Food Chemistry 110 (2008) 946–952

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Interfacial composition and stability of emulsions made with mixtures of commercial sodium caseinate and whey protein concentrate

Aiqian Ye *

Riddet Centre, Massey University, Private Bag 11 222, Palmerston North, New Zealand
Fonterra Research Centre, Fonterra Co-operative Group Limited, Dairy Farm Road, Private Bag 11 029, Palmerston North, New Zealand

ARTICLE INFO

Article history:
Received 21 November 2007
Received in revised form 17 December 2007
Accepted 29 February 2008

Keywords:
Oil-in-water emulsion
Adsorption
Creaming stability
Sodium caseinate
Whey protein concentrate

ABSTRACT

The interfacial composition and the stability of oil-in-water emulsion droplets (30% soya oil, pH 7.0) made with mixtures of sodium caseinate and whey protein concentrate (WPC) (1:1 by protein weight) at various total protein concentrations were examined. The average volume-surface diameter (d_{32}) and the total surface protein concentration of emulsion droplets were similar to those of emulsions made with both sodium caseinate alone and WPC alone. Whey proteins were adsorbed in preference to caseins at low protein concentrations (<3%), whereas caseins were adsorbed in preference to whey proteins at high protein concentrations. The creaming stability of the emulsions decreased markedly as the total protein concentration of the system was increased above 2% (sodium caseinate >1%). This was attributed to depletion flocculation caused by the sodium caseinate in these emulsions. Whey proteins did not retard this instability in the emulsions made with mixtures of sodium caseinate and WPC.

1. Introduction

Milk proteins are well-known surfactants and hence are used as ingredients in a wide range of formulated food systems (Mulvihill, 1992). Milk proteins, either individual molecules or in the form of aggregates, become adsorbed rapidly at the new oil/water interface during emulsification (Walstra & Smulders, 1997). However, the state of the protein in the bulk solution influences its adsorption behaviour and the composition of the protein interfacial layer, which subsequently influences the stability of the emulsion (Dalgleish, 1995; Damodaran, 2004; Dickinson, 2001; Dickinson & McClements, 1995; Dickinson & Parkinson, 2004; McClements, Monahan, & Kinsella, 1993; Parkinson & Dickinson, 2004; Srinivasan, Singh, & Munro, 1996).

Sodium caseinate, a widely used food ingredient produced from milk casein, exists in aqueous solution at neutral pH as a mixture of casein monomers (α_{s1}-, α_{s2}-, β- and κ-casein) (Mulvihill, 1992) and small casein aggregates (so-called 'sub-micelles') (Pepper & Farrell, 1982). In contrast to the caseins, the whey proteins (β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins) are characterised by well-defined three-dimensional structures held together by disulphide bridges; these proteins are much more rigid than the caseins (Kinsella, 1984).

In oil-in-water emulsions stabilised by milk proteins, some competitive adsorption of the milk proteins at the interface occurs during emulsification (Dickinson, Hunt, & Dalgleish, 1991), which may cause differences in the composition of the protein layer. Most investigations of the adsorption behaviour of proteins and the properties of emulsions made with milk proteins have involved simple model systems using purified proteins (Dickinson, 2001). However, because mixtures of milk proteins are generally used in food emulsions, it is of interest to look at more complex mixtures of proteins. For example, β-casein, which is more surface-active than the other caseins, was shown to adsorb in preference to α_{s1}-casein in emulsions stabilised by a model mixture of β-casein and α_{s1}-casein (Dickinson & Stainsby, 1988). However, no preference for α_{s1}-casein or β-casein in sodium-caseinate-stabilised emulsions was observed by Hunt and Dalgleish (1994). Euston, Singh, Munro, and Dalgleish (1996) and 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 the emulsions. At low protein concentration, β-casein was adsorbed in preference to α_s-casein, whereas a larger amount of α_s-casein than of β-casein was present at the interface at high protein concentrations. High amounts of κ-casein at the surface were observed by Srinivasan, Singh, and Munro (2000), in contrast to other reports (Hunt & Dalgleish, 1994). The structure of the adsorbed caseins on the surface is not clear.

In emulsions formed with caseinate and whey protein, Hunt and Dalgleish (1994) reported that there was no preferential adsorption between caseinate and whey protein at low concentrations, but that the amount of caseinate at the surface was much greater than the amount of whey protein at high concentrations. In addition, no preferential adsorption between β-lactoglobulin (β-lg) and α-lactalbumin (α-la) was reported by some workers (Dickinson, Flint,

* Tel.: +64 6 350 4649x7564; fax: +64 6 350 4660.
E-mail address: aiqian.ye@fonterra.com

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.091

& Hunt, 1989; Euston et al., 1996; Hunt & Dalgleish, 1994). However, Closs, Le Meste, Courthaudon, Colas, and Lorient (1993) found that β-lg was adsorbed in preference to α-la in emulsions formed with whey proteins, or with mixtures of β-lg and α-la. More information on the structure of the individual proteins in mixed films containing several different proteins is needed, especially when proteins are prone to self-assembly in concentrated solutions.

Very low creaming stability, observed in emulsions made with relatively high sodium caseinate concentration, has been attributed to depletion flocculation, with respect to unbound or non-adsorbed caseinate sub-micelles (Dickinson & Golding, 1997; Srinivasan et al., 2000; van Dam, Watts, Campbell, & Lips, 1995). However, the creaming stability of emulsions made with mixtures of caseinate and whey protein is not known. It would be of interest to determine whether the flocculation is affected by the presence of non-aggregated proteins.

This work examined the influence of protein concentration on the interfacial protein composition and the stability of emulsions made with mixtures of sodium caseinate and whey protein concentrate (WPC).

2. Materials and methods

2.1. Materials

Sodium caseinate (Alanate 180) and WPC (ALACEN 342) were obtained from Fonterra Co-operative Group Ltd, Auckland, New Zealand. 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, USA), unless otherwise specified.

2.2. Emulsion preparation

Protein solutions of different concentrations were prepared by adding the sodium caseinate and WPC powder to Milli-Q water (Millipore Corp., Bedford, MA), and then stirring for 60 min at room temperature to ensure complete dispersion. The pH of the solutions was 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 homogenised in a two-stage valve homogeniser (Rannie a/s, Roholmsvej 8, DK 2620 Albertslund, Denmark) at 20 MPa for the first stage and 4 MPa for the second stage. At least two separate emulsions were prepared for each treatment.

2.3. Determination of average droplet size

A Malvern MasterSizer MSE (Malvern Instruments Ltd, Malvern, Worcestershire, UK) was used to determine the average diameter of the emulsion droplets. The parameters that were used to analyse the droplet size distribution were defined by the presentation code 2NAD. The relative refractive index (N), i.e., the ratio of the refractive index of the emulsion droplets (1.456) to that of the dispersion medium (1.33), was 1.095. The absorbance value of the emulsion droplets was 0.001. Droplet size measurements are reported as the Sauter-average diameter, d_{32} ($= \sum n_i d_i^3 / \sum n_i d_i^2$, where n_i is the number of droplets with diameter d_i). Mean droplet diameters were calculated as the average of duplicate measurements.

2.4. Determination of surface protein concentration and composition

The emulsions were centrifuged at 45,000g 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 deionised water and re-centrifuged at 45,000g for 40 min. The subnatant was filtered sequentially through 0.45 and 0.22 μm filters (Millipore Corp.). The filtrates were analysed separately for total protein using the Kjeldahl method (1026 Distilling Unit and 1007 Digestor Block, Tecator AB, Hoganas, Sweden). The surface protein concentration (mg/m^2) was calculated from the surface area of the oil droplets, determined by MasterSizer, and the difference between the amount of protein used to prepare the emulsion and that measured in the subnatant after centrifugation.

The composition of the protein adsorbed at the surface of the emulsion droplets was determined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Ye and Singh (2000). A certain amount of cream was spread on to 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 SDS gels previously prepared on a Miniprotean 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 individual proteins and expressing the individual whey proteins and individual caseins as a fraction of the sum total.

Analysis of 12 separate emulsions, made with 3.0% sodium caseinate, 3.0% WPC and 30% soya oil, showed that the variations were ±0.02 μm for d_{32}, ~4% for surface protein concentration, ~4% for α_s-casein, ~4% for β-casein, ~5% for κ-casein, ~4% for α-la, and ~4% for β-lg.

2.5. Creaming stability

The method and the calculations used to determine the stability rating have been described by Ye and Singh (2000). Immediately after preparation, emulsions (30 g) were transferred into centrifuge tubes and maintained at 20 °C for 24 h. The samples were then centrifuged at 185g 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:

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

Analysis of six separate emulsions, made with 3.0% sodium caseinate and 30% soya oil, showed that the variation for the stability rating was ~5%.

2.6. Confocal laser microscopy

A Leica (Heidelberg, Germany) confocal scanning laser microscope 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 could reach the detector system) was used to determine the microstructure of the emulsions. Emulsions were made as described above. About 3 ml of sample was taken in a test tube, Nile Blue (fluorescent dye) was mixed through and then the sample was placed on a microscope slide. The slide was then covered with a coverslip and observed under the microscope.

3. Results and discussion

3.1. Average droplet size and interfacial composition of emulsions

The average droplet size (d_{32}) of emulsions made with sodium caseinate, WPC, and mixtures of sodium caseinate and WPC (1:1

by weight), as a function of protein concentration, is shown in Fig. 1. The d_{32} values of emulsions made with mixtures of sodium caseinate and WPC were almost identical to those of emulsions made with sodium caseinate alone and WPC alone at a given protein concentration. The average droplet sizes (0.50 ± 0.1 μm) slightly changed with the protein concentration in the range 1–6%. The droplet sizes of the emulsions were larger at low protein concentrations (below 1.0%).

Fig. 2 shows that the total surface protein concentrations and the surface concentrations of individual caseins or whey proteins changed with a change in the concentration of sodium caseinate or WPC when used alone to form the emulsion. In emulsions made with sodium caseinate, the total surface protein concentration increased gradually with an increase in the protein concentration (~2.6 mg/m^2 at 5% caseinate). At caseinate concentrations ⩽2%, β-casein was preferentially adsorbed at the droplet surface; however, for caseinate concentrations >2%, α_s-casein was adsorbed in preference to the other caseins (Fig. 2a). At all concentrations, κ-casein appeared to be less readily adsorbed. The total surface protein concentrations of emulsions made with WPC were slightly higher, compared with those of emulsions made with sodium caseinate, when the protein concentrations were <3.0% (Fig. 2b). At 3.0%, the surface concentrations were very similar (~2.0 mg/m^2) for both caseinate-stabilised emulsions and WPC-stabilised emulsions. Beyond this point, the sodium-caseinate-stabilised emulsions had higher surface concentration than the WPC-stabilised emulsions. Similar trends have been reported (Fang & Dalgleish, 1993; Hunt & Dalgleish, 1994), although the surface concentration values were lower in this study. The surface concentrations of α-la and β-lg in the emulsion droplets formed with WPC changed with WPC concentration (Fig. 2b), but the relative proportions of α-la (~18%) at the interface were slightly lower, and the relative proportions of β-lg (~82%) were slightly higher, than those in the original WPC. The proportions of α-la and β-lg in the original WPC used in this experiment were ~23% and ~77% respectively. This indicated that there was a slight preference for the adsorption of β-lg. This result is in agreement with Closs et al. (1993), who found that β-lg was adsorbed in preference to α-la on the oil droplets in emulsions formed with whey proteins or mixtures of β-lg and α-la. However, no preferential adsorption between β-lg and α-la was observed in other work (Dickinson et al., 1989; Euston et al., 1996; Hunt & Dalgleish, 1994).

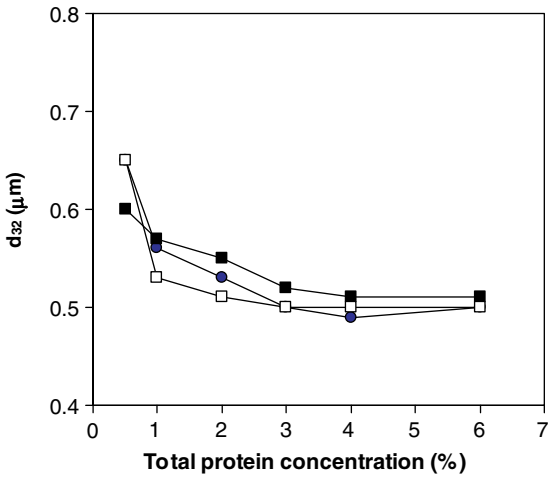

Fig. 1. Average droplet size (d_{32}) of emulsions made with a binary mixture of sodium caseinate and WPC (1:1 by weight) (●), sodium caseinate (■), and WPC (□), in 30% soya oil, pH 7.0, as a function of protein concentration. Each data point is the average of determinations on three separate emulsions.

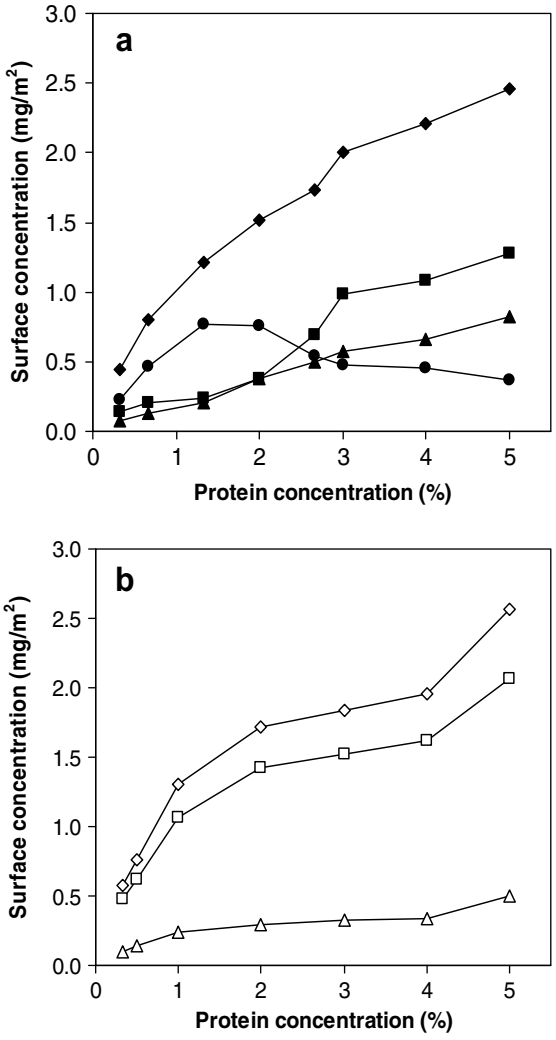

Fig. 2. Surface protein concentrations of α_s-casein (■), β-casein (●), κ-casein (▲), β-lg (□), and α-la (▽), in the cream phase of emulsions made with various concentrations of sodium caseinate (a) and WPC (b), in 30% soya oil, pH 7.0. Total surface protein concentrations (♦, ◇).

The proportions and the surface concentrations of caseins and whey proteins at the surface of emulsions made with mixtures of sodium caseinate and WPC as a function of protein concentration are shown in Fig. 3. Whey proteins adsorbed in preference to caseins at protein concentrations below 3%. In contrast, the proportions of whey proteins at the surface were lower than those of caseins at protein concentrations >3% (Fig. 3a).

The total surface protein concentration increased gradually with an increase in the protein concentration used to make emulsions from 0.5% to 4%, and then increased markedly at protein concentrations >4%.

The surface casein concentration increased gradually from ~0.3 to ~0.8 mg/m^2, as the total protein concentration increased from 0.5% to 4%, and then increased markedly at protein concentrations >4%. The surface whey protein concentration increased markedly at total protein concentrations <1% and then remained almost constant at ~0.8 mg/m^2 for total protein concentrations from 1% to 4%. Beyond 4%, the surface whey protein concentration increased again, but the increase was less than the increase in the surface casein concentration (Fig. 3b). This indicated that whey proteins adsorbed in preference to caseins, when low protein concentrations were used to make the emulsions. However, it reached sufficient at interface at >1% total protein used to form emulsions; this

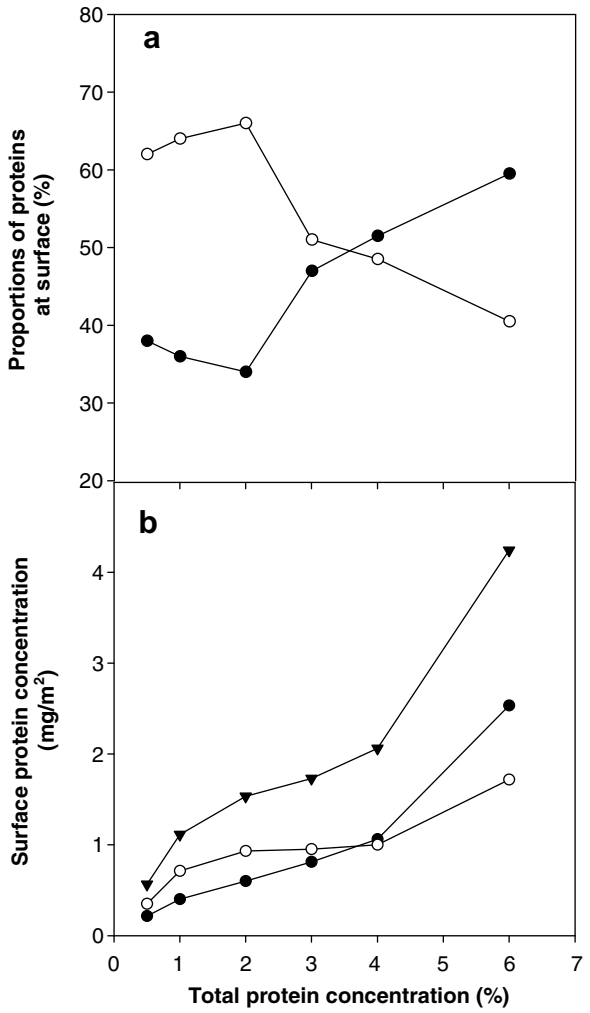

Fig. 3. Changes in the relative proportions (a) and surface concentrations (b) of caseins (●) and whey proteins (○), as well as total surface protein concentration (▼) at the droplet surface, in emulsions made with a binary mixture of sodium caseinate and WPC (1:1 by weight), in 30% soya oil, pH 7.0, as a function of the protein concentration in the mixture. Each data point is the average of determinations on two separate emulsions.

was the same as the adsorption behaviours of whey protein in the emulsions formed with whey protein alone (Hunt & Dalgleish, 1994). Thus the increase in total surface protein concentration at protein concentrations between 1% and 4% may be attributed to the increase in the surface casein concentration. Hunt and Dalgleish (1994) suggested that a protein concentration of 1% is sufficient to provide monolayer coverage of the nascent interfacial area during homogenisation. The addition of more protein to the system increases the surface protein concentration only slightly. The increase in the surface casein concentration is likely to be a result of closer packing of the adsorbed casein proteins in the monomolecular layer (Dalgleish, 1995; Hunt & Dalgleish, 1994; Srinivasan et al., 1996). Nylander and Wahlgren (1994) suggested that the adsorbed casein molecules form a rather tenuous, extended layer on oil/water interfaces.

At total protein concentrations >4%, the marked increase in the total surface protein concentration occurred as a result of increases in the concentrations of both casein and whey protein at the interface, although the increase in surface casein was greater than the increase in surface whey protein (Fig. 3b). A similar trend of preferential adsorption of caseins in emulsions made with binary mixtures of sodium caseinate and whey protein at high protein concentration has been observed by Hunt and Dalgleish (1994).

The sharp increase in surface protein concentration with the addition of more protein seems to suggest the formation of a secondary layer of adsorbed protein around the emulsion droplet. A similar result was reported by Srinivasan et al. (1996) in emulsions made with sodium caseinate; they suggested that an increase in caseinate concentration may cause the formation of casein aggregates in solution, which may subsequently be adsorbed at the interface, resulting in high values of surface casein concentration. Otherwise, Dickinson and McClements (1995) considered that, at low bulk protein concentration, an individual protein molecule arriving at the interface can unfold unimpeded by the presence of any surrounding molecules. In a concentrated system, on the other hand, an adsorbing molecule will probably have insufficient time to unfold before other protein molecules adsorb nearby. These neighbouring molecules in the adsorbed layer restrict subsequent unfolding through a combination of steric hindrance and electrostatic repulsion at the oil/water interface, and so the free energy gained by an isolated region is correspondingly reduced.

Fig. 4a shows the proportions of individual proteins on the surface of emulsion droplets made with mixtures of sodium caseinate and WPC. At low total protein concentrations (⩽1%), the proportions of adsorbed β-lg and α-la were higher than those in the original protein solutions, whereas the proportions of all caseins were

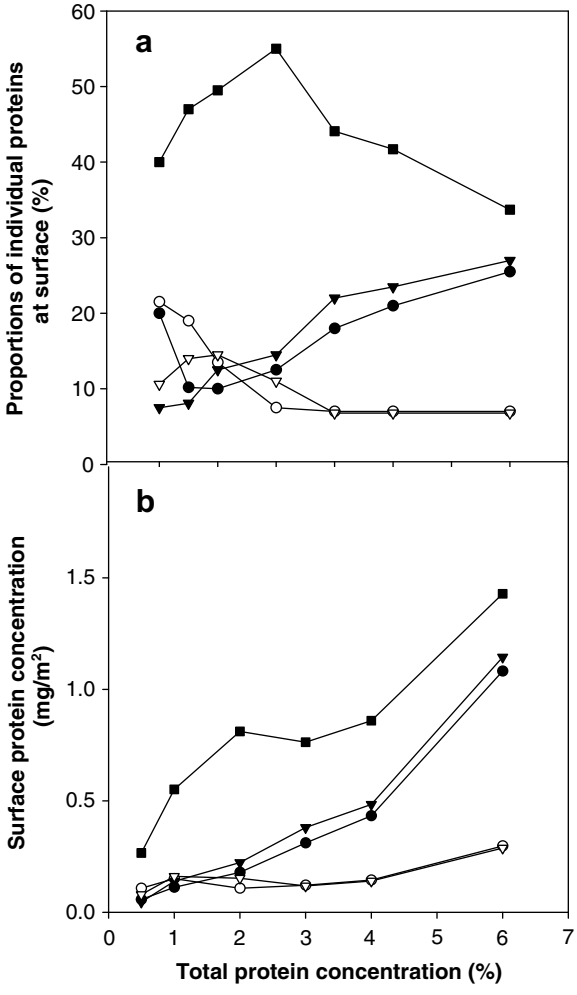

Fig. 4. Changes in the relative proportions (a) and surface concentrations (b) of α_s-casein (●), β-casein (○), κ-casein (▼), α-la (▽), and β-lg (■), at the droplet surface of emulsions made with a binary mixture of sodium caseinate and WPC (1:1 by weight), in 30% soya oil, pH 7.0, as a function of the protein concentration in the mixture. Each data point is the average of determinations on two separate emulsions.

lower than those in the original protein solutions used to make the emulsions, with β-casein being present in greater proportion. The proportion of β-lg increased to a maximum at 2.0% total protein concentration, and then decreased; α-la and β-casein decreased and then remained constant beyond 3% total protein, whereas α_s-casein and κ-casein increased with an increase in concentration of the protein mixture.

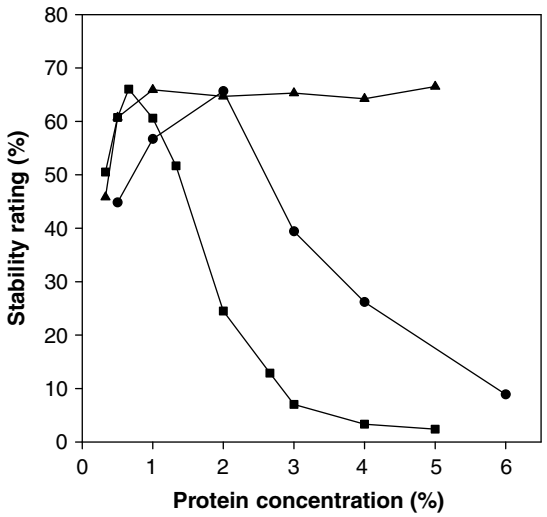

Fig. 5. Stability rating (%) of emulsions made with a binary mixture of sodium caseinate and WPC (1:1 by weight) (●), sodium caseinate alone (■), and WPC alone (▲), in 30% soya oil, pH 7.0, as a function of protein concentration. Each data point is the average of determinations on two separate emulsions.

The changes in estimated surface concentration of these individual proteins in emulsions formed with binary protein mixtures as a function of protein concentration in the mixtures is shown in Fig. 4b. The surface concentrations of α_s-casein and κ-casein increased with an increase in total protein concentration in the mixtures; those of β-casein and α-la remained almost constant and were very low. The surface concentration of β-lg increased markedly with an increase in the total protein concentration to 2%, remained constant from 2% to 4%, and then increased again with further increase in the protein concentration in the mixture.

The different amounts of individual proteins adsorbed at the surface (Fig. 4) may be attributed mainly to different states of protein molecular structure at the surface. Greater adsorption of whey proteins at low concentration may be due to less spreading of the globular whey protein molecules on the surface; in particular, β-lg may adsorb at the surface as a dimer structure (Mackie, Mingins, & Dann, 1993). Greater adsorption of caseins (α_s- and κ-) at high protein concentration may be because they adsorb as aggregated structures on the surface, whereas β-casein and α-la may adsorb in a monomolecular state.

The concentration dependence of the competitive adsorption behaviour of α_{s1}-casein and β-casein in sodium caseinate emulsions is curious. Because of its high surface activity (Dickinson & Stainsby, 1988), the preferential adsorption of β-casein appears to exist only at low concentrations, when caseins exist as monomers. With increasing protein concentration, the caseins aggregate to form various complexes of different compositions and sizes (Lucey, Srinivasan, Singh, & Munro, 2000; Pepper & Farrell, 1982). β-Casein may lose its competitive ability because of its self-aggregation to form micelles or through the formation of complexes with other caseins. Therefore, the surface composition of emulsions

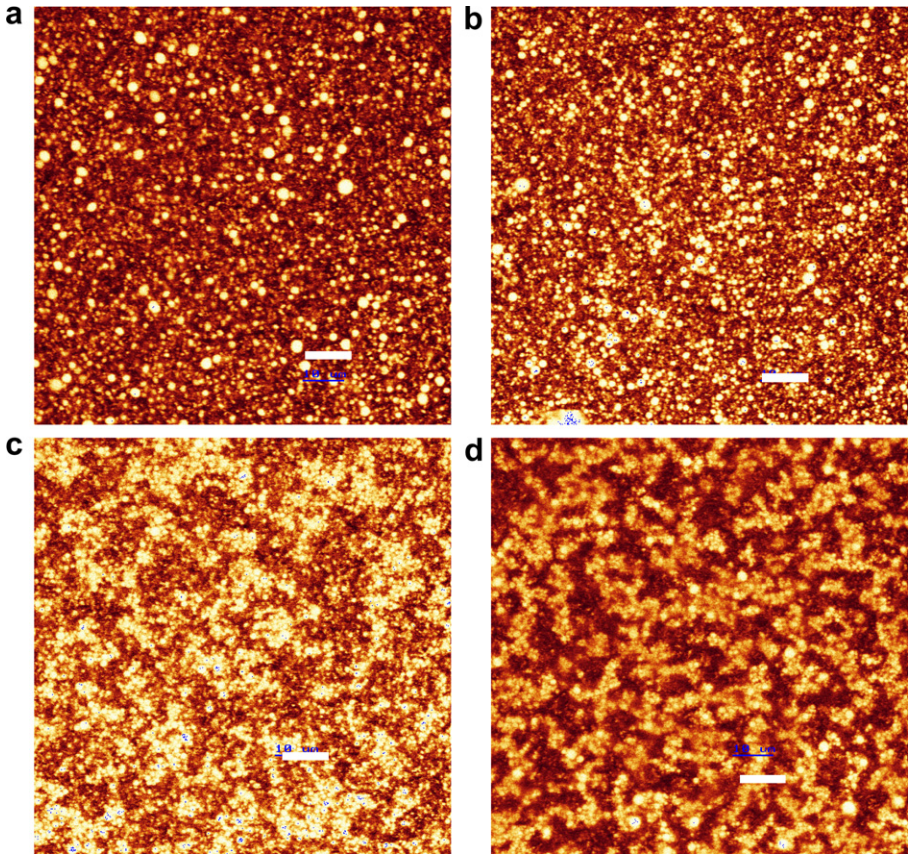

Fig. 6. Confocal micrographs of oil-in-water emulsions (30% soya oil, pH 7.0) made with binary mixtures of sodium caseinate and WPC (1:1 by weight), of total protein concentration 1% (a), 2% (b), 3% (c), and 4% (d).

formed using a relatively high sodium caseinate concentration is likely to be determined by the surface activities of the casein aggregates and complexes. Although extensive information on the surface activity and hydrophobicity of individual caseins is available, little is known about how these characteristics are modified when casein molecules undergo self-association under different conditions.

3.2. Creaming stability of emulsions

The stability rating of emulsions formed with mixtures of sodium caseinate and WPC (1:1 by weight), as a function of total protein concentration, is shown in Fig. 5. For comparison, the stability ratings of emulsions made with sodium caseinate alone and WPC alone are also shown. The stability of the emulsions formed with protein mixtures reached a maximum at 2% and then decreased markedly as the total protein concentration increased further. This trend in stability with concentration was similar to that of sodium-caseinate-stabilised emulsions, but the plot was shifted to higher concentration (about two-fold). The stability rating of the 4% binary protein mixture emulsion was similar to that of the 2% sodium caseinate emulsion. In addition, the stability ratings obtained at 3% and 6% binary protein concentration were comparable with those obtained at 1.5% and 3% sodium caseinate concentration (Fig. 5). This indicated that the stabilities of the emulsions would be similar for emulsions made with sodium caseinate alone and a binary protein mixture (sodium caseinate and WPC) containing identical concentrations of sodium caseinate, and that the whey protein in the system did not affect the stability of the emulsions.

Low stabilities of emulsions made with relatively high concentrations of sodium caseinate have been reported (Dickinson & Golding, 1997 and Srinivasan et al., 2000), and have been attributed to depletion flocculation caused by non-adsorbed sodium caseinate in the aqueous phase (Dickinson & Golding, 1997). Dickinson and Golding (1997) reported that the extent of deletion flocculation is dependent mainly on the size and concentration of unbound or non-adsorbed protein; Radford and Dickinson (2004) found that the caseinate particles (15–20 nm) and 2–3% concentration in the aqueous phase were responsible for the initial discernible flocculation. The results in the present study suggest that depletion flocculation of emulsions caused by non-adsorbed caseinate in the aqueous phase will occur when the caseinate in the aqueous phase reaches a critical concentration, whether or not whey protein exists in the system; whey protein, involved in the formation of the emulsions, did not affect the depletion flocculation phenomenon.

3.3. Confocal micrographs of emulsions

Fig. 6 shows confocal micrographs of emulsions made with binary mixtures of sodium caseinate and WPC (1:1 by weight) at various concentrations. In emulsions containing 1% or 2% protein, the emulsion droplets appeared to be homogeneous with no sign of flocculation (Fig. 6a and b). Emulsions containing 3% or 4% protein showed large numbers of small particles aggregated together and separated from the aqueous phase to form a network structure (Fig. 6c and d). The aggregated network structure, with respect to the depletion flocculation formed with high concentrations of caseinate in emulsions, has been described in previous work (Dickinson & Golding, 1997; Srinivasan et al., 2000; Ye & Singh, 2001).

4. Conclusions

The interfacial composition and the stability of emulsions made with mixtures of sodium caseinate and WPC (1:1 by weight) were dependent on the protein concentration used to form the emulsion. At low protein concentrations (<3%), the surface contained higher proportions of whey proteins than caseins. In contrast, caseins were adsorbed in preference to whey proteins at high protein concentrations. The different amounts of adsorbed individual proteins at the surface could be attributed mainly to different states of protein molecular structure at the surface, which was related to the concentration at the surface. An increase in the creaming stability of emulsions with an increase in the protein concentration was observed at low concentrations. However, the creaming stability decreased markedly as the total protein concentration of the system was increased above 2% (sodium caseinate >1%). This was attributed to depletion flocculation, caused by sodium caseinate occurring in these emulsions. The whey proteins in the system did not retard this instability in emulsions made with mixtures of sodium caseinate and WPC.

References

Closs, B., Le Meste, M., Courthaudon, J. L., Colas, B., & Lorient, D. (1993). Interactions between whey proteins and lipids in emulsions. In E. Dickinson & P. Walstra (Eds.), *Food colloids and polymers: Stability and mechanical properties* (pp. 571–575). Cambridge: The Royal Society of Chemistry.

Dalgleish, D. G. (1995). Structures and properties of adsorbed layers in emulsions containing milk proteins. In E. Dickinson & D. Lorient (Eds.), *Food macromolecules and colloids* (pp. 23–34). Cambridge: The Royal Society of Chemistry.

Damodaran, S. (2004). Adsorbed layers formed from mixtures of proteins. *Current Opinion in Colloid & Interface Science, 9*, 328–339.

Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability and rheology. *Colloids and Surfaces B: Biointerfaces, 20*, 197–210.

Dickinson, E., Flint, F. O., & Hunt, J. A. (1989). Bridging flocculation in binary protein stabilized emulsions. *Food Hydrocolloids, 3*, 389–397.

Dickinson, E., & Golding, M. (1997). Depletion flocculation of emulsions containing unadsorbed sodium caseinate. *Food Hydrocolloids, 11*, 13–18.

Dickinson, E., Hunt, J. A., & Dalgleish, D. G. (1991). Competitive adsorption of phosvitin with milk proteins in oil-in-water emulsions. *Food Hydrocolloids, 4*, 403–414.

Dickinson, E., & McClements, D. J. (1995). *Advances in food colloids*. Glasgow: Blackie Academic & Professional.

Dickinson, E., & Parkinson, E. L. (2004). Heat-induced aggregation of milk protein-stabilized emulsions: Sensitivity to processing and composition. *International Dairy Journal, 14*, 635–645.

Dickinson, E., & Stainsby, G. (1988). Emulsion stability. In E. Dickinson & G. Stainsby (Eds.), *Advances in food emulsions and foams* (pp. 1–44). London: Elsevier Applied Science.

Euston, S. E., Singh, H., Munro, P. A., & Dalgleish, D. G. (1996). Oil-in-water emulsions stabilized by sodium caseinate or whey protein isolate as influenced by glycerol monostearate. *Journal of Food Science, 61*, 916–920.

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.

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.

Kinsella, J. E. (1984). Milk proteins: Physicochemical and functional properties. *CRC Critical Reviews in Food Science and Nutrition, 21*, 197–262.

Lucey, J. A., Srinivasan, M., Singh, H., & Munro, P. A. (2000). Characterization of commercial and experimental sodium caseinates by multiangle laser light scattering and size-exclusion chromatography. *Journal of Agricultural and Food Chemistry, 48*, 1610–1616.

Mackie, A. R., Mingins, J., & Dann, R. (1993). Preliminary studies of β-lactoglobulin adsorbed on polystyrene latex. In E. Dickinson & P. Walstra (Eds.), *Food colloids and polymers: Stability and mechanical properties* (pp. 96–112). Cambridge: The Royal Society of Chemistry.

McClements, D. J., Monahan, F. J., & Kinsella, J. E. (1993). Disulfide bond formation affects stability of whey protein isolate emulsions. *Journal of Food Science, 58*, 1036–1039.

Mulvihill, D. M. (1992). Production, functional properties and utilization of milk protein products. In P. F. Fox (Ed.), *Advanced dairy chemistry – 1. Proteins* (pp. 369–405). London: Elsevier Applied Science.

Nylander, T., & Wahlgren, N. M. (1994). Competitive and sequential adsorption of β-casein and β-lactoglobulin on hydrophobic surfaces and the interfacial structure of β-casein. *Journal of Colloid and Interface Science, 162*, 151–162.

Parkinson, E. L., & Dickinson, E. (2004). Inhibition of heat-induced aggregation of a beta-lactoglobulin-stabilized emulsion by very small additions of casein. *Colloids and Surfaces B: Biointerfaces, 39*, 23–30.

Pepper, L., & Farrell, H. M. (1982). Interactions leading to formation of casein submicelles. *Journal of Dairy Science, 65*, 2259–2266.

Radford, S. J., & Dickinson, E. (2004). Depletion flocculation of caseinate-stabilised emulsions: What is the optimum size of the non-adsorbed protein

nano-particles? *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 238*, 71–81.

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. (2000). The effect of sodium chloride on the formation and stability of sodium caseinate emulsions. *Food Hydrocolloids, 14*, 497–507.

van Dam, B. M. C., Watts, K. M., Campbell, I. J., & Lips, A. (1995). On the stability of milk protein-stabilized concentrated oil-in-water food emulsions. In E. Dickinson & D. Lorient (Eds.), *Food macromolecules and colloids* (pp. 215–222). Cambridge: The Royal Society of Chemistry.

Walstra, P., & Smulders, I. (1997). Making emulsions and foams: An overview. In E. Dickinson & B. Bergenstahl (Eds.), *Food colloids: Proteins, lipids and polysaccharides* (pp. 367–381). London: The Royal Society of Chemistry.

Ye, A., & Singh, H. (2000). Influence of calcium chloride addition on the properties of emulsions stabilized by whey protein concentrate. *Food Hydrocolloids, 14*, 337–346.

Ye, A., & Singh, H. (2001). Interfacial composition and stability of sodium caseinate emulsions as influenced by calcium ions. *Food Hydrocolloids, 15*, 195–207.