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Functional Properties of α_s-/κ- or β-Rich Casein Fractions

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ABSTRACT

The functional properties of a β-casein-enriched and an α_s-/κ-casein-enriched fraction obtained from ultrafiltration of sodium caseinate were compared to those of sodium caseinate. The α_s-/κ-casein-enriched fraction compared favourably to sodium caseinate with respect to solubility, viscosity and water sorption. However, the surface activity of the fraction was adversely affected and consequently its foaming capacity, foam stability and emulsion capacity, were reduced, but emulsion stability was increased. The β-casein-enriched fraction was as soluble as sodium caseinate, was less viscous and had similar water sorption properties. The fraction was more surface active than Na-caseinate and its foaming properties and emulsifying capacity were enhanced. However, emulsions formed from this fraction were less stable than emulsions prepared in either sodium caseinate or α_s-/κ-casein-enriched casein.

INTRODUCTION

The physico-chemical and functional properties of caseinates, especially sodium caseinate, are well established and have been compared extensively with those of the other principal functional food proteins, especially soy and whey proteins (see Mulvihill & Fox, 1989). Some functional properties, notably surface activity, viscosity and voluminosity, of the individual caseins have also been established (see Mulvihill & Fox, 1989). However, heretofore, individual caseins have been prepared on a scale, usually by ion-exchange chromatography, which severely limits the quantities of proteins available for studies on their properties.

Development of a technique for the separation of α_s-/κ- and β-caseins by ultrafiltration was described by Murphy and Fox (1990). The β-casein prepared by this method was ~80% homogeneous, the principal contaminants being γ-caseins; likewise, the α_s-/κ-casein fraction contained a reduced level of β-casein (~15%). Relatively large (500 g) quantities of these fractions can be prepared by ultrafiltration, thus creating an opportunity to assess some functional properties of these proteins by techniques not applicable heretofore.

MATERIALS AND METHODS

Casein fractionation

α_s-/κ- and β-rich casein fractions were prepared from sodium caseinate by ultrafiltration at 4°C, as described by Murphy and Fox (1990). The protein solutions were adjusted to ~pH 7 with NaOH and freeze-dried. The efficiency of fractionation was monitored by electrophoresis on polyacrylamide gels (Andrews, 1983).

The permeate was mainly (70%) β-casein, the principal contaminants being γ-caseins; the retenate contained essentially all of the α_{s1}, α_{s2} and κ-caseins and ~45% of the β-casein of the original sodium caseinate (i.e. β-casein represented ~15% of the total protein in the retentate (Murphy & Fox, 1990).

Functional properties

Protein solubility was measured using the method of Morr *et al.* (1985). Sufficient protein to give a final concentration of 1% was dispersed in 0·1 M NaCl. The pH was adjusted to values in the range 2·0–7·0 using 0·1 M HCl or NaOH and the dispersions stirred for 1 h at 20°C. Insoluble material was removed by centrifugation at 20 000 g for 30 min and filtration through Whatman No 1 filter paper. Protein was determined by the macro-Kjeldahl method.

$$\text{Protein Solubility (\%)} = \frac{\text{Protein in supernatant (\%)} \times 100}{\text{Protein in sample (\%)}}$$

Viscosity was measured using a Brookfield Viscometer, model LVT, equipped with a temperature-controlled chamber. Spindle No. 18 was used and readings taken after 1 min shearing at 12 rpm. The relative viscosity (η) was calculated taking the viscosity of water at 20°C to be 1·005 cp.

The water sorption isotherms were determined by drying samples, in triplicate, to equilibrium over phosphorus pentoxide and measuring the

water absorbed after equilibration in closed chambers, containing saturated salt solutions, of known relative humidities, at 20°C, i.e. LiCl (0·12), $MgCl_26H_2O$ (0·33), $Mg(NO_3)_26H_2O$ (0·52), NaCl (0·75), $(NH_4)_2SO_4$ (0·79), K_2CrO_4 (0·88), KNO_3 (0·94) and Na_2HPO_4 (0·98).

The adsorption behaviour of the three caseinates at the air–water interface was studied by the drop-volume technique as described by Tornberg (1977, 1978*a*). Protein dispersions were made up in double-distilled water, the pH adjusted to pH 7·0 and equilibrated overnight at 4°C. After filtration through Whatman No 1 filter paper, the density of the sample was measured at 25°C using a pyknometer with a volume of 25 ml at 20°C. Surface tension time-dependent measurements were performed at $25 \pm 0{\cdot}1$°C. The time necessary for the proteins to reduce the surface tension to such an extent that a drop of known volume, corresponding to a certain γ-value, becomes detached was measured. A plot of the surface tension as a function of time was constructed.

Foaming properties (capacity and stability) were compared using the sparging method described by Waniska and Kinsella (1979). Protein samples (0·25 and 0·35%, w/v) at pH 7·0 and 20°C were sparged with compressed air at a rate of 20 ml/min. The time needed to form 70 ml of foam, the volume of protein solution required to form the foam and the amount of liquid drained from the foam over time, were recorded. Whipping was also used to evaluate the foaming properties using a modified version of the method described by Patel *et al.* (1988). A Philips food mixer was used to whip 100 ml of a 0·5% (w/v) protein solution, pH 7·0, at 20–25°C. The volume of foam formed and drainage over time were recorded up to 30 min.

Emulsification stability was assessed on the basis of emulsion globule size, amount of protein adsorbed onto the fat surface, i.e. protein load, and stability rating. Emulsions were prepared from 30 g of 2·5% (w/v) protein solution, pH 7·0, and 20 g soya bean oil, using a valve homogenizer at $25 \pm 0{\cdot}5$°C, maintained by circulating the emulsion through a cooling coil (Tornberg & Lundh, 1978). Mean globule size was measured by a spectroturbidimetric method (Walstra, 1965, 1968). Protein load was determined according to Tornberg (1978*b*) and creaming stability (stability rating) determined by measuring the % change in fat in the lower aqueous phase after creaming for 24 h (Tornberg, 1978*c*).

Emulsifying capacity was measured over a range of protein concentrations (0·25–0·75%, w/v, pH 7·0) using a commercial food blender (Waring type) (Mohanty *et al.*, 1988). Increasing amounts of coloured (Sudan III) corn oil were added to 100 ml of the protein solution. After each addition, the mixture was homogenized at low speed for 1 min and then at high speed for a further 1 min. Temperature was maintained at 20°C and the end point detected by a change in the visual appearance of the emulsion.

RESULTS AND DISCUSSION

Solubility

Solubility is a primary functional requirement of dairy proteins and is used to assess the potential usefulness of preparations. The effect of pH on the solubility of sodium caseinate, α_s-/κ-casein-enriched caseinate and β-casein-enriched caseinate, was investigated to assess whether processing by ultrafiltration could have a detrimental effect on the derived caseinates. As expected, minimum solubility was observed at pH 3·5–4·5 and the solubility of all samples was greater than 90% above pH 5·5 (Fig. 1). At pH values < pH 3·5, α_s-/κ-casein-enriched caseinate was more soluble than sodium caseinate and therefore potentially more useful as a constituent of fluid acid foods.

Viscosity

Viscosity of the three protein preparations increased logarithmically with increasing protein concentration (Fig. 2A), as found by others, e.g. Hayes *et al.* (1968) and Hermansson (1975). Hayes *et al.* (1968) described a linear relationship between log viscosity and the reciprocal of absolute temperature for 20% sodium caseinate and this was verified using 10% protein solutions for the preparations studied (Fig. 2B). The viscosity of the

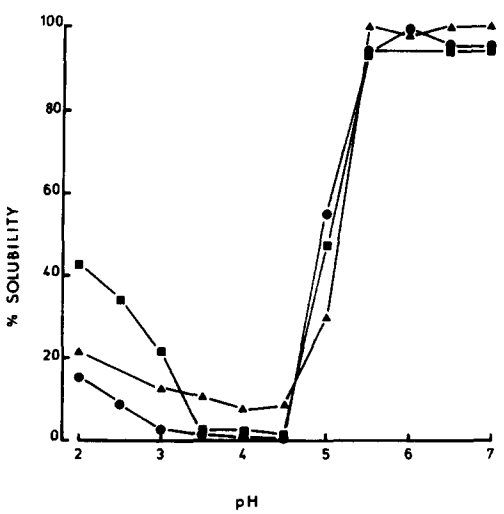

Fig. 1. Effect of pH on solubility of sodium caseinate (●—●), α_s-/κ-casein-enriched caseinate (■—■) and β-casein-enriched caseinate (▲—▲). Values represent single measurements.

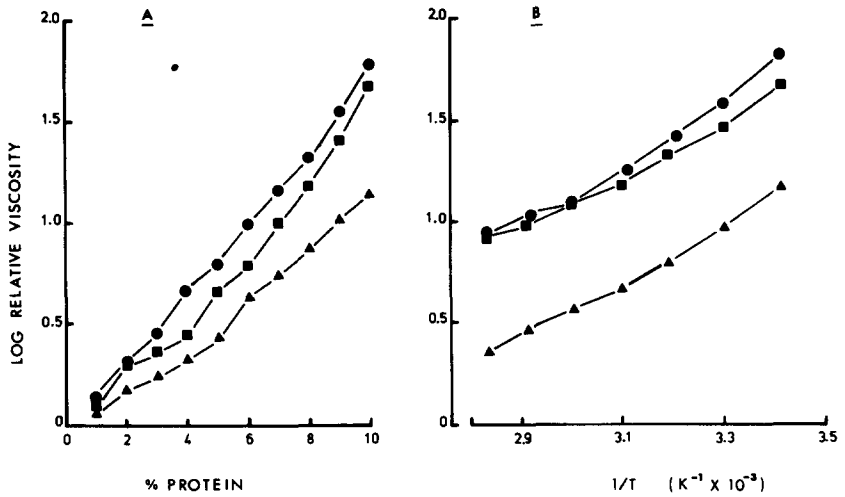

Fig. 2. Effect of concentration at 20°C (A) and temperature at 10% protein (B) on the viscosity of sodium caseinate (●—●), α_s-/κ-casein-enriched caseinate (■—■) and β-casein-enriched caseinate (▲—▲). Values are the mean of duplicate measurements.

β-casein-enriched caseinate was lower than that of sodium caseinate at all concentrations and temperatures used. Although it would facilitate processing, the relatively low viscosity of the β-caseinate may be a disadvantage in certain applications, e.g., emulsion stability and as a textural component of foods. The viscosity of the α_s-/κ-casein-enriched caseinate was similar to that of sodium caseinate at any particular concentration and temperature.

Water sorption

Water sorption isotherms were similar for all three preparations (Fig. 3). At equilibrium relative humidities > 0·8, the α_s-/κ-casein-enriched casein absorbed slightly more water than either sodium caseinate or β-casein-enriched caseinate. However, this small difference is unlikely to adversely affect storage stability or quality of this protein.

Surface activity

The time-dependences of surface tension at the air–water interface of 10^{-1}, 10^{-2} and 10^{-3}% (w/v) solutions of sodium caseinate, α_s-/κ-casein-enriched caseinate and β-casein-enriched caseinate at pH 7·0 and 25°C are shown in Fig. 4. The experiment was performed in duplicate but the results of a single experiment are reported; the results of the duplicate experiments were very similar. β-Casein-enriched caseinate was the most surface active of the three

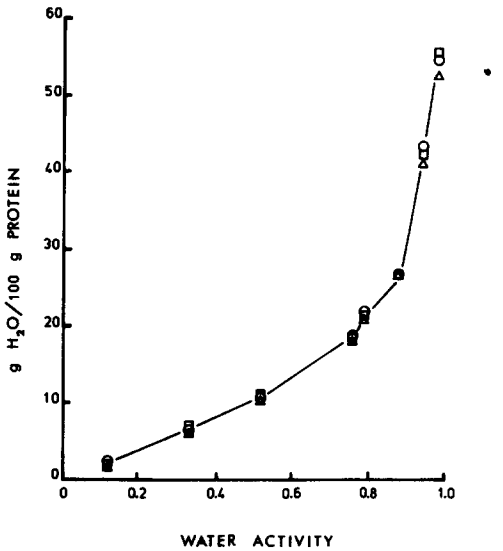

Fig. 3. Water sorbtion isotherm of sodium caseinate (○—○), α_s-/κ-casein-enriched caseinate (□—□) and β-casein-enriched caseinate (△—△). Values are the mean of triplicate measurements.

proteins at all concentrations used while the α_s-/κ-casein-enriched caseinate was least surface active.

The concentration dependence of the surface tension decay may be evaluated from Fig. 5 in which the surface pressure obtained after 40 min (π_{40} min) was plotted against initial sub-phase concentration. At high concentrations, i.e. 10^{-1} and 10^{-2}% (w/v), surface activity was high for all samples. However, the inherent trend of β-casein-enriched caseinate > sodium caseinate > α_s-/κ-casein-enriched caseinate held true. At 10^{-3}% (w/v), the difference was more obvious with the α_s-/κ-caseinate displaying little or no surface activity while the β-casein-enriched sample was still quite effective as a surface tension depressor. These results agree with the findings of Dickinson *et al.* (1985) who concluded that the difference in surface activity between β-casein and sodium caseinate was due mainly to the presence of less surface active casein (α_s- and κ-) in the latter. Mitchell *et al.* (1970) also found that caseins reduced surface tension in the order β-casein > α_{s1}-casein > κ-casein. The surface active properties at the air–water interface of β-casein and its proteolytic fragments have been compared (Wilson *et al.*, 1989). The results of the present study show that at 10^{-1}% (w/v) the surface activity of the β-casein-enriched caseinate compared very favourably with that found by Wilson *et al.* (1989) for pure β-casein. However, at 10^{-2}% (w/v) the β-casein-enriched caseinate decreased surface tension more rapidly and attained a higher π_{40} min than pure β-casein. This was probably due to the presence of γ-casein in the β-casein-enriched

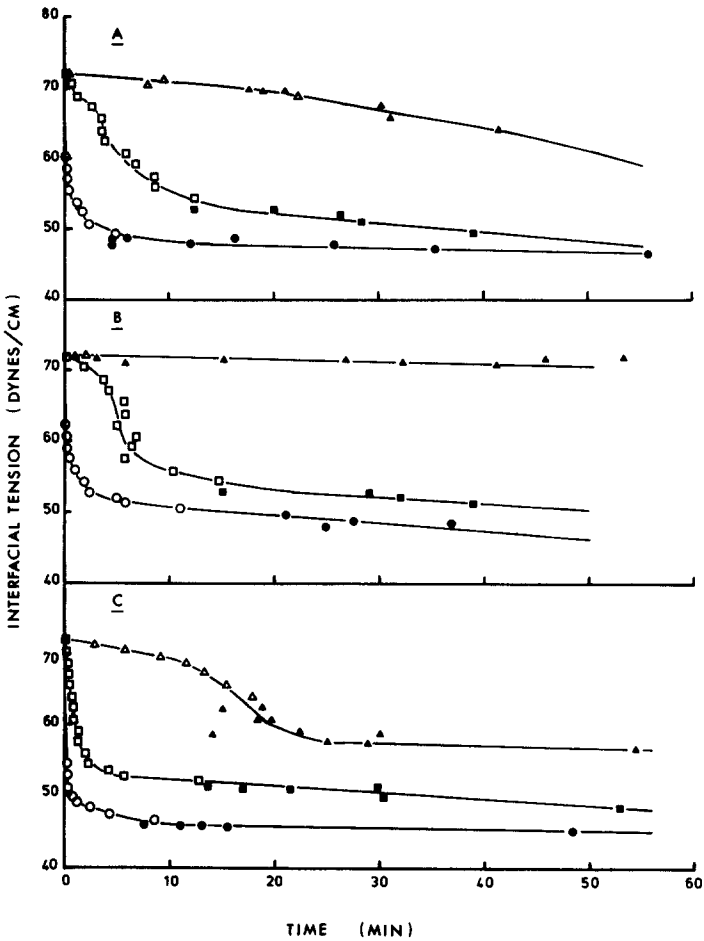

Fig. 4. Time-dependence of surface tension at the air–water interface for (A) sodium caseinate, (B) α_s-/κ-enriched caseinate and (C) β-casein-enriched caseinate. ○—●, 10^{-1}%; □—■, 10^{-2}%; △—▲, 10^{-3}%; ○, □ and △, represent the mean of four values and ●, ■ and ▲ single values. All samples were prepared in double-distilled water and adjusted to pH 7·0.

caseinate. At this concentration, Wilson *et al.* (1989) found that all proteolytic fragments of β-casein examined were more effective than β-casein at reducing surface tension at the first rate-determining step and the surface pressure attained after 40 min to be higher also.

Foaming properties

The extent to which the interfacial behaviour of the three protein preparations could be correlated to their ability to form stable foams was investigated. Two methods, bubbling/sparging and whipping, were used to

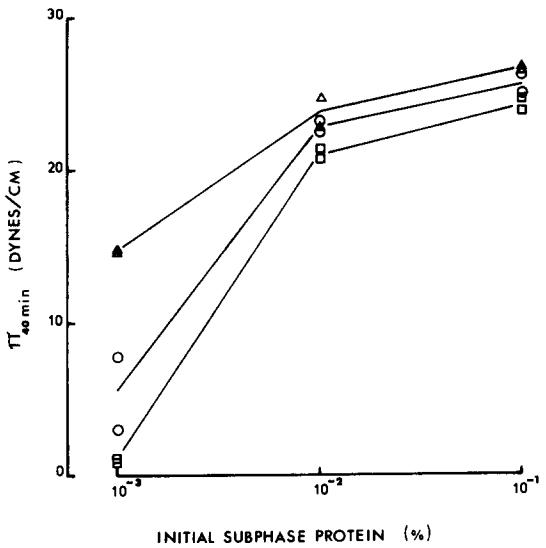

Fig. 5. The surface pressure attained after 40 min, π_{40} min, as a function of initial sub-phase concentration for (○—○) sodium caseinate, (□—□) α_s-/κ-casein-enriched caseinate and (△—△) β-casein-enriched caseinate.

introduce air, and the ability of the protein solutions to form a stable encapsulating interfacial film compared. Both foam capacity (measured by G_i) and foam stability have been found to increase with increasing ovalbumin concentration (Waniska & Kinsella, 1979). In the present study, foaming capacity was significantly higher at 0·35% protein compared to 0·25% protein for both sodium caseinate and α_s-/κ-casein-enriched

TABLE 1
Foaming Properties of Sodium Caseinate, α_s-/κ-Casein-Enriched Caseinate and β-Casein-Enriched Caseinate

[Protein] (% w/v)	*G_i = % gas initially in 70 ml foam*	
	0·25%	*0·35%*
Sodium caseinate	92·5 ± 0·7	97·6 ± 2·7
α_s-/κ-casein-enriched caseinate	86·5 ± 6·8	94·2 ± 0·7*
β-casein-enriched caseinate	99·5 ± 4·6**	93·8 ± 2·4*

Protein samples were dissolved in distilled water and adjusted to pH 7·0. Foams were formed by sparging with compressed air at 20°C. Values are the mean, ± sd, of five values and when compared to sodium caseinate.
* indicates significance at 5% level.
** indicates significance at 1% level.

$$G_i = \frac{\text{(Volume foam formed} - \text{Volume liquid in foam initially (ml)} \times 100}{\text{Gas flow rate (ml/min)} \times \text{Time required to form the foam (min)}}$$

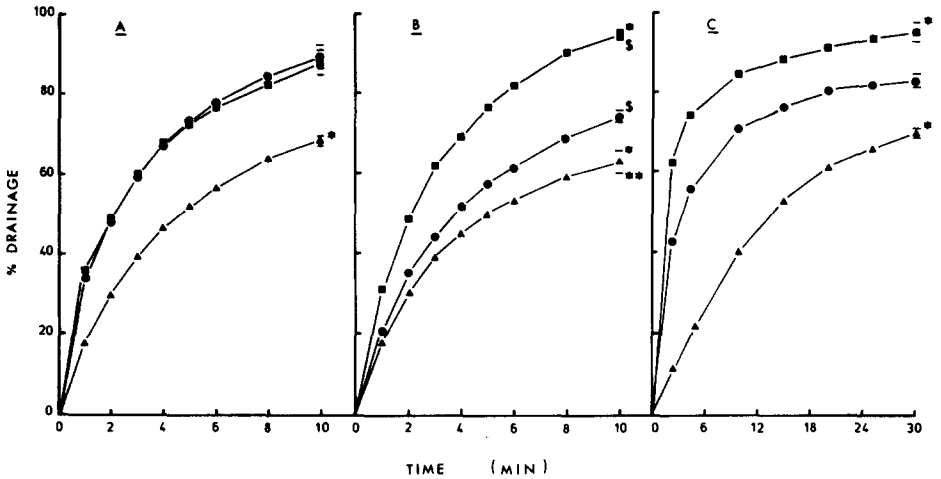

Fig. 6. Stability of foams formed, by sparging with compressed air at 20°C, from sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲) at 0·25% (A) or 0·35% (B) protein, pH 7·0.

$$\% \text{ Drainage} = \frac{\text{Volume liquid drained from foam}}{\text{Initial volume of liquid in the foam}} \times 100$$

(c) Stability of foams formed, by whipping 0·5% protein solutions, pH 7·0 at 20°C, from sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲).

$$\% \text{ Drainage} = \frac{\text{Volume liquid drained} \times 100}{\text{Sample volume}}$$

Values are the mean (± sd) of five measurements. *, Significant at 0·1% level when compared with sodium caseinate. **, Significant at 1·0% level when compared to corresponding drainage in (A). $, Significant at 0·1% level when compared to corresponding drainage in (A).

caseinate but significantly lower for the β-casein-enriched caseinate ($P < 0{\cdot}05$) (Table 1). On the other hand, the foams formed from 0·35% solutions of sodium caseinate and the β-enriched caseinate were more stable than those prepared from 0·25% solutions whereas the α_s-/κ-casein-enriched caseinate foam was less stable at the higher concentration. At both concentrations, the β-casein-enriched caseinate foam was the most stable with the α_s/κ-caseinate foams being the least stable (Figs 6A and B).

Foams were also formed by whipping to evaluate the samples under conditions which simulate commercial conditions more closely, e.g. in the preparation of meringues, souffles, etc. The foamability of the β-casein-enriched caseinate was significantly higher than that of sodium caseinate which, in turn, was higher than that of the α_s-/κ-casein-enriched caseinate (Table 2). The same trend was observed for the stability of the foams formed. In terms of both foam volume stability and foam liquid stability (100% liquid drainage), β-casein-enriched caseinate foams performed best, with

TABLE 2
Foaming Properties of Sodium Caseinate, α_s-/κ-Casein-Enriched Caseinate and β-Casein-Enriched Caseinate

	Sodium casinate	*α_s-/κ-casein-enriched caseinate*	*β-casein-enriched caseinate*
% Foam Expansion (FE)	402 ± 20·5	315 ± 8·7*	585 ± 10*
% Foam Volume Stability (FVS)	82·1 ± 1·1	6·4 ± 2·8*	89·8 ± 0·3*
% Foam Liquid Stability (FLS)	17·0 ± 1·4	5·2 ± 2·2*	30·2 ± 0·8*

Protein samples were dissolved in distilled water and adjusted to pH 7·0 prior to use. Foams were formed by whipping 0·5% (w/v) protein solutions at 20–25°C.
Values are the mean ± sd of five values and when compared to sodium caseinate, * indicates significance at 0·1% level.

$$\% \text{ FE} = \frac{(\text{Initial volume of foam including liquid} - \text{Volume sample used})(\text{ml}) \times 100}{\text{Volume sample used (ml)}}$$

$$\% \text{ FVS} = \frac{\text{Foam volume at 30 min (ml)}}{\text{Initial volume of foam including liquid (ml)}} \times 100$$

$$\% \text{ FLS} = 100 - \% \text{ Liquid drainage}$$

sodium caseinate foams next; the α_s-/κ-casein-enriched caseinate foams were extremely unstable (Fig. 6C). All foaming properties determined for sodium caseinate in the present study compared favourably to values quoted by Patel *et al.* (1988). These workers also studied the foaming properties of bovine serum albumin (BSA); β-casein-enriched caseinate solution foamed as well and the foams formed were as stable as the BSA foams prepared by Patel *et al.* (1988).

Thus, the foaming properties of sodium caseinate, α_s-/κ-casein-enriched caseinate and β-casein-enriched caseinate correlated well with predictions based on the interfacial behaviour of the samples, i.e. the most surface active sample (β-caseinate) had highest foamability and stability and the α_s-/κ-casin-enriched caseinate, depleted of the more surface active β-casein, performed very badly in terms of foaming properties.

Emsulsifying properties

The effect of protein concentration (0·25 to 0·75%, w/v) on the volume of oil emulsified was determined to assess the emulsion capacity of the protein preparations. As protein concentration increased, the volume of oil emulsified increased (Fig. 7A). However, the rate of this increase was greatest for the β-casein-enriched caseinate and least for the α_s-/κ-casein-enriched caseinate. The results for sodium caseinate are in general

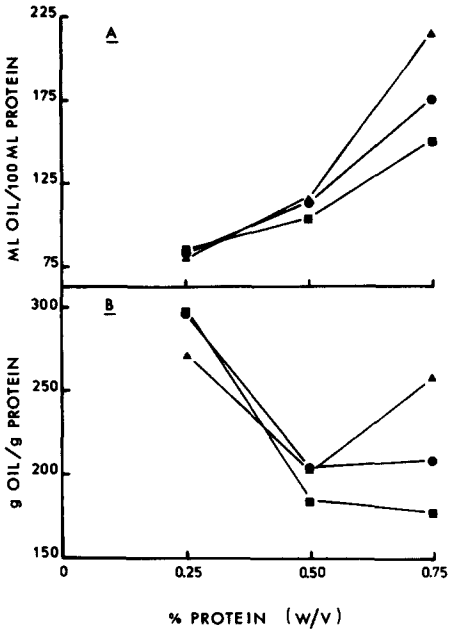

Fig. 7. Effect of protein concentration on (A) the volume of oil emulsified by sodium caseinate and (B) the emulsifying capacity of sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲). Values are the mean of duplicate measurements.

agreement with those of Murphy (1988) but the amounts of oil emulsified by the protein were slightly higher in the present study. A plot of the emulsifying capacity (E.C., g oil emulsified per g protein) against protein concentration (Fig. 7B) showed that at 0·25% (w/v) protein concentration, the E.C. of the β-casein-enriched caseinate was lowest but at 0·75% (w/v) the order for the E.C. was β-casein-enriched caseinate > sodium caseinate > α_s-/κ-casein-enriched caseinate. Murphy (1988) showed that there is a critical concentration of sodium caseinate [>0·75% (w/v)] above which the E.C. increases significantly. The slight increase in E.C. between 0·5 and 0·75% (w/v) protein found in the present study is consistent with this although the critical concentration was less than 0·75% (w/v) for the β-casein-enriched caseinate (Fig. 7B).

At protein concentrations above 0·5% (w/v), the E.C. of the preparations correlated well with predictions based on their surface activity. The least surface active α_s-/κ-casein-enriched caseinate had the lowest E.C., sodium caseinate was intermediate and the most surface active caseinate, the β-caseinate-enriched caseinate, had the highest E.C. Nakai *et al.* (1980) also showed a good correlation between effective hydrophobicity and surface tension, interfacial tension and emulsifying properties. However, at 0·25%

(w/v) protein concentration this correlation did not hold true, the β-casein-enriched caseinate showing the lowest E.C. A possible explanation is the lower viscosity of this sample compared to the other two caseinates (Fig. 2). Also, Schmidt and Payens (1972) found that β-casein does not associate below a critical micelle concentration. At higher β-casein concentrations, the ability of the protein-covered fat globules to associate may lead to the formation of a gel-like network with increased viscosity. The viscosity of the 0·75% (w/v) β-casein-enriched caseinate emulsion, prior to phase inversion, was visibly greater than that of the emulsions formed in sodium caseinate or the α_s-/κ-casein-enriched caseinate at this concentration. Murphy (1988) found that highly aggregated proteins, such as micellar caseins, have a low capacity to emulsify oil at high protein concentrations but that high-calcium caseinate, although aggregated, had a much higher E.C. at the same concentration. He attributed this, and the very high viscosity found, to the cross-linkages of the protein-covered fat globules by Ca^{2+}.

Emulsions were prepared, using 2·5% (w/v) protein solutions and soya oil in a ratio of 3:2, in a valve homogenizer at different power inputs. The sodium caseinate, α_s-/κ-casein-enriched caseinate and β-casein-enriched caseinate-stabilized emulsions, were characterized and compared with respect to fat globule size, i.e. total fat surface area, the amount of protein adsorbed on the fat surface, i.e. protein load, and finally the ability of the

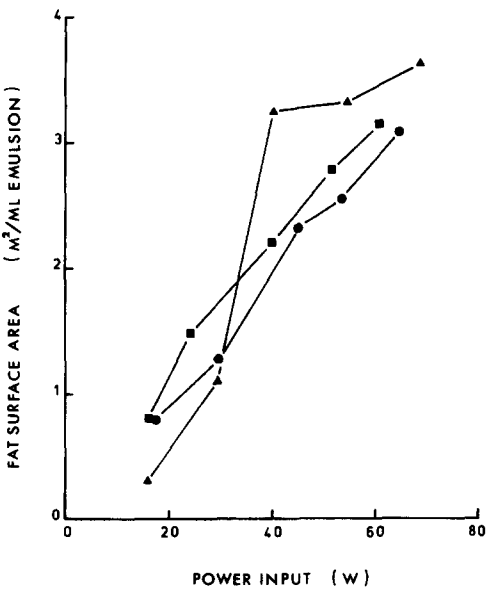

Fig. 8. Fat surface area created on emulsification as a function of power input for sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲) stabilized emulsions. Values represent single measurements.

caseinates to form a stable emulsion. Each experiment was performed in duplicate, which compared favourably, but the results from only a single series of emulsions are reported. Results obtained throughout for sodium caseinate were in close agreement with those of Murphy (1988).

The fat surface area (FSA) increased as the power input increased for all protein-stabilized emulsions examined (Fig. 8). The rate of this increase was similar for the sodium caseinate and α_s-/κ-casein-enriched stabilized emulsions with the latter preparation forming slightly smaller fat globules at all power inputs. At lower power inputs (<40W) the FSA created by the β-casein-enriched caseinate was lower than those for the other two samples but at power inputs >40W the reverse was true, with fat globules formed by this preparation being the smallest of those analyzed.

The total amount of protein adsorbed on the fat surface increased as power input increased for the three protein-stabilized emulsions (Fig. 9A). Although the FSA formed by sodium caseinate was less than that by the α_s-/κ-casein-enriched caseinate at all power inputs, the amount of protein adsorbed on the fat surface of the latter was smaller. At higher power inputs, i.e. >60W, levels of protein adsorbed were in the order β-casein-enriched caseinate > sodium caseinate > α_s-/κ-casein-enriched caseinate. The total amount of protein adsorbed on the fat surface was plotted against FSA (Fig. 9B). Protein adsorption increased with increasing FSA for the protein-stabilized emulsions analyzed. The rate of this increase was similar for sodium caseinate and α_s-/κ-casein-enriched caseinate stabilized emulsions

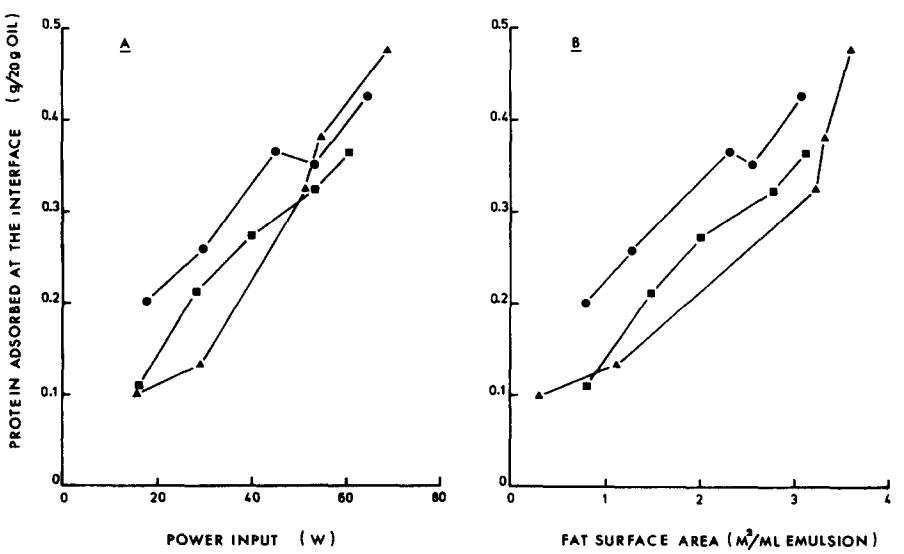

Fig. 9. Total protein adsorbed on emulsification as a function of power input (A) and fat surface area (B) for sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲) stabilized emulsions. Values represent single measurements.

but at FSA > 3 m^2/ml emulsion the amount of protein adsorbed on the β-casein-enriched caseinate-stabilized fat globules increased dramatically.

From these results it can be concluded that, as expected, FSA increased with increasing power input as smaller globules were formed. The greater FSA, and consequently the smaller fat globules, formed by the α_s-/κ-casein-enriched caseinate, may be due to the higher κ-casein content. Many workers have reported that the smaller naturally-occurring casein micelles contain a larger proportion of κ-casein (Schmidt, 1980) and size-determinations on synthetic micelles clearly revealed a decrease in micellar size with increasing κ-casein content (Schmidt, 1979). Furthermore, Boyd *et al.* (1973) reported that films of α- and β-caseins showed no detectable surface viscosity at any surface concentration whereas κ-casein formed viscous films at higher concentrations. This suggested that the more highly structured κ-casein, which is also capable of forming intermolecular disulphide bonds, formed a more cohesive film than the other caseins which have random-coil structures.

The emulsifying behaviour of the β-casein-enriched caseinate in a valve homogenizer was different from that of the other two caseinates. At power inputs < 30 W, fat globule size was larger and less protein was adsorbed at the interface than for the α-rich or Na-caseinate. At higher power inputs, the opposite was true with smaller fat globules being formed and larger amounts of protein adsorbed for the β-rich casein (Figs 8 and 9). These results might be explained by assuming a critical concentration below which β-casein association does not occur (Schmidt & Payens, 1972). Murphy (1988) also concluded that the more aggregated the protein the greater the adsorption onto the fat surface.

Protein load, i.e. mg protein/m^2 fat surface, as a function of power input and FSA are plotted in Fig. 10. Protein load decreased slightly with increasing power input or FSA for the α_s-/κ-casein-enriched caseinate stabilized emulsion indicating that adsorption of protein from the bulk phase was dominant. The sodium caseinate-stabilized emulsions had the highest protein load at most power inputs, and fat surface areas. The sharp decrease in protein load in the power input range 15 to 50 W (FSA range 1 to 2·5 m^2/ml) indicated that the newly formed surface of the sodium caseinate-stabilized emulsion was due to a combination of protein adsorption from the bulk phase and rearrangement of protein molecules already adsorbed. At higher power inputs, adsorption from the bulk phase dominated, as for the α_s-/κ-casein-enriched-caseinate stabilized emulsions. The situation was more complicated in the case of the β-casein-enriched caseinate. Rearrangement of adsorbed protein dominated at low power inputs/FSA, with adsorption from the bulk phase dominating at FSA between 1·0 and 3·0 m^2/ml emulsion. Above an FSA of 3·0 m^2/ml emulsion, the sharp

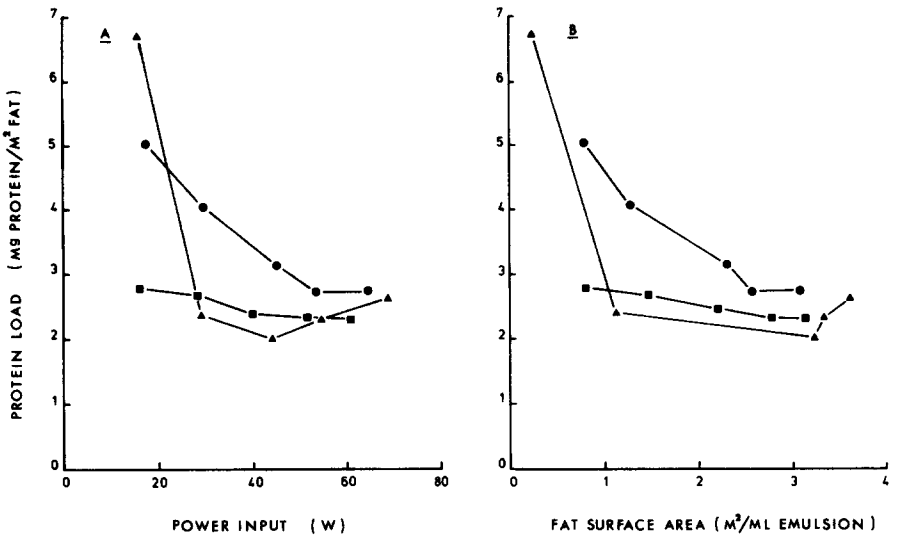

Fig. 10. Protein load as a function of power input (A) and fat surface area (B) for sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲) stabilized emulsions. Values represent single measurements.

increase in protein load relative to FSA may be due to increased association of the β-casein by hydrophobic bonding above the critical micelle concentration (0·3–0·8 mg/ml, depending on μ; Schmidt & Payens, 1972).

To compare the caseinates with respect to their ability to stabilize emulsions, the change in fat content in the lower aqueous phase after standing for 24 h was measured and used to calculate stability rating (%) (Tornberg, 1978*c*). At all power inputs and fat surface areas, the stability rating for the emulsions was in the order α_s-/κ-casein-enriched caseinate > sodium caseinate > β-casein-enriched caseinate (Fig. 11). Stability rating increased with increasing power input and FSA. However, at power inputs above 40 W and FSA above 3 m^2/ml emulsion, the stability ratings for the β-casein-enriched caseinate-stabilized emulsions, though above 90%, did not increase. The emulsions formed at low power inputs were found to be very unstable (S.R. < 40%). Murphy (1988) suggested that the more aggregated the protein, the greater its ability to stabilize emulsions. Assuming that association of β-casein occurs in the protein-covered fat globule, the results presented do not support this. As expected, the decreased fat globule size in β-casein-enriched caseinate-stabilized emulsions formed at power inputs > 40 W did not lead to greater stability. Though forming larger fat globules at comparable power inputs, both the α_s-/κ-casein-enriched caseinate and sodium caseinate had higher stability ratings than the β-enriched-caseinate. The viscosities of all emulsions formed were measured using a Brookfield viscometer. At all power inputs, the viscosity was in the order sodium

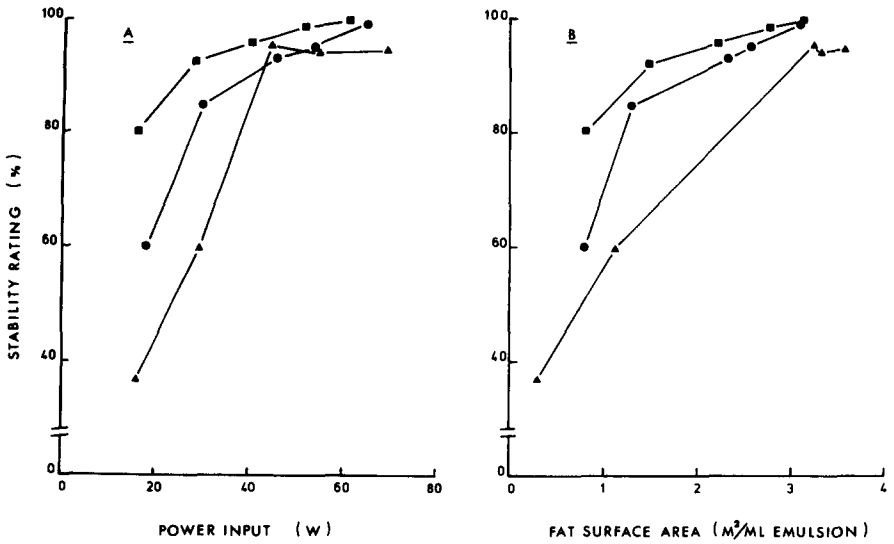

Fig. 11. Effect of power input (A) and fat surface area (B) on the stability rating of sodium caseinate (●), α_s-/κ-casein-enriched caseinate (■) or β-casein-enriched caseinate (▲) stabilized emulsions. Values represent single measurements.

caseinate > α_s/κ-casein-enriched caseinate > β-casein-enriched caseinate-stabilized emulsions. The lower stability ratings found for β-casein could therefore be attributed to the low viscosity. The higher stability ratings for the α_s-/κ-casein-enriched caseinate-stabilized emulsions suggest that other factors besides fat globule size and viscosity govern emulsion stability. Murphy (1988) suggested that the total amount of protein adsorbed during emulsification is an important factor but the total amount of protein adsorbed at the interface was lowest for the α_s-/κ-casein-enriched caseinate stabilized emulsion. Therefore, the nature of protein–protein interactions around the fat globules formed could be important. The increased κ-casein content of the most stable emulsion, through intermolecular disulphide bonds or increased hydrophobic association with other casein constituents, could lead to the formation of a more cohesive, stable film around the fat globules.

Thus, the emulsifying properties of sodium caseinate, α_s-/κ-casein-enriched caseinate and β-casein-enriched caseinate did not correlate well with predictions based on the interfacial behaviour of the proteins. The most surface active sample, i.e. the β-casein-enriched caseinate, produced emulsions with the lowest stability and the surface activity of the preparation could not be used to predict either the capacity of the sample to form emulsions or the type of emulsion formed. The least surface active sample, i.e. α_s-/κ-casein-enriched caseinate, had the highest ability to

stabilize emulsion, suggesting that factors other than the ability of proteins to reduce surface tension are important.

The economic viability of fractionating sodium caseinate by ultrafiltration would depend on the potential of the fractions obtained as functional food components. The results of this study indicate that the solubility, viscosity and water sorption properties of the α_s-/κ-casein-enriched and β-casein-enriched caseinates were not significantly affected. The β-casein-enriched fraction showed improved foaming properties and increased capacity for emulsification but the stability of emulsions stabilized by β-enriched caseinate was lower than those of emulsions formed in Na-caseinate or α_s-/κ-enriched caseinate. On the other hand, reducing the content of β-casein resulted in an α_s-/κ-casein-enriched caseinate with enhanced ability to stabilize emulsions even though the surface activity was reduced as was its foaming capacity, foam stability and emulsion capacity.

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REFERENCES

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *J. Dairy Res.*, **50**, 45–55.

Boyd, J. V., Mitchell, J. R., Irons, L., Musselwhite, P. R. & Sherman, P. (1973). The mechanical properties of milk protein films spread at the air–water interface. *J. Colloid Interface Sci.*, **45**, 478–86.

Dickinson, E., Pogson, D. J., Robson, E. W. and Stanby, G. (1985). Time dependent surface pressures of adsorbed films of caseinate-gelatin at the oil–water interface. *Colloids and Surfaces*, **14**, 135–41.

Hayes, J. F., Southby, P. M. & Muller, L. L. (1968). Factors affecting the viscosity of caseinate in dispersions of high concentrations. *J. Dairy Res.*, **35**, 31–47.

Hermansson, A. M. (1975). Functional properties of proteins for food flow properties. *J. Text. Stud.*, **5**, 425–39.

Mitchell, J., Irons, L. & Palmer, G. J. (1970). A study of the spread and adsorbed films of milk proteins. *Biochim. Biophys. Acta*, **200**, 138–50.

Mohanty, B., Mulvihill, D. M. & Fox, P. F. (1988). Emulsifying and foaming properties of acidic caseins and sodium caseinate. *Food Chem.*, **28**, 17–30.

Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., Lewis, B. A. & Mangino, M. E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *J. Food Sci.*, **50**, 1715–18.

Mulvihill, D. M. and Fox, P. F. (1989). Caseins: Functional properties. In *Developments in Dairy Chemistry—4*. ed. P. F. Fox, Elsevier Applied Science Publishers, London, pp. 131–72.

Murphy, J. M. & Fox, P. F. (1991). Fractionation of sodium caseinate by ultrafiltration. *Food Chemistry*, **39**, 27–38.

Murphy, P. C. (1988). The surface active and emulsifying properties of casein and caseinates. MSc Thesis, National University of Ireland.

Nakai, S., Ho, L., Helbig, N., Kato, A. & Tung, M. A. (1980). Relationship between hydrophobicity and emulsifying properties of some plant proteins. *J. Can. Inst. Food Sci. Technol.*, **13**, 23–7.

Patel, P. D., Stripp, A. M. & Fry, J. C. (1988). Whipping test for the determination of foaming capacity of protein: A collaborative study. *Int. J. Food Sci. Technol.*, **23**, 57–63.

Schmidt, D. G. (1979). Properties of artificial casein micelles. *J. Dairy Res.*, **46**, 351–5.

Schmidt, D. G. (1980). Colloidal aspects of casein. *Neth. Milk Dairy J.*, **34**, 42–64.

Schmidt, D. G. & Payens, J. A. J. (1972). The evaluation of positive and negative contributions to the second virial coefficient of some milk proteins. *J. Colloid Interface Sci.*, **39**, 655–62.

Tornberg, E. (1977). A surface tension apparatus according to the Drop-Volume principle. *J. Colloid Interface Sci.*, **60**, 50–3.

Tornberg, E. (1978*a*). The application of the drop-volume technique to measurements of the adsorption of proteins at interfaces. *J. Colloid Interface Sci.*, **64**, 391–402.

Tornberg, E. (1978*b*). Functional characterization of protein stabilized emulsions. Emulsifying behaviour of proteins in a valve homogenizer. *J. Sci. Food Agric.*, **29**, 867–9.

Tornberg, E. (1978*c*). Functional characterization of protein stabilized emulsions: Creaming stability. *J. Food Sci.*, **43**, 1559–65.

Tornberg, E. & Lundh, G. (1978). Functional characterization of protein stabilized emulsions: Standardized emulsifying procedure. *J. Food Sci.*, **43**, 1553–8.

Walstra, P. (1965). Light scattering by milk fat globules. *Neth. Milk Dairy J.*, **19**, 93–109.

Walstra, P. (1968). Estimating globule size distribution of oil-in-water emulsions by spectroturbidimetry. *J. Colloid Interface Sci.*, **27**, 493–500.

Waniska, R. D. & Kinsella, J. E. (1979). Foaming properties of proteins: Evaluation of a column aeration apparatus using ovalbumin. *J. Food Sci.*, **44**, 1398–402, 1411.

Wilson, M., Mulvihill, D. M., Donnelly, W. J. & Gill, B. P. (1989). Surface active properties at the air–water interface of β-casein and its fragments derived by plasmin proteolysis. *J. Dairy Res.*, **56**, 487–94.