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Emulsifying properties of protein fractions prepared from heated milk

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Heated milk was separated into κ-casein/whey protein–rich and κ-casein–depleted fractions using centrifugation. The emulsifying properties of the two fractions were compared to those of sodium caseinate, whey protein isolate and casein micelles. Fat surface areas, which formed on emulsification at different power inputs, were essentially similar for both fractions and also for casein micelles, but were considerably lower than those for sodium caseinate and whey protein isolate. Protein load (mg protein/m^2 fat surface area) decreased with increasing fat surface area and followed the order: casein micelles > κ-casein–depleted micelles ≈ whey protein/κ-casein–rich fraction > sodium caseinate > whey protein isolate. Emulsions stabilized by either fraction and by casein micelles were more stable at low fat surface areas than those stabilized by sodium caseinate or whey protein isolate. Emulsification capacities of the two fractions were lower than those of the other three proteins. It appeared that the size of the protein aggregates is more important than their composition in determining the emulsifying properties.

INTRODUCTION

Milk proteins isolate, e.g. caseins/caseinates, whey protein concentrates and whey protein isolates, are used as functional food ingredients in a wide variety of food products. Caseinates have been found to be more surface active than whey proteins owing to their random structure and non-uniform distribution of hydrophobic and hydrophilic residues (Mitchell *et al.*, 1970; Tornberg, 1978*a,b*; Phillips, 1981). However, whey proteins form more stable emulsions than caseins. The functionality of individual caseins, i.e. α_{s1}, -α_{s2}-, β- and κ- also differs significantly, β-casein being the most surface active (see Mulvihill & Fox, 1989).

Heat treatment of milk at temperatures greater than 80°C for several minutes causes whey proteins to denature and form a complex with the casein micelles via interaction with κ-casein. The casein–whey protein complex can be isolated from heated milk by acidification and/or addition of $CaCl_2$, resulting in a product known as casein–whey protein co-precipitates, the functional properties of which have been studied by Southward & Goldman (1978) and Grufferty & Mulvihill (1991). Recent studies have shown that when milk is heated at a pH value above 6·9, κ-casein dissociates from the micelles and most of the κ-casein/whey protein complexes are present in the serum phase (Singh & Fox, 1985, 1986). Concentration of milk before heating increases the extent of heat-induced dissociation of micellar κ-casein and lowers the pH at which dissociation occurs (Singh & Creamer, 1991*a*). Following dissociation of micellar κ-casein, the size of the casein micelles increases markedly (Singh & Creamer, 1991*b*). Based on these observations, milk can easily be fractionated by centrifugation into κ-casein/whey protein–rich and κ-casein–depleted fractions which may have different functional properties.

This report describes the centrifugal separation of protein fractions from heated milk and compares the emulsifying properties of these fractions with sodium caseinate, whey protein isolate (WPI) and casein micelles.

MATERIALS AND METHODS

Low-heat skim-milk powder was obtained from the New Zealand Dairy Research Institute, Palmerston

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North. Whey protein isolate (WPI) was obtained from Lesueur Isolates (Biopro Lesueur Isolates, Lesueur, MN, USA).

All chemicals were of analytical-reagent grade and were obtained from BDH Chemicals New Zealand Ltd (Palmerston North, New Zealand) or Sigma Chemical Co. (St Louis, MO, USA).

Fractionation of milk protein

Low-heat skim-milk powder was reconstituted at 20% (w/v) in distilled water. Samples of reconstituted milk were adjusted to pH 6·85 with 1 M NaOH and heated at 90°C for 5 min in a thermostatically controlled water bath. After cooling to room temperature, the samples were centrifuged at 25 000 *g* for 30 min. The supernatants were pooled and dialysed against distilled water for 24 h at 5°C. Both the sediment and the dialysed supernatant fractions were freeze-dried.

Casein micelles were prepared by centrifuging raw skim milk at 100 000 *g* for 1 h at 20°C. The recovered pellet was freeze-dried. Sodium caseinate was isolated from raw skim milk as described by Mulvihill & Murphy (1991).

Formation of emulsions

Emulsions were prepared from 30 g protein solution (2·5% (w/v)) and 20 g soybean oil using a valve homogenizer incorporated into a recirculating emulsification system in which the power input could be varied and measured, as described by Tornberg & Lundh (1978). The emulsion was recirculated through the valve for 10 passes at a flow velocity of 235 ml/min. Emulsions were prepared at power inputs ranging from 0 to 85 W.

Characterization of emulsions

Determination of average globule size and fat surface area
A spectroturbidimetric method (Walstra, 1965, 1968) was used to determine the average globule size, using a Pye-Unicam SP 500 spectrophotometer (Pye-Unicam Ltd, Cambridge, UK), modified as recommended by Walstra (1965). The fat surface area was calculated as described by Mulvihill & Murphy (1991).

Determination of protein load
The amount of protein adsorbed onto the fat surfaces was determined by measuring the protein content of the subnatant after separation of the fat and aqueous phases by centrifugation at 40 000 *g* for 40 min. A portion (2 ml) of well-mixed subnatant from each centrifuged emulsion was recovered and diluted 30-fold with distilled water. The solution was then filtered through a 0·22 mm Millipore filter (to remove any fat globules present) and the protein adsorbed on the fat surfaces calculated from the difference between the initial and final protein contents of the aqueous phase and expressed as protein load.

Creaming stability
The creaming stability, or stability rating (SR), was based on the percent change in fat in the subnatant after creaming, as described by Mulvihill & Murphy (1991). The emulsions (18 g) were transferred to tubes (21 mm in diameter and with tapered, stoppered ends) and held for 24 h at room temperature. A sample (5 g) of emulsion from the lower phase was carefully removed using a syringe and analysed for fat by the Gerber method as used for cream (British Standard, 1955). The stability rating was calculated as:

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

Emulsifying capacity

Emulsifying capacity was measured at protein concentrations of 0·25 and 0·50% (w/v), pH 7·0, using a commercial food blender (Waring type) as described by Mohanty *et al.* (1988). A sample (100 ml) of protein solution was placed in the bowl of the blender and increasing amounts of coloured (Sudan III) corn oil were added. After each addition, the mixture was homogenized at low speed for 1 min followed by high-speed blending for a further 1 min. After each addition of oil, the temperature of the mixture was brought to 20°C. The end-point was detected by a change in the visual appearance of the emulsion.

Viscosity

The viscosity of emulsions was measured at 20°C using a Brookfield viscometer (Model LTV, Brookfield Labs, Staughton, MA, USA).

Polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the method of Laemmli (1970), in which the separating gel was composed of 14% acrylamide in tris/HCl buffer, pH 8·8, and the stacking gel was composed of 4% acrylamide in tris/HCl buffer, pH 6·8. The gel was stained with Coomassie Blue R in 25% (v/v) isopropanol and 10% acetic acid. After destaining in 1 : 1 : 8 acetic acid/isopropanol/water, the gel was scanned on a laser densitometer (LKB, Ultroscan XL, LKB Produkter AB, Bromma, Sweden).

RESULTS AND DISCUSSION

Characterization of protein fractions

Centrifugation of heated reconstituted concentrated milk at 25 000 *g* for 30 min sedimented about 60% of the total protein. SDS-PAGE showed that the supernatant contained a higher proportion of κ-casein and

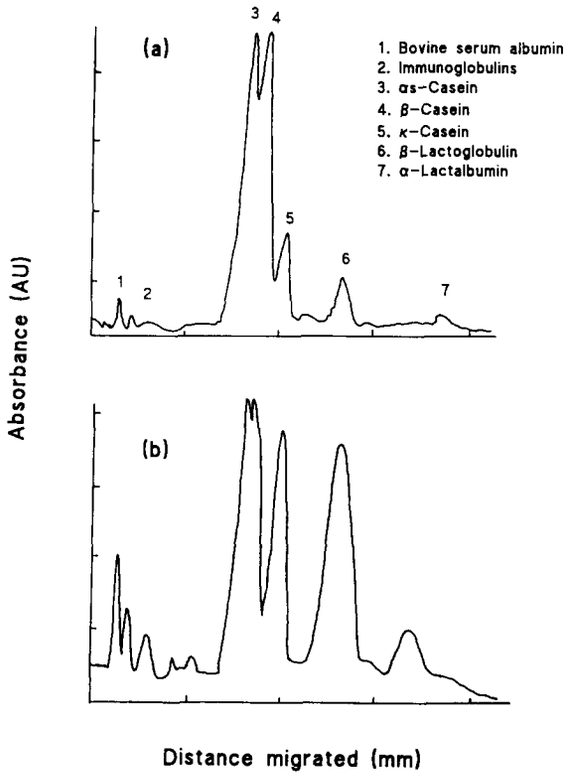

Fig. 1. Densitometric scans of electrophoretograms of the sediment (a) and the supernatant (b) fractions obtained by centrifugation (25 000 *g* for 30 min) of heated milk.

most of the whey proteins, while the sediment contained relatively little κ-casein (Fig. 1). The ratio of casein to whey protein in the supernatant was 1·31 : 1 as compared to 7·6 : 1 in the sediment. The ratios of α_s-, β- and κ-casein were 0·3 : 0·2 : 0·5 and 0·5 : 0·4 : 0·1 for supernatant and sediment, respectively.

The freeze-dried sediment contained about 70% (w/w) protein whereas the protein content of the freeze-dried supernatant was 64% (w/w). Generally, the sediment was less soluble than the supernatant, which may be due to the lower κ-casein content of the former. Both fractions were fully dispersed after stirring overnight at 5°C.

Emulsifying properties

The emulsions prepared using a valve homogenizer at different power inputs were characterized for fat globule size (i.e. the total surface area of the emulsion), the amount of protein adsorbed on the fat surface (i.e. protein load) and the ability of the protein to form a stable emulsion.

The fat surface area (FSA) increased for all emulsions as the power input increased, the extent of the increase being dependent upon the type of protein used to stabilize the emulsion (Fig. 2). The emulsions stabilized by supernatant, i.e. the whey protein/κ-casein–rich fraction, the sediment, i.e. κ-casein–depleted micelles, or casein micelles had similar FSA at power inputs below 60 W but at higher power inputs, emulsions stabilized by κ-casein–depleted micelles or the whey

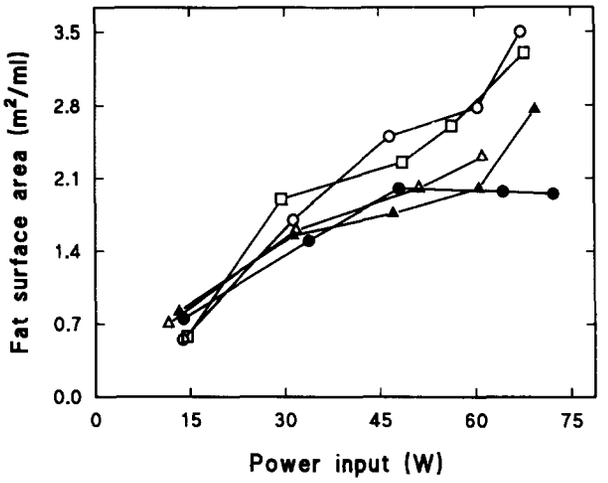

Fig. 2. Fat surface area created on emulsification as a function of power input for sodium caseinate (○), casein micelles (●), κ-casein-depleted micelles (Δ), whey protein/κ-casein–rich fraction (▲) or whey protein isolate (□) stabilized emulsions.

protein/κ-casein–rich fraction had greater FSAs than the casein micelle-stabilized emulsion. In general, FSA of emulsions stabilized by casein micelles, κ-casein–depleted micelles or the whey protein/κ-casein–rich fraction were lower than for those of emulsions stabilized by sodium caseinate or WPI, especially at power inputs above 30 W. The rate of increase in FSA with power input was similar for sodium caseinate or WPI-stabilized emulsions. The results obtained for sodium caseinate and WPI are in agreement with those of Tornberg (1978*a*) and Mulvihill and Murphy (1991).

The total amount of protein (g) adsorbed on the fat surface increased as the power input was increased for all the protein-stabilized emulsions, with the exception of those stabilized by casein micelles where the amount of protein adsorbed remained constant at 0·34 g over the entire range of power input (Fig. 3). This value was slightly lower than that (0·42 g) reported by Mulvihill and Murphy (1991). In the present study, casein micelles were freeze-dried which might have disrupted

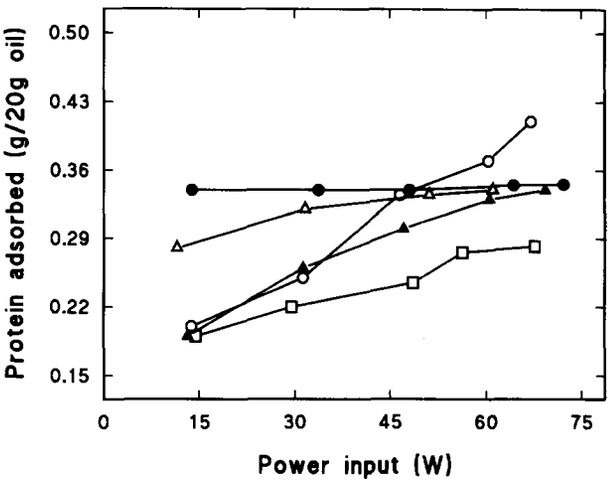

Fig. 3. Total protein adsorbed on emulsification as a function of power input for sodium caseinate (○), casein micelles (●), κ-casein–depleted micelles (Δ), whey protein/κ-casein–rich fraction (▲) or whey protein isolate (□) stabilized emulsions.

their structure. At lower power inputs (less than 40 W), the levels of protein adsorbed were in the order: casein micelles > κ-casein–depleted micelles > sodium caseinate ≈ whey protein/κ-casein–rich fraction > WPI, whereas at higher power inputs (>40 W), the levels of protein adsorbed were in the order: sodium caseinate > casein micelles > κ-casein–depleted micelles > whey protein/κ-casein–rich fraction > WPI.

The total amount of protein adsorbed on the fat surface as a function of increasing FSA is shown in Fig. 4. In general, the amount of protein adsorbed increased with increasing FSA for all the proteins, especially below 2·5 m^2/ml emulsion, except in casein micelles where protein adsorption was independent of the FSA.

Protein loads (mg protein/m^2 fat surface area) as a function of power input and FSA are shown in Figs 5 and 6. In general, protein loads decreased sharply as power input was increased to values in the range 15–30 W or as the fat surface area was increased from 0·5 to 1·5 m^2/ml emulsion but at higher power inputs or higher surface areas, protein loads changed only slightly. For emulsions stabilized by κ-casein-depleted micelles or whey protein/κ-casein-rich fraction, protein loads decreased with increasing power input or FSA to 3·5–4·0 m^2/ml. The minimum protein load for casein micelle-stabilized emulsions was 4·0 mg/m^2 at ~50 W. The protein loads for sodium caseinate and WPI were essentially similar and were lower than for the other three proteins. At the higher power inputs or FSA created on emulsification, protein loads ranged from 4·0 to 1·8 mg/m^2 and were in the order: casein micelles > κ-casein–depleted micelles ≈ whey protein/κ-casein–rich fraction > sodium caseinate > WPI.

From these results, it was apparent that aggregated protein systems, i.e. casein micelles, κ-casein–depleted micelles and the whey protein/κ-casein–rich fraction, had a lower ability to increase the FSA than highly dispersed protein systems, e.g. sodium caseinate or WPI. The decrease in protein load with increasing FSA probably reflected the spreading and rearrangement of protein molecules at the fat interface. At low FSA, multilayers of protein were probably formed at the fat interface, whereas at high FSA the layers of protein were much thinner, probably approaching monolayers. Therefore, the comparatively high protein loads obtained for all emulsions at low power inputs or small FSA were probably due mainly to coverage of the newly formed surface by a supply of protein from the bulk phase. At higher power inputs or large FSA, adsorption of protein from the bulk phase and spreading of protein already adsorbed were important.

The higher protein loads for the emulsions stabilized by the whey protein/κ-casein–rich fraction compared to those for sodium caseinate or WPI were probably due to higher concentrations of total protein being adsorbed at the fat interface, which may be due to differences in the sizes of protein aggregates in solution. It has been shown that the κ-casein/whey protein fraction consists of relatively large protein aggregates linked through disulphide bonds, with molecular

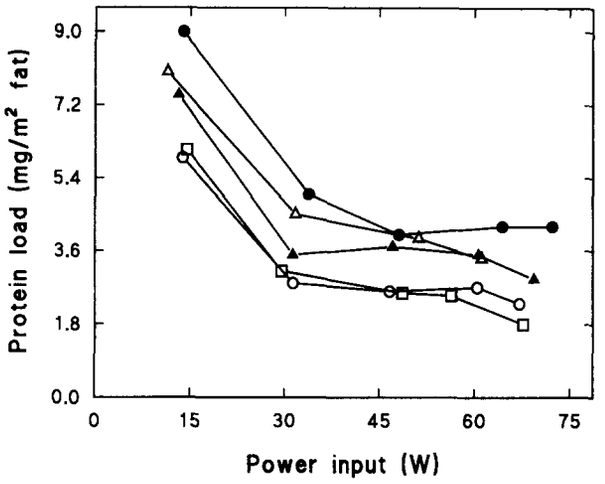

Fig. 5. Protein load as a function of power input for sodium caseinate (○), casein micelles (●), κ-casein-depleted micelles (Δ), whey protein/κ-casein-rich fraction (▲) or whey protein isolate (□) stabilized emulsions.

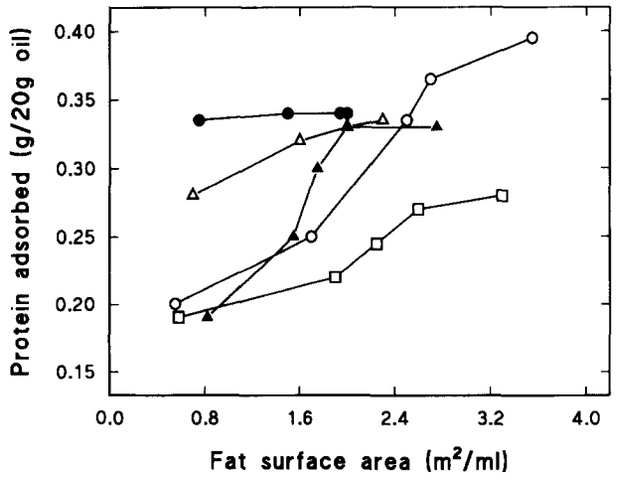

Fig. 4. Total protein adsorbed on emulsification as a function of fat surface area for sodium caseinate (○), casein micelles (●), κ-casein–depleted micelles (Δ), whey protein/κ-casein–rich fraction (▲) or whey protein isolate (□) stabilized emulsions.

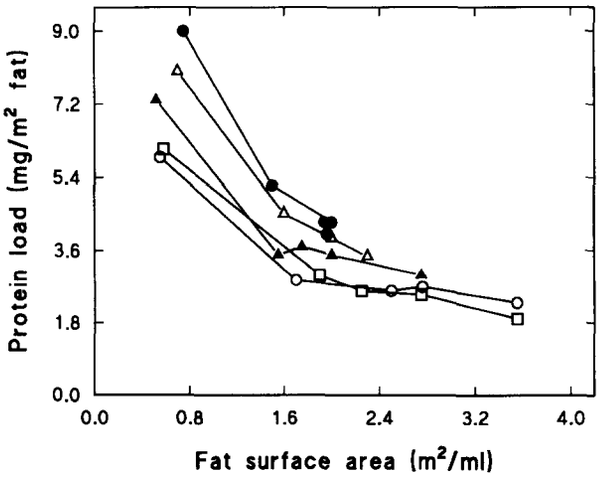

Fig. 6. Protein load as a function of fat surface area for sodium caseinate (○), casein micelles (●), κ-casein–depleted micelles (Δ), whey protein/κ-casein–rich fraction (▲) or whey protein isolate (□) stabilized emulsions.

weights greater than 2×10^6 (Singh & Creamer, 1991*b*). It might be expected that the concentration of protein at the interface would be higher for more aggregated proteins than for more highly dispersed proteins and, consequently, the greater the protein load for the former type of protein. The structure of aggregated protein and the type of bonding involved in holding the aggregates together may also be important, e.g. aggregates linked through covalent bonds are less likely to undergo spreading when adsorbed at the fat interface than those linked through non-covalent forces. A similar relationship between protein load and the aggregation state of protein has been demonstrated by Oortwijan and Walstra (1979) and Mulvihill and Murphy (1991). Oortwijan and Walstra (1979) reported protein loads of 2·5, 2·6 and 20 mg/m^2 in emulsions stabilized by whey protein, sodium caseinate and casein micelles, respectively. Mulvihill and Murphy (1991) reported highest protein loads for emulsions stabilized by casein micelles, lower protein loads for the less highly aggregated ethanol-precipitated casein and high calcium caseinate–stabilized emulsions, and lowest protein loads for the least aggregated low-calcium, sodium and ammonium caseinate–stabilized emulsions.

The stability ratings (SR), i.e. the percent change in fat in the subnatant of the protein-stabilized emulsions after 24 h, as a function of the power input are shown in Figs 7 and 8. The SR increased as the power input during emulsification was increased from 15 to 45 W or the FSA increased from 0·5 to 2·5 m^2/ml. At ~30 W or 1·5 mg/m^2 FSA, stability ratings were in the order: casein micelles > κ-casein–depleted micelles > κ-casein/whey protein–rich fraction > WPI > sodium caseinate. In general, the stability ratings of emulsions stabilized by casein micelles, κ-casein–depleted micelles or the whey protein/κ-casein–rich fraction were high at all FSA and were not influenced as much by increasing FSA as were emulsions stabilized by WPI or sodium caseinate. The viscosities of all emulsions formed were measured using a Brookfield viscometer (Model LVT,

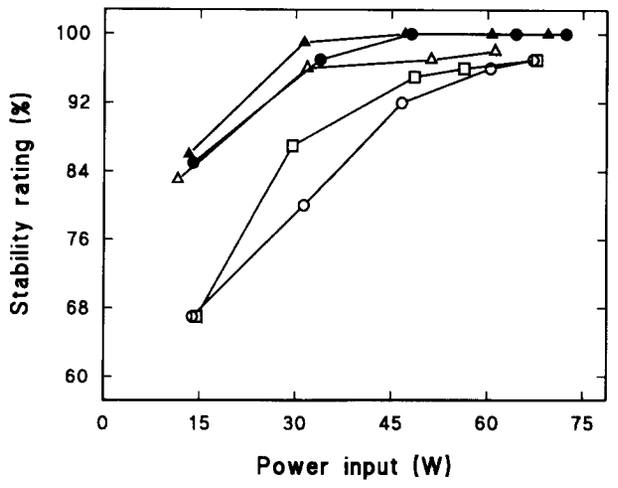

Fig. 7. Stability rating as a function of power input for sodium caseinate (○), casein micelles (●), κ-casein–depleted micelles (Δ), whey protein/κ-casein–rich fraction (▲) or whey protein isolate (□) stabilized emulsions.

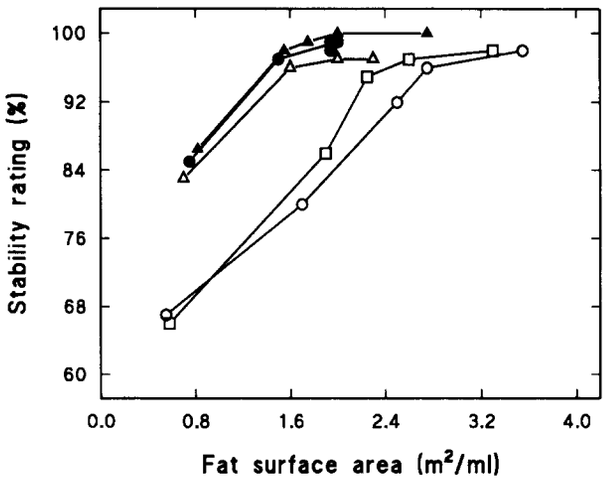

Fig. 8. Stability rating as a function of fat surface area for sodium caseinate (○), casein micelles (●), κ-casein–depleted micelles (Δ), whey protein/κ-casein–rich fraction (▲) or whey protein isolate (□) stabilized emulsion.

Brookfield Engineering Laboratories, Staughton, MA, USA). At all power inputs, the viscosity was in the order: whey protein/κ-casein–rich fraction > κ-casein–depleted micelles ≈ casein micelles > sodium caseinate > WPI. The lower stability ratings found for sodium caseinate and WPI-stabilized emulsions may be attributed to lower viscosity and less aggregated forms of these proteins compared to the other three proteins. Whey proteins form more stable emulsions than caseins, presumably because surface films of whey proteins are more viscous than surface films of caseins (Boyd *et al.*, 1973). Casein–whey protein co-precipitates, prepared from milk heated at neutral or alkaline pH values, form more stable emulsions than sodium caseinate (Southward & Goldman, 1978; Grufferty & Mulvihill, 1991). The presence of disulphide linkages between κ-casein and whey proteins in the whey protein/κ-casein–rich fraction could lead to the formation of a more cohesive, stable film around the fat globules.

Emulsifying capacity

In agreement with the results of previous investigations (Mulvihill & Murphy, 1991; Murphy & Fox, 1991), the volume of oil emulsified by all protein preparations was higher at higher protein concentrations (Table 1). At a protein concentration of 0·5% (w/v), the emulsification capacities were in the order: WPI > sodium caseinate > casein micelles ≈ κ-casein–depleted micelles > κ-casein/whey protein–rich fraction. It appears that highly aggregated proteins, which are unable to undergo conformational rearrangements because of constraints imposed by the covalent bonding or colloidal calcium phosphate bonding, have a lower capacity to emulsify oil than the less-aggregated protein such as sodium caseinate or WPI. The results for casein micelles and sodium caseinate are in general agreement with those reported by Mulvihill & Murphy (1991).

Thus, these results indicate that, although the protein composition of the whey protein/κ-casein–rich and κ-

Table 1. Emulsifying capacities (volume of oil emulsified/100 ml protein solution) of various protein preparations

Protein	Protein concentration (% (w/v))	Oil (ml/100 ml protein solution)
Whey protein/ κ-casein–rich fraction	0·25	69·0
	0·50	74·0
κ-Casein–depleted micelles	0·25	78·5
	0·50	82·0
Sodium caseinate	0·25	72·0
	0·50	92·0
Whey protein isolate	0·25	93·0
	0·50	120·0
Casein micelles	0·25	76·0
	0·50	85·5

Values are the mean of duplicate measurements.

casein–depleted micelles fractions isolated from heated milk were markedly different, their emulsifying properties did not differ significantly from each other. Both fractions showed emulsifying capacities and emulsion stabilizing abilities essentially similar to freeze-dried casein micelles but different from those of sodium caseinate or WPI. It would appear that the size of the protein aggregates in solution was more important for determining the emulsifying properties than the protein composition of the aggregates. Larger-sized protein aggregates, as in whey protein/κ-casein–rich and κ-casein–depleted micelle fractions or casein micelles, had low emulsifying capacities but produced more stable emulsions when compared with smaller-sized protein aggregates as in sodium caseinate or WPI.

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