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Emulsifying and Foaming Properties of Acidic Caseins and Sodium Caseinate

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ABSTRACT

The emulsifying capacity of casein was dependent on pH, decreasing from 325 to 264 g oil/g protein as pH increased from 1·5 to 3·5 and increasing from 251 to 268 g oil/g protein between pH 5·5 and 7·0; above pH 7·0 emulsifying capacity increased sharply to ~ 700 g oil/g protein at pH 8·5. The emulsifying capacity of sodium caseinate increased on addition of NaCl, up to 1 M, or $CaCl_2$, up to 20 mM, while the emulsifying capacity of acidic casein (pH 2·5) increased on addition of NaCl, up to 5 mM, or $CaCl_2$, up to 5 mM and thereafter decreased on further addition of either. The creaming stability of emulsions prepared in acidic casein increased as the pH increased from 2·0 to 3·0. Foaming capacities of caseins were in the order, acidic casein pH 2·0 > acidic casein pH 2·5 > sodium caseinate pH 7·0 > acidic casein 3·0, while foam stabilities were in the opposite order. Addition of NaCl, up to 20 mM, or $CaCl_2$, up to 10 mM, decreased foam capacity of acidic casein (pH 2·0) but increased foam stability.

INTRODUCTION

The amphipathic nature of proteins, arising from the mixture of polar and non-polar amino acid residues, causes them to concentrate at air/water or oil/water interfaces and to reduce surface or interfacial tension and hence to reduce the mechanical energy required to form a foam or emulsion. This

amphipathic character is influenced by the pH and the ionic nature of the environment of the protein and therefore these factors influence the emulsifying and foaming properties of protein solutions.

In studying the emulsifying properties of proteins, their capacity to emulsify oil (emulsifying capacity, EC) and the stability of prepared emulsions, as measured by various methods, are widely used. Pearson *et al.* (1965) studied the emulsifying properties of potassium caseinate and non-fat-dry milk (NFDM) at pH values from 5·4 to 10·4 and at various ionic strengths. The emulsifying capacity of potassium caseinate was highest at pH 10·4 and at an ionic strength of 0·05 and was lowest at pH 5·6, where the layer of interfacial protein was thickest. In contrast, the emulsifying capacity of NFDM was highest at pH 5·6 and lowest at pH 10·4. Tornberg & Hermansson (1977) found that the energy needed to form a stable emulsion of soyabean oil (40%, w/w) in sodium caseinate (2·5%, w/w, protein, pH 7·0) was reduced by addition of 0·2M NaCl and Sabharwal & Vakaleris (1972) found that addition of $CaCl_2$ to an emulsion of 4% coconut oil in 2% caseinate and 0·5% emulsifier (HLB-11) increased emulsion stability sharply.

Protein solubility appears to be the most important factor affecting emulsifying capacity. Crenweldge *et al.* (1974) found that the emulsifying capacity of protein solutions decreased as the pH approached the isoelectric point and then increased again on the acid side of the isoelectric point. These changes in emulsifying capacity with pH reflect changes in protein solubility and concentration.

Foams are formed by the entrapment of air by protein films. For foam formation, proteins must be in solution and must be capable of rapid migration and orientation to form an interfacial film around the gas to prevent contact and coalescence of the nascent bubbles. On adsorption at interfaces, proteins spread and unfold and various segments interact to form a continuous cohesive film. The mechanical and rheological properties of such films are important in relation to the formation and stabilization of foams (Benjamins *et al.*, 1975; Graham, *et al.*, 1976; Kinsella, 1982; Tornberg, 1978*a*) and these properties are also influenced by pH and the ionic nature of the environment of the protein.

The foaming properties of proteins are usually characterized in terms of foam volume, over-run, foam strength, drainage rate, break-down time and half-life period of the foam (Kinsella, 1981, 1982). Graham & Phillips (1976) correlated the foaming properties of β-casein, lysozyme and BSA with their behaviour at an air/water interface. Kitabake & Doi (1982) showed that there was no relationship between the surface activity of several proteins and their foamability but there was a good correlation between the rate at which proteins decreased surface tension and their foamability. Among the

proteins studied, sodium caseinate and α_{s1}-casein had some of the highest values for foamability. Foamability and foam stability are not, however, necessarily synonymous and Henson *et al.* (1970) found that proteins differ markedly in the rate at which they aggregate at an interface. Although the rate of aggregation differed with pH and ionic strength of the buffer, the caseins (α_s, β, κ) were always very resistant to aggregation; this may be particularly important in the stabilization of foams.

Casein is widely used as a functional surface active protein in the food industry (Southward & Walker, 1980), usually as soluble sodium caseinate. Therefore, the emulsifying and foaming properties of Na-caseinate have been widely studied (Fox & Mulvihill, 1983).

Casein is also soluble at pH values on the acid side of its isoelectric point (Mohanty *et al.*, 1988); however, no studies on its emulsifying and foaming properties at these pH values have been reported. The objectives of this study were to determine these properties of acidic casein (pH < 3·5) and to compare them to sodium caseinate at pH 7·0 and also to determine the effects of added NaCl and $CaCl_2$ on these properties.

MATERIALS AND METHODS

Isoelectric casein and sodium caseinate were the same samples used previously (Mohanty *et al.*, 1988).

Soyabean oil was a commercial grade product, obtained from a local supplier.

Chemicals

All chemicals used were of reagent grade obtained from B.D.H. Chemicals Ltd, Poole, Dorset, Great Britain.

Determination of emulsifying capacity

Aqueous caseinate solutions at concentrations ranging from 0·25 to 2% (w/v) protein (at 0·25 unit intervals) were adjusted to pH 7·0 and equilibrated for 10 min at room temperature (20°C). The emulsifying capacities of individual samples were determined using a commercial food blender (Waring type) as follows.

A sample (100 ml) of caseinate solution was placed in the bowl of the blender. Initially, 50 ml of soyabean oil (coloured with Sudan-III at a concentration of 40 mg/litre) was added to the casein solution and the

mixture homogenized at low speed for 1 min followed by high speed blending for a further 1 min. Additional portions (10 ml) of oil were added and homogenized, as described above, after each addition. When the end point was approached, the incremental additions of oil were reduced to 1 ml.

After each addition of oil, the temperature of the sample was brought to 20°C by standing the homogenizer in cold water.

Dispersions of isoelectric casein (0·25%, w/v, protein) were adjusted to pH values in the range 1·5–8·5 (at 0·5 unit intervals) using 0·1M HCl or 0·1M NaOH. After stirring, the pH values of the samples were checked and re-adjusted if necessary. The samples were maintained at 4°C for 24 h, after which the pH was readjusted if necessary.

The emulsifying capacity of each protein solution was determined in a similar manner except that the volume of sample used was 200 ml. The effects of $CaCl_2$ (up to 60 mM) or NaCl (up to 1000 mM) on the emulsifying capacity of acidic casein (0·25%, w/v, protein, pH 2·5) and sodium caseinate (0·25%, w/v, pH 7·0) were determined as above.

The end point was apparent as: a sudden drop in viscosity, a change in the visual appearance of the emulsion, and by a change in the typical sound of the blender.

The emulsifying capacity of individual samples was expressed as: g of oil emulsified per g of protein.

Preparation of emulsions using a valve homogenizer and determination of emulsion stability

Acidic casein dispersions (2·5%, w/v, protein) in distilled water were prepared and adjusted to pH 2·0, 2·5 or 3·0 at 20°C with 0·2M HCl.

Soyabean oil (20 g) was added to 30 g of acidic casein solution and emulsified in a valve homogenizer as described by Tornberg & Lundh (1978). The flow velocity was set to 250 ml/min and the total solution was recirculated through the valve for 10 passes. Emulsions were prepared with the pressure drop across the valve ranging from 20 to 60 MPascals (MPa).

The method used to determine the stability of emulsions was adapted from Tornberg (1978*b*). The emulsions were transferred to tubes (height × width = 16 × 2·1 cm) and held for 24 h at room temperature (20°C). A sample (5 g) of emulsion from the lower aqueous phase was taken using a syringe and its fat content determined by the Gerber method.

The extent of creaming, or creaming stability, was calculated as:

$$\% \text{ Stability rating} = \frac{\text{Per cent fat in lower aqueous phase}}{\text{Per cent fat in original emulsion}} \times 100$$

Determination of foaming capacity and stability

Method I

A sample of isoelectric casein/caseinate, equivalent to 2 g protein, was dispersed in distilled water and the pH adjusted to 2·0, 2·5, 3·0 (acidic casein) or 7·0 (caseinate) using 2M HCl or 2M NaOH. The final volume of individual samples was made to 200 ml with distilled water and the solutions equilibrated at 20°C for 5 min. The foaming properties were assessed by whipping the samples at 20°C in a commercial food blender (Waring type) for 5 min at top speed. The foam was transferred immediately to a 500 ml graduated cylinder and the initial volumes of foam and liquid noted. Overrun (%) was calculated as:

$$\frac{\text{foam volume (ml)}}{\text{original volume of protein solution (ml)}} \times 100$$

The stability of the foam was determined by measuring the volume of liquid drained after set elapsed times (at intervals of 1 min for the first 10 min and 10 min thereafter).

Method II

The method of Waniska & Kinsella (1979), using a column aeration apparatus, was used to assess foaming properties.

Solutions of isoelectric casein/caseinate (0·25%, w/v, protein) in distilled water were prepared and adjusted to pH 2·0, 2·5, 3·0 or 7·0 using 2M HCl or 2M NaOH. The samples were equilibrated for 15 min before assay. Protein solution in the sparging chamber was sparged with compressed air at a constant flow rate (20 ml/min) until foam volume reached 70 ml; sparging was then stopped and the volumes of liquid drained from the foam after 1, 2, 3, 4, 5 and 10 min recorded.

The following equations were employed to calculate various parameters of foaming:

$$G_i = \frac{100(70 - V_i)}{(FR) \times (T_F)}$$

$$V_r = V_i - V_d$$

$$D_{10} = \frac{100(V_r)}{V_i}$$

where: G_i = per cent of sparged gas initially in 70 ml of foam, FR = flow rate (ml/min) of air, T_F = time (min) to fill the column with foam, V_i = volume of liquid in the foam initially, V_r = volume of liquid retained in the foam after 10 min, V_d = volume of liquid drained from foam after 10 min and D_{10} = per cent of liquid in the foam after 10 min.

RESULTS

Emulsifying properties

To determine the most suitable protein concentration to use in emulsifying capacity studies, a series of determinations were made in which the protein concentration of sodium caseinate (pH 7·0) solutions was varied from 0·25 to 2% (w/v) at 0·25% (w/v) increments. The results showed that when solutions containing <0·25% (w/v) protein were used it was difficult to determine the end point while, with solutions containing >2·0% (w/v) protein, viscous, heavy emulsions which clung to the sides of the mixer were formed; also at these high protein concentrations, the capacity of the mixer was exceeded. It was therefore decided to use 0·25% (w/v) protein solutions in further emulsifying capacity experiments as it was easy to observe the end-point using this protein concentration.

Effect of pH on emulsifying capacity of caseins

The emulsifying capacity of casein in the pH range 1·5 to 3·5 and 5·5 to 8·5 was studied. The results (Fig. 1) show that on the acid side of the isoelectric point the emulsifying capacity of casein was slightly better than that of caseinate up to pH 7·0. Emulsifying capacity decreased from 325 g oil/g protein at pH 1·5 to 264 g oil/g protein at pH 3·5. Between pH 5·5 and 7·0, the emulsifying capacity increased from 251 to 268 g oil/g protein but above pH 7·0 emulsifying capacity increased dramatically to ~700 g oil/g protein at pH 8·5. The casein was insoluble >pH 3·5 and <pH 5·5 and consequently no emulsification studies were made in this pH range.

Effect of salts ($CaCl_2$ and NaCl) on the emulsifying capacity of acidic casein, pH 2·5 and sodium caseinate, pH 7·0

The emulsifying capacity of sodium caseinate (0·25%, w/v, protein, pH 7·0) increased linearly from 268 g oil/g protein at 0 mM $CaCl_2$ to 286 g oil/g protein at 2 mM $CaCl_2$ (Fig. 2(a)). When $CaCl_2$ was added to the acidic casein solution (0·25%, w/v, protein, pH 2·5), the emulsifying capacity increased from 288 g oil/g protein at 0 mM $CaCl_2$ to 307 g oil/g protein at 5 mM $CaCl_2$ (Fig. 2(b)). Addition of further $CaCl_2$ decreased emulsifying capacity to 255 g oil/g protein at 60 mM $CaCl_2$. Addition of >2 mM $CaCl_2$ to sodium caseinate solutions or >60 mM $CaCl_2$ to acidic casein solutions caused precipitation.

The emulsifying capacity of sodium caseinate (0·25%, w/v, protein, pH 7·0) increased sharply from 268 g oil/g protein to 294 g oil/g protein on addition of NaCl up to 100 mM and increased slightly thereafter to 309 g oil/g protein at 1000 mM NaCl (Fig. 3(a)). Addition of NaCl (up to 5 mM) to the

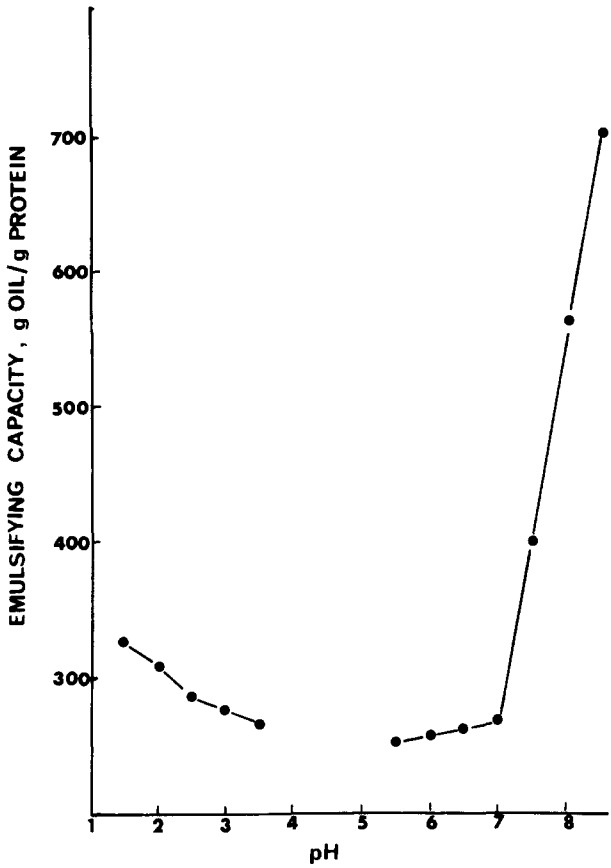


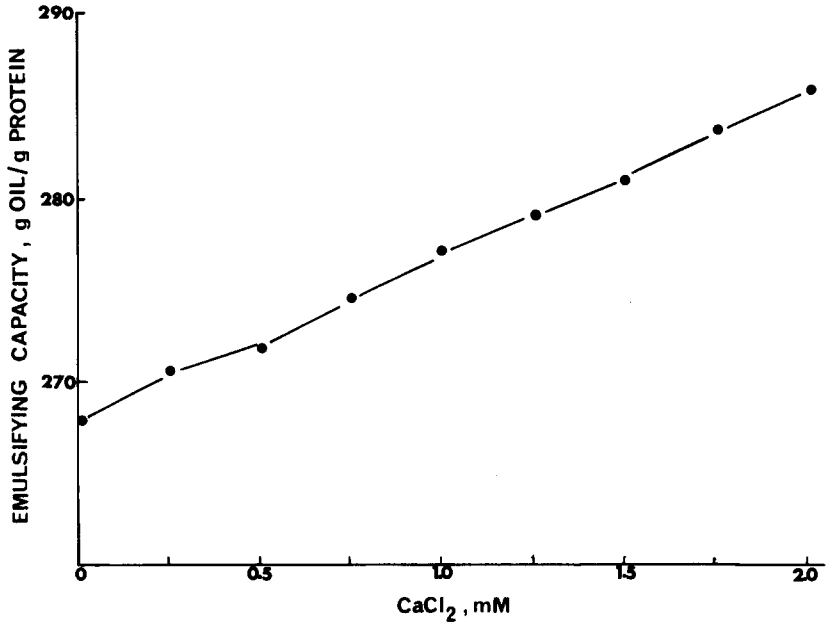
Fig. 1. Effect of pH on the emulsifying capacity of casein (0·25%, w/v, protein). Each value plotted is the mean of three replicates with a maximum standard deviation of ±3·4 g oil/g protein.

acidic casein solution (0·25%, w/v, protein, pH 2·5) increased emulsifying capacity from 288 g oil/g protein without NaCl to 294 g oil/g protein at 5 mM NaCl (Fig. 3(b)). Addition of further NaCl to acidic casein solutions caused a decrease in emulsifying capacity. Addition of >1000 mM NaCl to sodium caseinate solutions or >100 mM NaCl to acidic casein solutions caused precipitation.

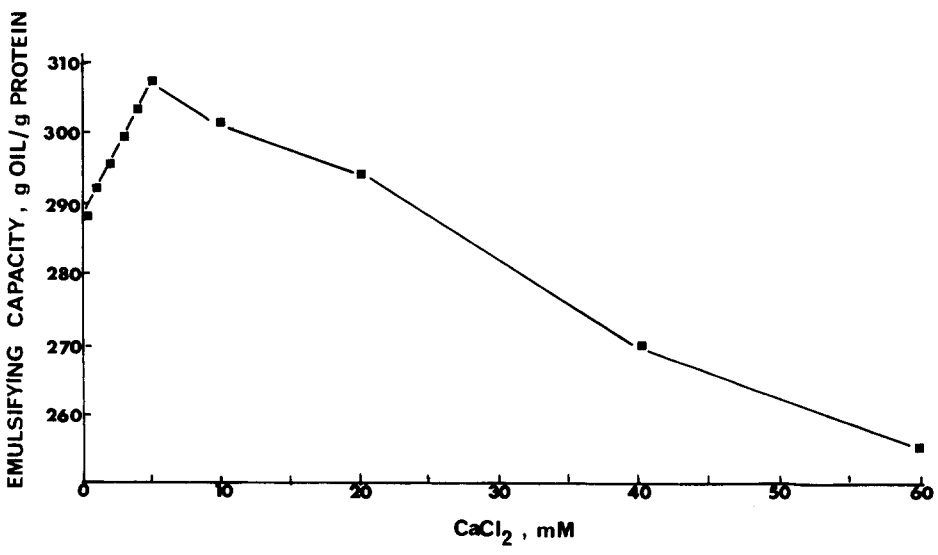
Stability of emulsions prepared using acidic casein

Emulsions consisting of acidic casein solution and soyabean oil were prepared using a valve homogenizer and characterized to determine their creaming stabilities.

The results (Fig. 4) show that the stability rating of the emulsions increased with increasing pressure drop due to the higher energy input to the

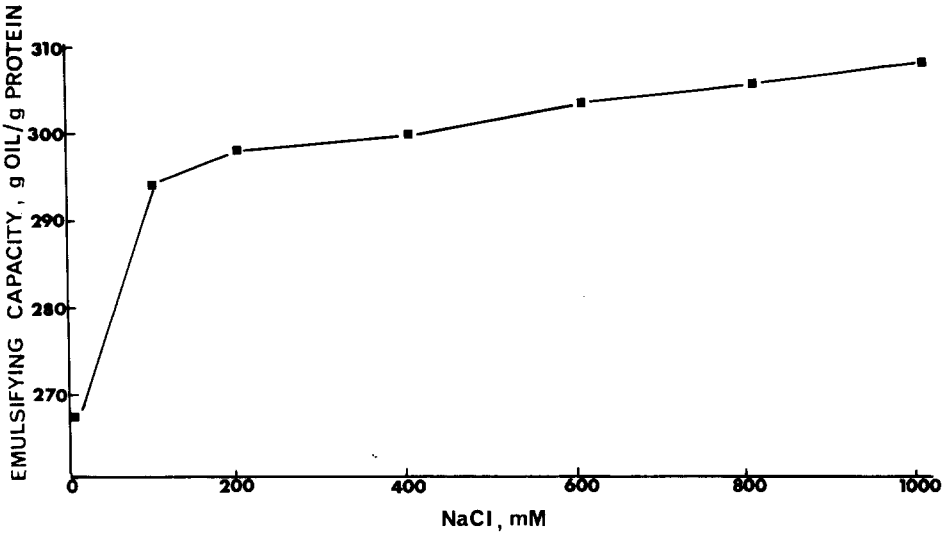


(a)

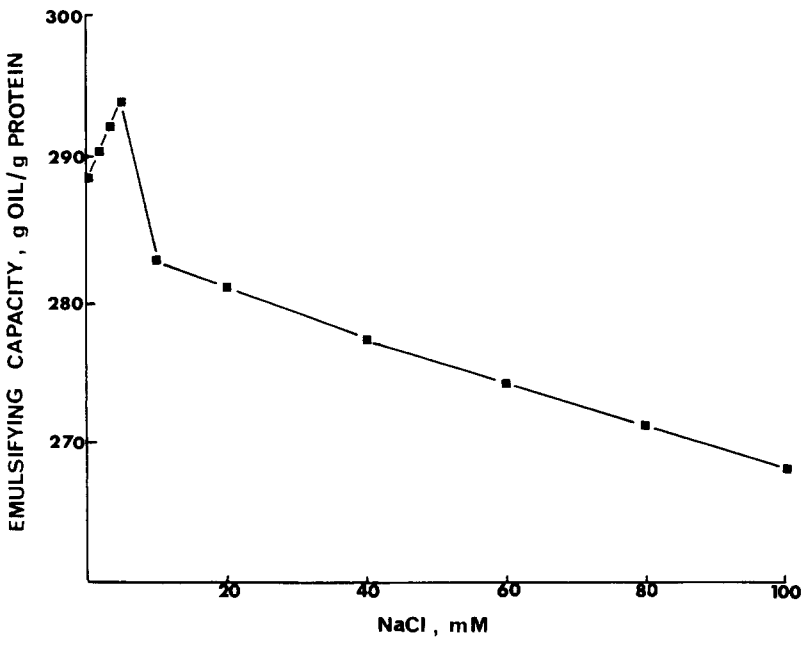


(b)

Fig. 2. Effect of $CaCl_2$ on the emulsifying capacity of (a) sodium caseinate (0·25%, w/v, protein, pH 7·0) and (b) acidic casein (0·25%, w/v, protein, pH 2·5). Each value plotted is the mean of three replicates.



(a)



(b)

Fig. 3. Effect of NaCl on the emulsifying capacity of (a) sodium caseinate (0·25%, w/v, protein, pH 7·0) and (b) acidic casein (0·25%, w/v, protein, pH 2·5). Each value plotted is the mean of three replicates.

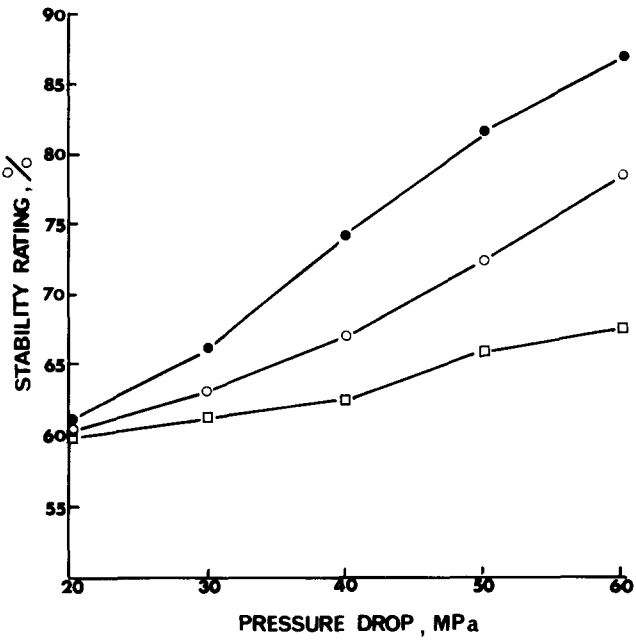


Fig. 4. Stability rating (%) of emulsions prepared in acidic casein at pH 2·0 (□), 2·5 (○), or 3·0 (●) as a function of pressure drop across the emulsifying valve.

system which results in a smaller average globule size. At low pressure drop (20 MPa) the effect of pH on the creaming stability of emulsions containing acidic casein was not very evident; however, on increasing pressure drop (to 60 MPa) the differences in stability rating became very apparent. Decreasing the pH of acidic caseins from 3·0 to 2·0 decreased the stability rating of acidic casein emulsions at any pressure drop.

Foaming properties

Using the whipping method, experiments were conducted to determine the foaming capacity (% over-run) and foam stability with and without added $CaCl_2$ or NaCl.

In the absence of added salts, foaming capacities (Table 1) and volumes of liquid drained from the foam (Fig. 5) were in the order acidic casein, pH 2·0 > acidic casein, pH 2·5 > sodium caseinate, pH 7·0 > acidic casein, pH 3·0, while foam stabilities were in the order acidic casein, pH 3·0 > sodium caseinate, pH 7·0 > acidic casein pH 2·5 > acidic casein pH 2·0.

Addition of $CaCl_2$ (up to 10 mM) to acidic casein (pH 2) decreased over-run substantially while addition of NaCl (10 and 20 mM) decreased over-run slightly (Table 2). In the presence of either salt the volume of liquid

TABLE 1

Foam Volumes (expressed as % over-run) Obtained Following Whipping, at 20°C, of 1% (w/v) Solutions of Acidic Casein at pH 2·0, 2·5 or 3·0 or Sodium Caseinate at pH 7·0

Samples	*Over-run* (%)
Acidic casein, pH 2·0	162·5 ± 9·3
Acidic casein, pH 2·5	140·0 ± 8·7
Acidic casein, pH 3·0	120·0 ± 6·5
Sodium caseinate, pH 7·0	137·5 ± 7·5

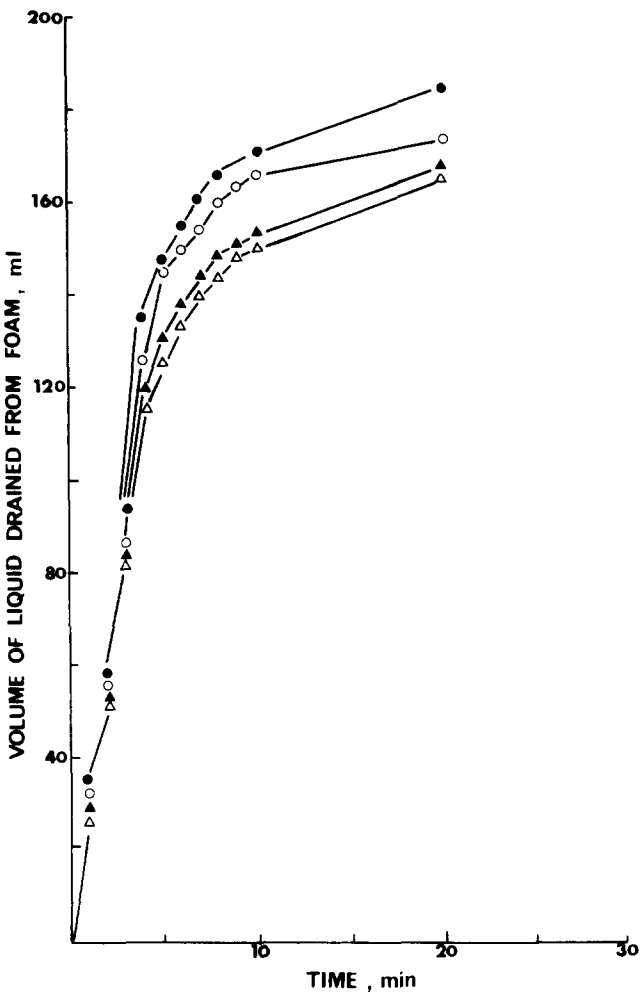


Fig. 5. Stabilities, as measured by volume of liquid drained over time, of foams prepared by whipping, at 20°C, 1% (w/v) solutions of acidic casein, pH 2·0 (●), acidic casein, pH 2·5 (○), acidic casein, pH 3·0 (△) or sodium caseinate, pH 7·0 (▲).

TABLE 2

Foam Volumes (expressed as % over-run) and Foam Stabilities (As Measured by Volume of Liquid Drained after 30 min) Obtained Following Whipping, at 20°C, 1% (w/v) Solutions of Acidic Casein (pH 2·0) with Added $CaCl_2$ or NaCl

Concentration (mM)	*Over-run (%)*	*Volume of liquid (ml) drained after 30 min*
$CaCl_2$		
0	162·5 ± 9·3	190
5	120 ± 7·6	185
10	110 ± 6·1	180
NaCl		
0	162·5 ± 9·3	193
10	115 ± 8·2	190
20	110 ± 7·0	187

drained from the foam decreased slightly with increasing salt concentration (Table 2).

Using a sparging method V_i, V_r, G_i and D_{10} were determined (Table 3). The G_i values for acidic casein (pH 2·0, 2·5 and 3·0) were much lower than that for sodium caseinate (pH 7·0). The D_{10} values were in the order acidic casein, pH 3·0 < sodium caseinate, pH 7·0 < acidic casein, pH 2·5 < acidic casein, pH 2·0. The percentages of liquid drained from the foams over time were in the order acidic casein, pH 2·0 > acidic casein, pH 2·5 > sodium caseinate, pH 7·0 > acidic casein pH 3·0.

Using both methods (whipping and sparging), it was observed that the stabilities of acidic casein foams were in the order pH 3·0 > 2·5 > 2·0 and that foams prepared using sodium caseinate (pH 7·0) were more stable than those from acidic caseins at pH 2·0 or 2·5, but less stable than the foam from acidic casein, pH 3·0.

TABLE 3

Foaming Properties of Acidic Casein Solutions (pH 2·0, 2·5 or 3·0) and Sodium Caseinate (pH 7·0) as Determined by Sparging 0·25% (w/v) Protein Solutions at 20 C

pH	*Volume of liquid (ml) in the foam initially (V_i)*	*Volume of liquid (ml) retained in the foam after 10 min (V_r)*	*Per cent sparged gas entrapped initially in 70 ml of foam (G_i)*	*Per cent liquid drainage from foam after 10 min (D_{10})*
3·0	12·0	5·8	74·4	51·7
2·5	11·9	4·3	70·7	63·9
2·0	11·5	3·7	56·4	67·8
7·0	12·0	5·6	90·6	53·3

DISCUSSION

The results show that the emulsifying and foaming properties of acidic caseins were of the same order as those of sodium caseinate (pH 7·0) and that these properties were influenced by addition of NaCl or $CaCl_2$. The study confirms the findings of Crenweldge *et al.* (1974) that pH influences the emulsifying properties of proteins; however, the shape of the emulsifying capacity–pH curve is quite different from the shape of the solubility–pH curve (Mohanty *et al.*, 1987). It therefore appears that the effect of pH is not solely an effect on protein solubility but may also exert an influence by controlling the thickness of the adsorbed protein film. The surface potential barrier to adsorption increases as pH moves from the isoelectric point; therefore, at pH values further from the isoelectric point, surface coverage decreases and emulsifying capacity increases, as observed. However, because of this surface coverage effect the stability of emulsions decreased as pH moved further from the isoelectric point due to the formation of less condensed and less densely packed films.

The improvement in the emulsifying properties of sodium caseinate on addition of NaCl observed by Tornberg & Hermansson (1977) or on addition of $CaCl_2$ as observed by Sabharwal & Vakaleris (1972) was also observed in this study. However, while low concentrations (< 5 mM) of NaCl or $CaCl_2$ increased the emulsifying capacity of acidic casein (pH 2·5), higher concentrations had the opposite effect.

The increased stability of acidic casein foams with increasing pH may be due to decreased electrostatic repulsion between protein molecules resulting in closer packing of protein at the air–water interface and increased protein–protein interaction giving stronger protein films that are less permeable to gas. Kinsella (1976), who studied the effect of pH on the stability of foams produced from a range of proteins, came to similar conclusions.

The improvement in the stability of foams prepared from acidic casein solutions on adding salts (either $CaCl_2$ or NaCl) may also be due to the formation of more dense protein films. Mita *et al.* (1977), who studied the foaming properties of gluten solutions (1%, w/w, pH 3·5) containing up to 1M added NaCl, reported that salt caused a more compact protein conformation at an air–water interface, resulting in increased foam stability.

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