium were ~94, ~1.58 and ~0.07%, respectively. 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 by mixing appropriate amounts of calcium caseinate solutions (0.5 to 5.0 wt.%) and soya oil to give 30% oil in the final emulsion, as described by Srinivasan, Singh, and Munro (2000). The mixture was then homogenized in a two-stage valve homogenizer (Rannie a/s, Roholmsvej 8, DK 2620 Alberslund, Denmark) at 207 bar for the first stage and 34 bar for the second stage. The emulsions were sealed in glass bottles and then heated at 121 °C for 15 min in an autoclave. This heat treatment was similar to many retort treatments used in the food industry. In some cases, the caseinate solutions were heated at 121 °C for 15 min in an autoclave, and then mixed with soya oil (to give 30% oil in the final emulsion) followed by homogenization. Emulsions were prepared at least in duplicate.

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 analyze 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) to that of dispersion medium (1.33). The absorbance value used was 0.001.

2.4. Determination of surface protein concentration and composition

The surface protein concentration and composition in emulsions was determined as described by Ye et al. (2000). The reproducibility of these methods was determined by analyzing nine separate emulsions made with 2.5% calcium caseinate and 30% soya oil. Variations were approximately ± 0.02 μm for d_{32}, $\pm 4\%$ for surface protein concentration, $\pm 5\%$ for α_{s1}-casein, $\pm 4\%$ for β-casein and $\pm 7\%$ for κ-casein.

2.5. Determination of microstructure

A Leica (Heidelburg, Germany) confocal scanning laser microscope with a 100 mm oil immersion objective and an Ar/Kr laser with an excitation line of 488 nm (in such a way that only fluorescent wavelength band can reach the detector system) was used to determine the microstructure of emulsions. Emulsions were made as described above and Nile Blue (approx. 0.1%) was added with through mixing. A sample was then placed on a microscopy slide with a coverslip for observation.

2.6. Determination of creaming stability

Emulsions were poured into a centrifuge tube and maintained at 20 °C. After 24 h, the samples were centrifuged at low speed (180 *g*) at 20 °C for 15 min, using a Sorvall RC5C centrifuge (DuPont Company, USA). After centrifugation, 5 ml of the lower phase was carefully removed with a syringe for fat determination by the Mojonnier method. The following equation was used to determine the stability rating

$$\text{Stability Rating} = \frac{\%\ \text{Fat in lower phase}}{\%\ \text{Fat in original emulsion}} \times 100\%$$

3. Results and discussion

3.1. Emulsion formation

The droplet size distributions of emulsions, made with 0.5 and 1.0% calcium caseinate, were broad with bimodal characteristics (Fig. 1a) but became monomodal when caseinate concentrations were increased to 2% or above. Heat treatment of emulsions made using $\leqslant 2.0\%$

calcium caseinate caused a major shift in the particle size distributions towards higher particle size ranges (Fig. 1b). For example, the emulsions formed with 0.5 and 1.0% protein and heated at 121 °C for 15 min had some particles >80 μm (Fig. 1b); many of these particles could not be measured accurately with the MasterSizer using a 45 mm lens. Emulsions formed with higher concentrations of calcium caseinate showed considerable broadening of the particle size distribution after heat treatment. Generally, similar trends were observed when emulsions were formed with heated calcium caseinate solutions (Fig. 1c), although the size distributions, particularly at lower caseinate concentrations, were markedly different.

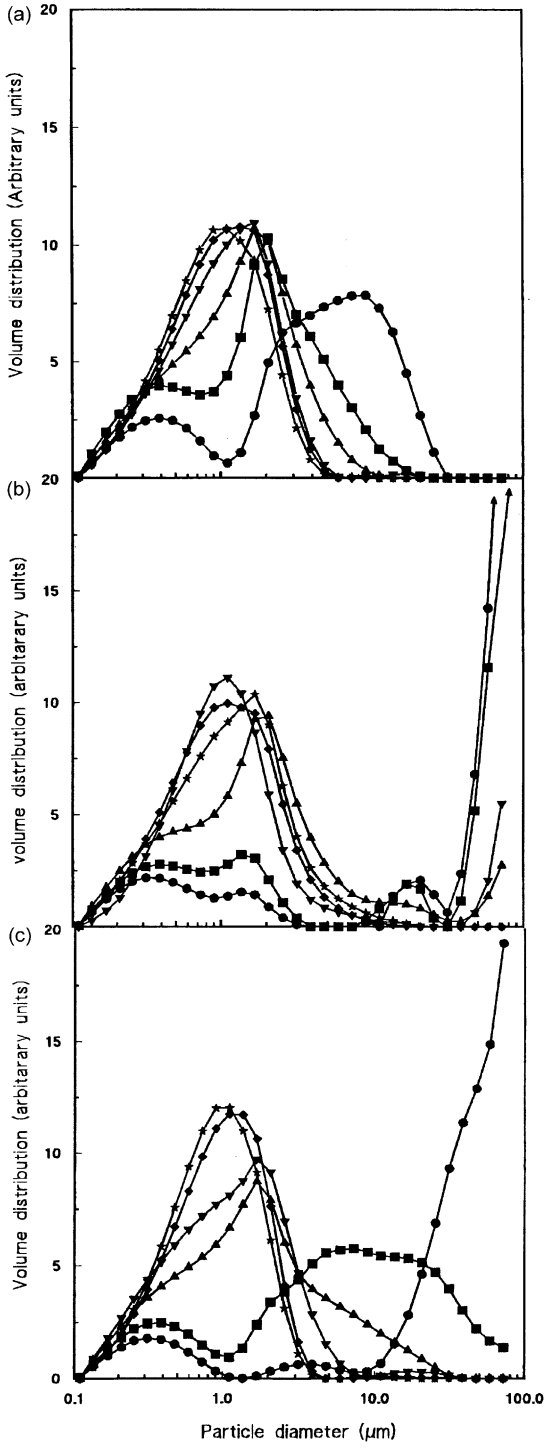

Fig. 1. Particle size distributions in unheated emulsions (a), emulsions heated at 121 °C for 15 min (b), or emulsions formed from calcium caseinate solutions that had been heated at 121 °C for 15 min (c). The emulsions contained 30% oil and 0.5% (●); 1.0% (■); 2.0% (▲); 3.0% (▼); 4.0% (◆); 5.0% (⋆) calcium caseinate.

When the emulsions (heated after homogenization) were diluted with 0.02% EDTA (which chelates calcium and thus causes the disintegration of calcium caseinate) and 2% SDS (which breaks the hydrophobic interactions and displaces the protein from the oil–water interface), the size distribution of droplets became narrower (0.1 to ~5 μm), particularly for 0.5 and 1.0% calcium caseinate emulsions (Fig. 2). Similar results were obtained when caseinate solutions were heated before emulsion formation (data not shown).

The average diameter of droplets, in both the unheated and heated calcium caseinate emulsions, decreased with an increase in calcium caseinate concentration (Fig. 3A). Heat treatment of emulsions, made using >2.0% calcium caseinate, had no significant effect on the average droplet diameter, but the droplet diameter of emulsions made with 0.5 and 1.0% calcium caseinate increased markedly after heat treatment. The average droplet diameters in emulsions formed from heat-treated calcium caseinate solutions were similar to those in heated emulsions (Fig. 3a).

Heat treatment of calcium caseinate solutions, before homogenization, or emulsions after homogenization, also resulted in an increase in the surface protein coverage at all caseinate concentrations (Fig. 3b). At 0.5 and 1.0% calcium caseinate, the surface protein coverage in emulsions, formed with heated calcium caseinate solutions, was slightly higher than that in heated emulsions but at higher calcium caseinate concentrations the reverse was true.

3.2. SDS-PAGE of heated calcium caseinate solutions

SDS-PAGE of heated and unheated calcium caseinate solution (2%) (Fig. 4a) showed that the intensity of all casein bands decreased after heating. The α_{s2}-casein band had disappeared completely and the intensity of the α_{s1}-casein band was very low. There was a corresponding increase in the intensity of high molecular weight protein material (labelled as X in Fig 4a) that remained on the top of the resolving and stacking gels after heating. Simultaneously, there was an increase in the low molecular weight casein degradation products (labelled as Y in Fig. 4a).

Comparisons of the SDS-PAGE patterns in Fig. 4a with those in heated sodium caseinate solutions (Srinivasan, Singh, & Munro, 2002), indicate that heat treatment

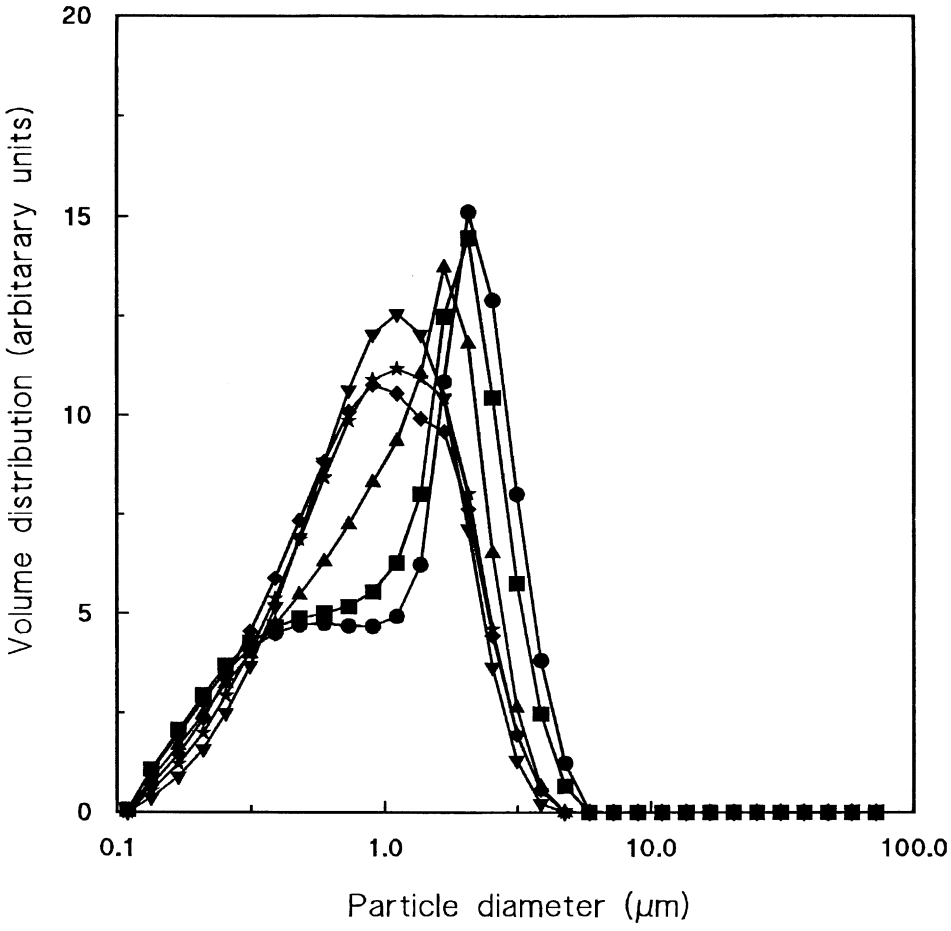

Fig. 2. Effect of dispersion of heated emulsions in a dissociating buffer (0.02% EDTA, 2% SDS) on the particle size distributions. Emulsions were formed with 0.5% (●); 1.0% (■); 2.0% (▲); 3.0% (▼); 4.0% (◆); 5.0% (⋆) calcium caseinate.

of calcium caseinate solution caused more extensive polymerization of caseins than heat treatment of sodium caseinate solutions. It is also interesting to note that α_{s1}- and α_{s2}-caseins were more sensitive to heating in calcium caseinate solutions than in sodium caseinate solutions (Srinivasan et al., 2002).

3.3. SDS-PAGE of various phases from heated calcium caseinate-stabilized emulsions

The SDS-PAGE patterns of the cream phase, obtained by centrifugation (45,000 *g* for 40 min) of heated calcium caseinate emulsions, are shown in Fig. 4b. In agreement with Srinivasan et al. (1999), in the unheated calcium caseinate-stabilized emulsions, the relative proportions of α_{s1}-casein adsorbed at the droplet surface, (i.e. in the cream phase) were higher than those of any other caseins. When the emulsions were heated (121 °C for 15 min), the intensities of the α_{s1}- and α_{s2}-casein bands at the droplet surface were markedly reduced. Some of the protein material could not be resolved and remained at the top of the stacking and resolving gels. As in the case of heated sodium caseinate emulsions (Srinivasan et al., 2002), accumulation of considerable amounts of fast moving peptides (labelled as Y) at the droplet surface was also noted. All the major casein bands were visible at all concentrations, although the intensity of the α_{s1}-casein band was relatively low compared with unheated emulsions.

The SDS-PAGE patterns of the subnatants, obtained after centrifugation of heated calcium caseinate emulsions, are shown in Fig. 4c. It is interesting to note that the subnatant (unadsorbed protein) contained mainly β- and κ-caseins and the α_{s1}- and α_{s2}-casein bands were hardly visible, which may suggest that these caseins were either on the interface or had polymerized to form large aggregates that sedimented during centrifugation.

The SDS-PAGE patterns of the sediments, obtained after centrifugation of heated calcium caseinate emulsions, are shown in Fig. 4d. The sediment contained relatively high proportions of high molecular weight protein material that could not be resolved and remained at the top of the stacking and resolving gels. Surprisingly, the sediment also contained considerable

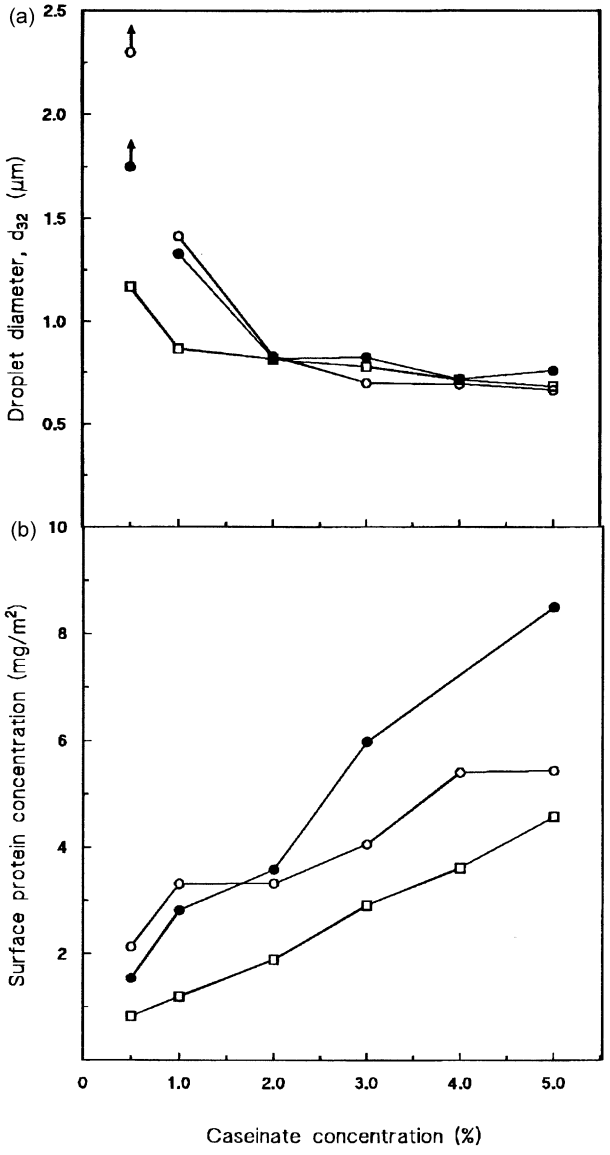

Fig. 3. Average droplet diameter (d_{32}) (a) and surface protein coverage (mg/m^2) (b) as a function of caseinate concentration in emulsions containing 30 wt.% soya oil and varying amounts of calcium caseinate. Unheated emulsions (□), emulsions heated at 121 °C for 15 min (●) or emulsions formed with caseinate solutions heated at 121 °C for 15 min (○). Data represent averages of two determinations. Arrow indicates that the actual d_{32} may be higher, as a significant number of droplets in the emulsions were found to be outside the Mastersizer range.

amounts of faster moving peptides. At least three bands, labelled P, Q and R, arising from the heat-induced degradation of caseins could be clearly seen (Fig. 4d).

3.4. SDS-PAGE of various phases from emulsions formed with heated calcium caseinate solutions

The SDS-PAGE patterns of the cream phase, obtained from emulsions formed with heated (121 °C for 15 min) calcium caseinate solutions, were somewhat similar to those obtained for heated emulsions (Fig. 4e). However, at all caseinate concentrations, when emulsions were formed with heated calcium caseinate solutions, some of the protein material was polymerized and was not resolved in the gels. The intensity of this unresolved material was much greater than that in heated calcium caseinate emulsions. As in the case of heated calcium caseinate emulsions, the intensities of slower moving aggregates (labelled as X) increased and the intensity of α_{s1}- and α_{s2}-casein bands at the droplet surface were markedly reduced compared to the unheated solutions. The three bands labelled as P, Q and R, were clearly visible at the droplet surface and their intensities were greater than in the heated calcium caseinate solutions or in heated emulsions. The electrophoretic patterns of the subnatant (unadsorbed protein) and the sediments were similar (results not shown) to those for the heated emulsions (Fig. 4d).

Calcium caseinate, in solution, contains some relatively large casein aggregates induced by calcium binding with caseins. During emulsification, these large casein aggregates are adsorbed on the interface, giving high protein surface coverage (Srinivasan et al., 1999). On heating calcium caseinate solution or emulsions, the caseins were further polymerized to form even larger aggregates; adsorption of these larger aggregates at the droplet surface would increase the surface protein coverage.

Binding of calcium to caseinate effectively reduces the negative charge, which diminishes the electrostatic repulsions between the caseinate chains (Cruijsen, 1996), leading to aggregation of caseinate particles. It has been shown that heat treatments of sodium caseinate at very high temperatures causes cleavage of negatively-charged phosphoserine residues; e.g. sodium caseinate heated at 120 °C for 20 min lost 15% of its serine phosphate (Belec & Jenness, 1962). Therefore, it is likely that on heating at high temperatures, the negative charge on the caseinate gradually diminishes, as a result of dephosphorylation, causing further aggregation of caseins in the presence of calcium.

When the emulsions formed with calcium caseinate are heated, it appears that similar polymerization and degradation reactions occur at the emulsion droplet surface. In addition, serum (unadsorbed) caseinate may interact with adsorbed casein to form polymerized products.

3.5. Stability of emulsions

The changes in the creaming stabilities of unheated and heated emulsions or emulsions, formed with heated calcium caseinate solutions, are shown in Fig. 5. The stability rating of unheated calcium caseinate emulsions increased rapidly with an increase in protein concentration from 0.5 to 2.0%. An increase in caseinate concentration beyond 2% had no further significant effect on stability rating.

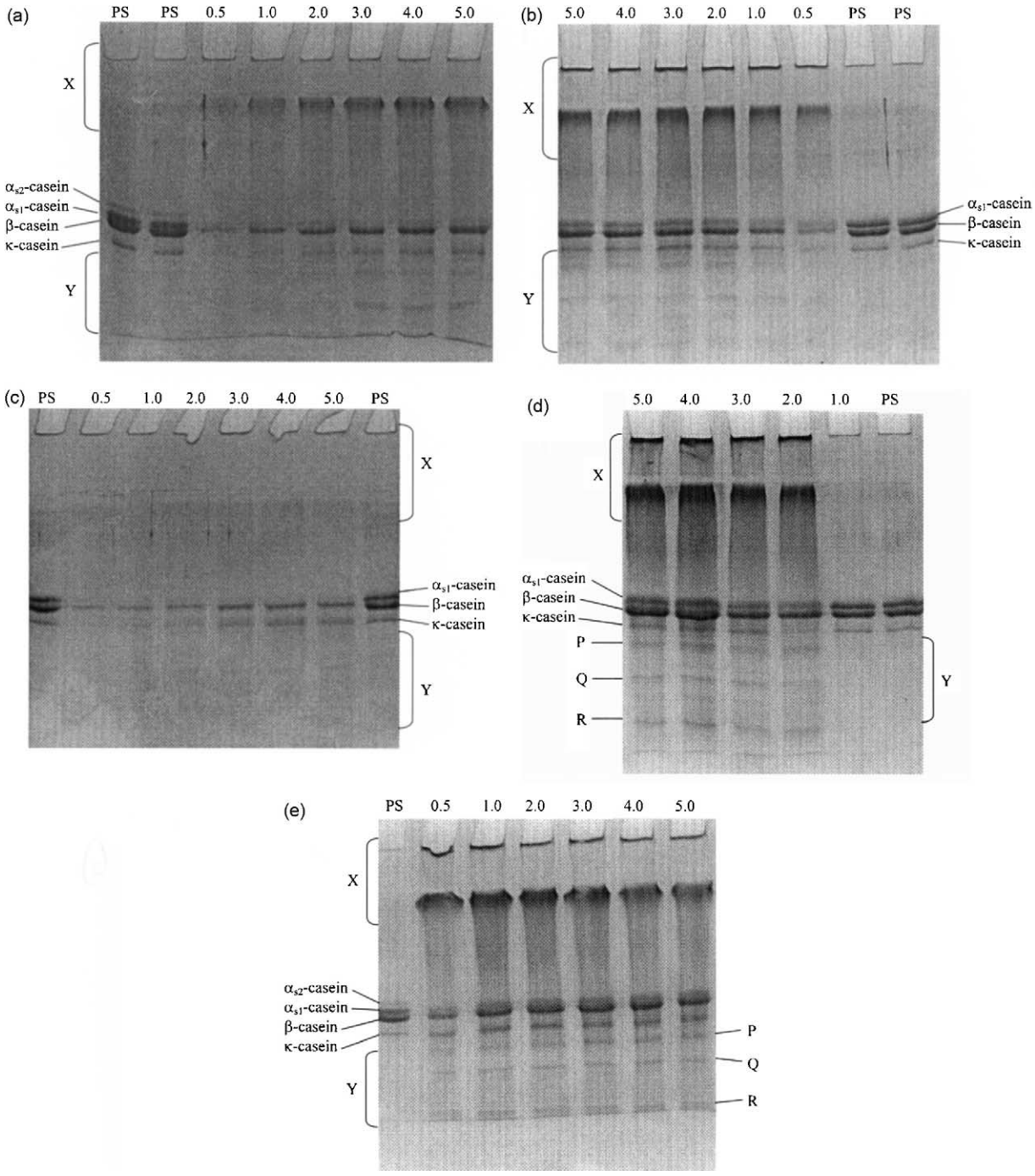

Fig. 4. SDS-PAGE patterns of calcium caseinate solutions and emulsions heated at 121 °C for 15 min before or after homogenization. (a) Heated calcium caseinate solutions. (b) Cream phase of heated calcium caseinate emulsions. (c) Subnatant obtained from heated calcium caseinate emulsions. (d) Sediment obtained from heated calcium caseinate emulsions. (e) Cream phase of emulsions formed with heated calcium caseinate solutions. PS is unheated calcium caseinate solution (2 wt.%). Calcium caseinate concentrations are noted above the lanes.

The creaming profiles of heated calcium caseinate emulsions were different from those of unheated emulsions. The stability rating of heated emulsions increased almost linearly with an increase in caseinate concentration from 1 to 4%, with no further increase at higher caseinate concentrations. The stability rating values of the heated emulsions were higher than those of the unheated emulsions at caseinate concentrations $\geqslant 3.0\%$. However, when the caseinate concentration was $<2\%$, the stability rating values of the unheated emulsions were slightly higher than those of the heated emulsions (Fig. 5).

The creaming profiles of heated emulsions and of emulsions formed with heated (121 °C for 15 min) calcium

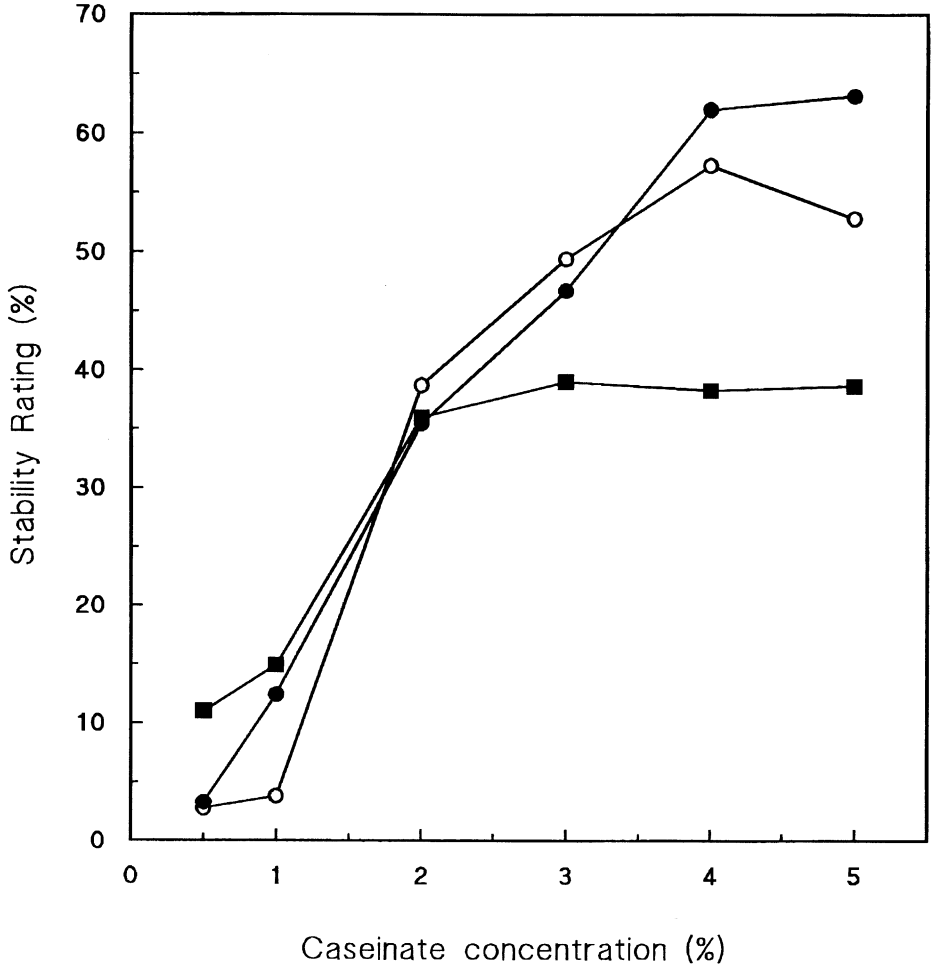

Fig. 5. Creaming stability rating of emulsions made with various concentrations of calcium caseinate (30% oil); unheated (■); emulsions heated at 121 °C for 15 min (○); calcium caseinate heated (121 °C for 15 min) prior to emulsion formation (●).

caseinate solutions were similar except that, at 4 and 5% caseinate, the stability ratings of emulsions formed with heated calcium caseinate solutions were slightly higher.

The increase in creaming stability in calcium-caseinate-stabilized emulsions with increasing protein concentration can be attributed to a combination of factors, including a decrease in droplet diameter (Fig. 3a) and an increase in viscosity of the continuous phase.

Fig. 6 shows the microstructures of calcium caseinate-stabilized emulsions heated at 121 °C for 15 min. Comparing the confocal micrographs of unheated emulsions and heated calcium caseinate emulsions, it appears that heat treatment caused extensive flocculation of oil droplets in emulsions made with 0.5 (Fig. 6a) or 1.0% caseinate concentration (data not shown). The extent of flocculation in 0.5% caseinate emulsions was considerably higher than that in 1.0% caseinate emulsions. This could have been mainly due to bridging flocculation between oil droplets (i.e. sharing of protein molecules/aggregates by two droplets) as the number of protein molecules/particles available for adsorption would decrease due to polymerization. This flocculation was probably responsible for the decrease observed in creaming stability rating (Fig. 5). When the calcium caseinate concentrations in the emulsions were 2.0% (Fig. 6c and d) or 3.0% (data not shown), the droplets seemed to be homogeneous and more individual droplets were observed. There were only slight differences in the appearance of droplets between heated and unheated emulsions made under these conditions.

It was not immediately apparent why the creaming stabilities of heated emulsions or emulsions made with heated calcium caseinate at concentrations $>2.0\%$ are greater than those of unheated emulsions. One possibility is that casein peptides, formed by heat-induced degradation, provide better emulsification. Alternatively, heat treatment, through its effect on aggregation, may increase the viscosity of the continuous phase. The increase in surface protein load observed after heating may increase the density of the droplets, so that there is less density difference between the aqueous phase and the droplets.

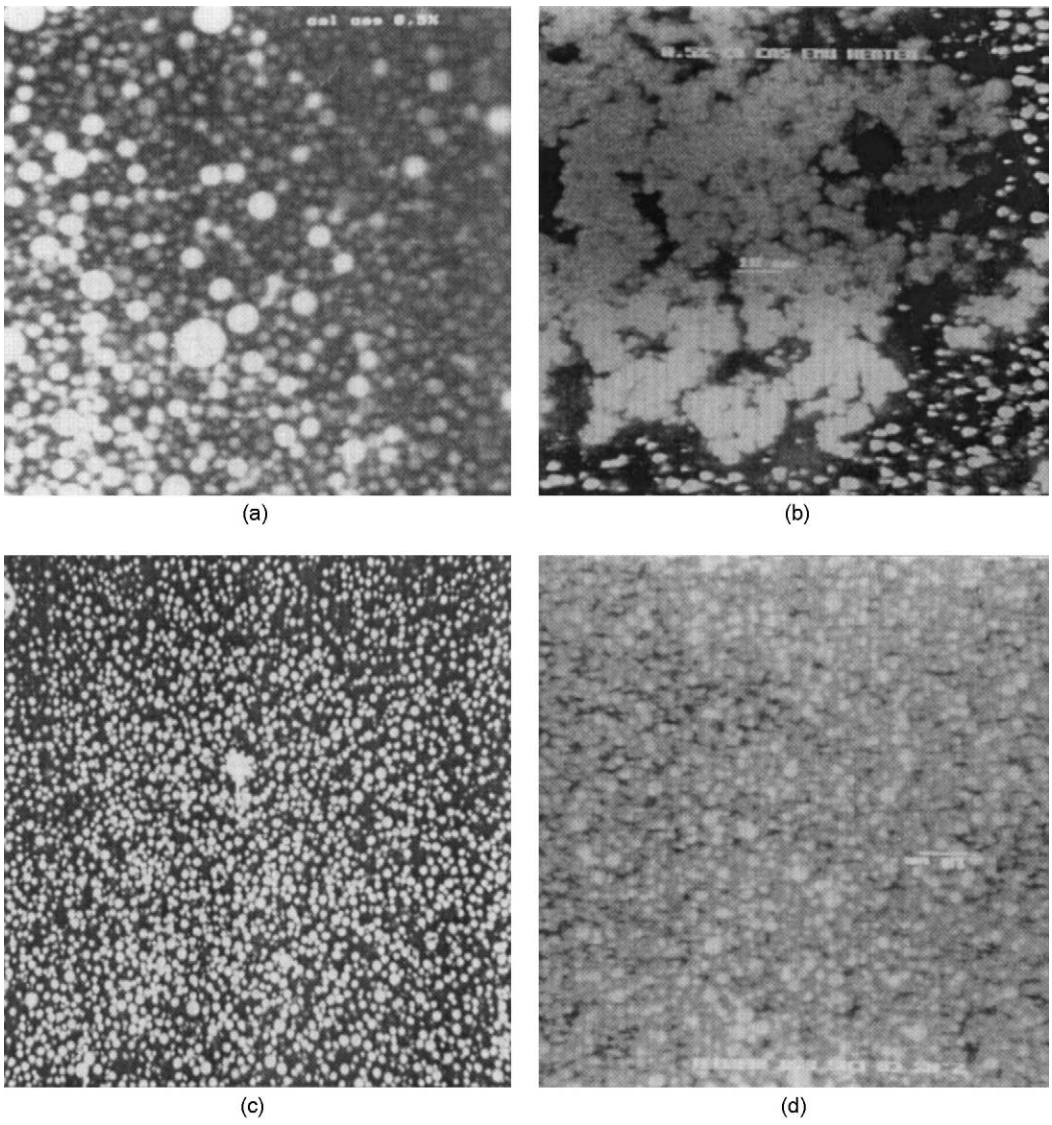

Fig. 6. Confocal micrographs of emulsions formed with 0.5% unheated calcium caseinate (a); 0.5% caseinate emulsion heated at 121 °C for 15 min (b); unheated 2.0% calcium caseinate emulsion (c) and 2% caseinate emulsion heated at 121 °C for 15 min (d).

Overall, the creaming stability and flocculation behaviour of calcium caseinate emulsions were different from that of sodium caseinate emulsions (Srinivasan et al., 2002). Sodium caseinate emulsions containing >2.0% protein showed flocculation of oil droplets, due to depletion interactions, which resulted in extensive creaming. The absence of depletion flocculation in calcium caseinate emulsions at the same protein concentrations is due to the greater degree of casein aggregation in calcium caseinate solution. These aggregates are probably too large to cause depletion flocculation as the solutions of large aggregates tend to have a relatively low osmotic pressure. Heat treatment (121 °C for 15 min) did not significantly affect the extent of depletion flocculation in sodium caseinate emulsions (Srinivasan et al., 2002), but improved the creaming stability of both types of emulsions.

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