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Concentration of Proteins from Single Component Solution Using a Semibatch Foaming Process

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ABSTRACT

A laboratory-scale foam separation system was employed to examine the enrichment and recovery of six proteins: sodium caseinate, β-casein, bovine serum albumin (BSA), β-lactoglobulin, α-lactalbumin, and chymotrypsinogen A. In this report we present experimental data which demonstrate the effectiveness of the separation process in extracting proteins from single component solutions. In particular, we have examined the effects of: 1) the solution pH at a fixed air flow rate and initial protein concentration, 2) superficial air velocity at fixed values of pH and protein concentration, and 3) protein concentration at the optimum pH and at a given superficial air velocity. The maximum enrichment of BSA was obtained at its isoelectric point (pH 4.8), and for other proteins better enrichment was achieved at a pH higher than their isoelectric point. The lower the superficial velocity in the 0.079–0.92 cm/s range the higher the enrichment for all the proteins except for α-lactalbumin and chymotrypsinogen A (for these proteins enrichment was insensitive to the superficial velocity). The higher enrichment was also obtained by foaming at a smaller initial protein concentration (in the 30–120 mg/L range).

INTRODUCTION

The separation of proteins from a culture medium or from industrial process streams is usually carried out by adsorption, ion exchange, chromatography, ultrafiltration, and precipitation. However, these processes require elaborate

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apparatus and have problems in continuous operations and in scaling up for commercial applications. Foam separation is a promising technique for the extraction of proteins, and it has the advantages of relatively low capital and operating costs if compared to other separation processes (1–5). This is based on the preferential adsorption of surface-active solutes like proteins at the gas–liquid interfaces. This type of separation is more effective for the recovery of proteins from dilute solutions (typically <1 wt%) because the change in surface tension of the solution is very high at low solute concentrations (6). The other advantage is that there is no need for the addition of foreign materials and/or thermal energy, except for the addition of an acid or a base to achieve a desired pH state. Thus, contamination by exogenous agents and thermal denaturation of proteins are minimized. This gives special advantage for processing dairy, food, and biochemical/biological products which are usually heat sensitive and need restrictive treatment.

Foam fractionation was used to concentrate acids from its mixture with salts (7, 8). It has also been used to separate a specific enzyme from a multicomponent mixture or crude culture filtrates (9–14).

The foam fractionation processes and their potential application were reviewed by Okamoto and Chou (15), Somasundaran and Anatharpadmanathan (16), and Wilson and Clarke (17). An overview of foam separation was covered by Lemlich (18), and the stability of protein foams was presented in detail by Halling (19). Much of the previous work is on systems where a foaming agent was added to a solute or a mixture of solutes. Some studies have been done with systems where stable foams can be generated without adding any surfactants or similar chemicals. These characteristics of the naturally foaming substances need to be further studied, especially to determine the selective separation conditions.

The main objective of this paper was to evaluate the performance of foam fractionation for the extraction of various dairy proteins from aqueous solutions. This was fulfilled by determining enrichment, recovery, and separation factors in a laboratory-scale foam fractionation cell for six different single component proteins: (a) sodium caseinate, (b) β-casein, (c) BSA, (d) β-lactoglobulin, (e) α-lactalbumin, and (f) chymotrypsinogen A. The effectiveness of separation has been determined by protein enrichment factors in foam as a function of the following variables:

- pH of feed solution
- Initial protein concentration
- Superficial air velocity

MATERIALS AND METHODS

The chemicals sodium hydroxide (NaOH) and hydrochloric acid (HCl), both AR grade, were purchased from BDH (England). The proteins α-lactal-

bumin (L-6010, 1995), β-lactoglobulin (L-7880, 1995), β-casein (C-6905, 1996), and chymotrysinogen A (C-4879, 1994) were obtained from Sigma Chemical Co. (USA). BSA (PSB 100063, 1995) was from Life Technologies Ltd. (New Zealand). Sodium caseinate (BA G0009-G00012, 1995) was a gift from the New Zealand Dairy Board.

Surface Tension Measurements

The surface tension of the protein solutions was measured using a tensiometer (Cambridge Instrument Company Ltd., England) and following ASTM method D971-91 (20). A detailed procedure of the measurements is described below.

Preparation of Apparatus

A 14% chromic acid stock solution (maximum concentration 5 g/L) heated to 60°C was used for cleaning the glass Petri dishes. After chromic acid cleaning, the Petri dishes were rinsed thoroughly with tap water and then given a final rinse with distilled water.

The platinum ring was cleaned using a repetitive process (2 to 3 times) of heating over a small Bunsen burner using a hot flame until the ring was red hot, and rinsing with acetone.

Preparation of Test Solutions

Protein solutions ranging in concentration from 5 to 150 mg/L were prepared. For each concentration the pH was adjusted using NaOH and HCl for buffering of protein solutions.

Measurement of Surface Tension of Samples

The surface tension of water was measured and taken as the reference point.

To determine the surface tension of the solution, the platform of the tensiometer was lowered simultaneously as the torque of the ring system was increased in order to maintain the torsion arm in the zero position. The value at which the platinum ring broke free of the surface of the solution was recorded as the surface tension of the solution (dyn/cm). The measurement was repeated with the same solution to determine a reproducible result.

Foaming Process and Experimental Procedure

The experimental setup consisted of a standard laboratory glass column with a glass frit having a porosity range of 16–40 μm fused into its base. A

modified PVC U-bend was used as part of the foam collection unit. Foam passing through a PVC tube attached at the end of the U-bend was collected in a plastic beaker. The air pressure was controlled using a Norgen (Germany) 0–1.6 bar pressure regulator connected to the compressed air supply. The pressure was set to 0.8 bar in all experiments. Air flow rate from the pressure regulator was controlled using a rotameter (GGC-Elliot, England). The details of the foam separation column are shown in Fig. 1. The dimensions of the column are listed in Table 1.

The protein solution fed into the column is contacted with gas bubbles rising from the bottom of the column. As bubbles rise through the liquid pool,

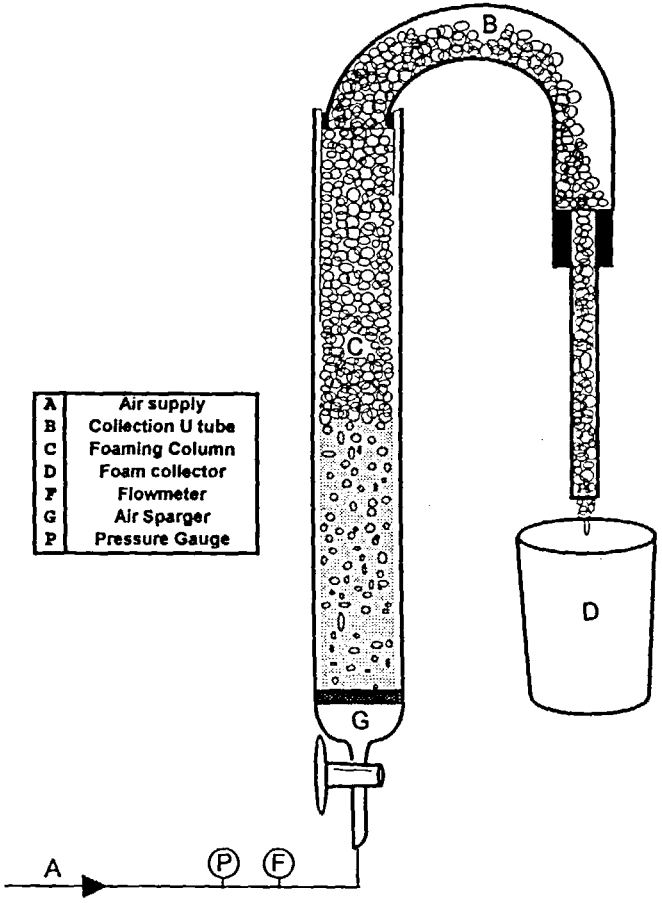

FIG. 1 Schematic diagram of a foam separation column.

TABLE 1
Foaming Column Dimensions

Overall column length	770 mm
Height from frit to top	609 mm
Length from frit to bottom	157.5 mm
I.D. of column	52 mm
O.D. of column	56 mm
I.D. of tail	6 mm
Glass frit pore size	porosity No. 3 (16–40 μm)
Glass frit thickness	3.5 mm
I.D. of PVC U bend	40 mm
I.D. of clear PVC Tube	13 mm

they are entrained with the process solution. At the foam–liquid interface, bubbles join with each other and form foam. Continuous air flow from the bottom of the column forces the foam to rise toward the top of the column. As the foam rises, the liquid entrained in the foam drains off due to gravity, and as a result the foam collected at the top of the column is highly concentrated in protein.

The stepwise procedure for performing the separation experiment is:

1. A 600-mL solution of target protein at a desired concentration was charged into the column. The solution temperature was kept in the 18–20°C range. The pH was adjusted by adding either 0.1 M HCl or 0.1 M NaOH solution and measuring the pH with a PHM-64 Research pH meter (Radiometer Co., Copenhagen).
2. Air from the compressed air line was allowed to enter at the bottom below the glass frit at a desired air flow rate. Air bubbling through the liquid produced fine bubbles and stable foam. The latter was carried over and collected in a beaker.
3. After foaming ceased, the collected foam was allowed to collapse and the liquid volume was measured.
4. The samples of feed, foam, and residual solution were analyzed for their protein concentrations.

Analysis of Proteins

The concentration of protein in the feed, foam, and residual liquid was determined by a standard BioRad protein assay (21) using a dye reagent. The absorbance of the samples was measured at a wavelength of 595 nm using a Shimadzu UV-160 Spectrophotometer (Shimadzu, Kyoto, Japan).

It is noted that UV absorbances of less than 1 were used when producing a standard curve and measuring samples, as the curve loses linearity after this point. The equation of the line was used to determine the concentration

from the absorbance of the solution. Samples containing a high concentration of protein were diluted with distilled water before the absorbance was measured.

Performance Criteria

Foam Separation

To characterize the efficiency of the foam separation process, the following factors were used:

$$\text{Ef (protein enrichment)} = \frac{\text{protein concentration in foam liquid } (C_P)}{\text{protein concentration in initial solution } (C_F)} \quad (1)$$

$$\text{Sf (protein separation)} = \frac{\text{protein concentration in foam liquid } (C_P)}{\text{protein concentration in residue liquid } (C_R)} \quad (2)$$

$$\text{RP (percentage recovery)} = \frac{\text{mass of protein in foam liquid } (m_P)}{\text{mass of protein in initial solution } (m_F)} \quad (3)$$

For the production of concentrated proteins, high enrichment factors and percentage recoveries are desirable. For stripping of proteins from process streams, high separation factors are a measure of good performance.

Theoretical Calculation of Surface Tension

The surface tension at various pH values and concentrations were tested using a modified form of the Szyszkowski equation (22):

$$\gamma_s = \gamma_w - A \log\left(1 + \frac{C_F}{B}\right) \quad (4)$$

where γ_s and γ_w represent the surface tension values of the solute (protein) and water, respectively, under identical conditions. A and B are the parameters to be obtained by fitting the experimental data with Eq. (4), and C_F is the concentration of the protein solution.

RESULTS AND DISCUSSION

Surface Tension Measurements

The effect of protein concentration on the surface tension at various solution pH values are shown in Fig. 2: (a) sodium caseinate, (b) β-casein, (c) BSA,

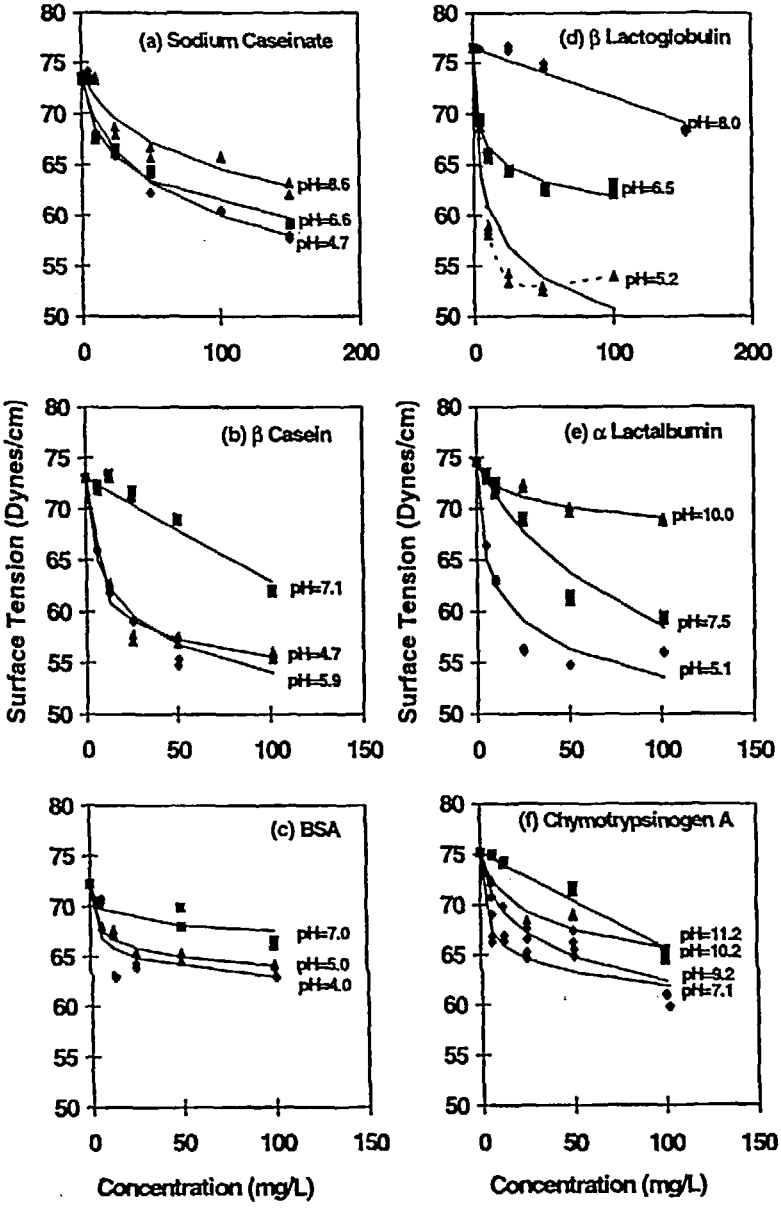

FIG. 2 Effect of protein concentration on the surface tension of protein solutions.

TABLE 2
Key Properties and Experimental CMC Values for the Proteins

Protein	MW (kD)	mg/L	p*I*	pH
Sodium caseinate	24.5	35	4.7	8.6
β-Casein	25	35	4.7	5.9
BSA	67	25	4.8	5.0
α-Lactalbumin	14.2	35	5.1	5.1
β-Lactoglobulin	36.6	35	5.2	6.5
Chymotrypsinogen A	25	30	9.2	9.2

(d) β-lactoglobulin, (e) α-lactalbumin, and (f) chymotrypsinogen A. The slope of the graphs are negative, indicating the proteins tend to accumulate at the gas–liquid interface according to Gibbs' equation (23) and eventually escape the system as foam. The slope of the surface tension concentration decreases with the increase in concentration, and it approaches zero at very high concentration, resulting in a very small amount of adsorption. The surface tension of the solutions attains a constant value, which is called the critical micelle concentration (CMC). This can be taken as a measure of the surface activity of a protein for the gas/liquid interface. The CMC values along with some properties of the proteins are listed in Table 2. These values suggest that BSA, with the largest molecular weight, is the most active protein. A protein with a higher molecular weight contains longer chains and offers more active sites for adsorption, and this lowers the CMC. This value also depends on the solution pH relative to the isoelectric pH.

The surface tension of the solutions has not leveled off at higher pH (higher than the isoelectric point), indicating the CMC has not been reached. The surface tension of protein solutions at higher pH approaches that of distilled water because of weaker electrostatic attractions when the solution is at a pH away from the isoelectric point.

The experimental data of surface tension for all proteins were analyzed and the predictions of Eq. (4) are plotted in Fig. 2. The values of the parameters *A* and *B* obtained for best fit lines are listed in Table 3. Except for the surface tension measurements at low pH and concentration (especially for the proteins BSA, β-lactoglobulin, and α-lactalbumin), the agreement between the predicted curve and experimental data was good.

The effects of protein concentration and feed solution pH on foam concentration are described in the next few paragraphs. It is noted that the experiments were conducted under semibatch conditions for a period averaging 20–25 minutes. As a result, as time proceeds the concentration of the remaining solution decreases and this becomes insufficient to produce stable foam.

Effect of Feed Solution pH

The effect of pH on enrichment of the six proteins is shown in Fig. 3. For sodium caseinate, at a feed concentration of 35 mg/L and foamed at a superficial air velocity of 0.52 cm/s, protein enrichment did not vary greatly with pH. The maximum enrichment was at pH 8, and at pH below 7 unstable foams were formed, although the isoelectric point (p*I*) of sodium caseinate is 4.7. This result is not consistent with the predictions of Gibbs theory.

Enrichment increased sharply from pH 6 to attain maximum at pH 7 for β-casein at a concentration of 40 mg/L and a superficial air velocity of 0.52 cm/s. Enrichment factors remained about the same up to pH 10, and beyond this pH insufficient foaming occurred. Again, β-casein did not foam at its isoelectric point (about pH 4.7), although the slope of the surface tension–concentration plot (Fig. 2b) suggests a negative value (i.e., a tendency for protein accumulation at the liquid–gas interface).

BSA enrichment decreased slightly from pH 4.2 to 4.4 and then sharply increased toward pH 4.8 (the isoelectric point) at a concentration of 25 mg/L and a superficial velocity of 0.079 cm/s. This behavior can be explained

TABLE 3
Parameters *A* and *B* for Surface Tension Curve Fitting of Six Proteins: (a) Sodium Caseinate, (b) β-Casein, (c) BSA, (d) β-Lactoglobulin, (e) α-Lactalbumin, and (f) Chymotrypsinogen A

Protein	pH	Parameter *A* (Eq. 4)	Parameter *B* (Eq. 4)
(a) Sodium caseinate	4.7	12.4	8.77
	6.6	8.2	3.1
	8.6	10.9	16.2
(b) β-Casein	4.7	5.93	0.12
	5.9	9.51	1.03
	7.0	1662	7000
(c) BSA	4	3.23	0.14
	5	2.90	0.16
	7	1.70	0.19
(d) β-Lactoglobulin	5.2	10.3	0.32
	6.5	4.97	0.11
	8.0	223	1923
(e) α-Lactalbumin	5.1	9.06	0.48
	7.5	24.1	27.4
	10.0	3.44	2.63
(f) Chymotrypsinogen A	7.1	4.53	0.12
	9.2	8.51	3.23
	10.2	6.50	3.55
	11.2	449	2028

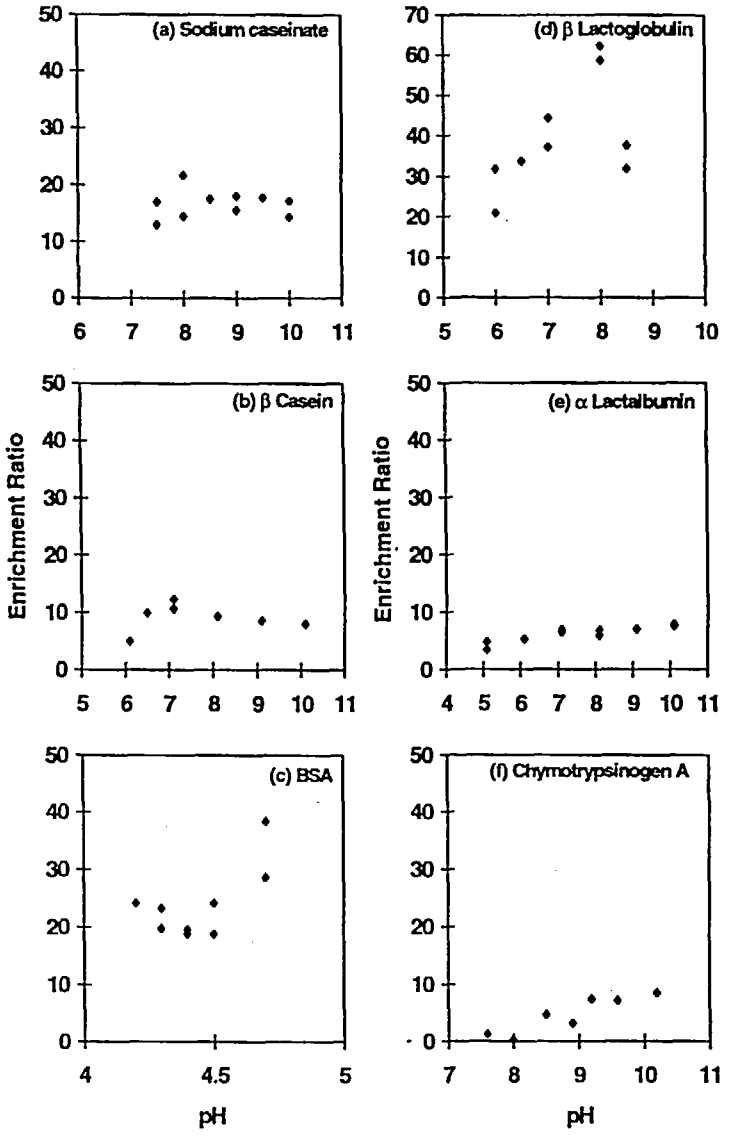

FIG. 3 Effect of solution pH on enrichment of the proteins mentioned in Fig. 2.

adequately with the surface tension values measured (Fig. 2c) and those reported by Schnepf and Gaden (2). The slope of the surface tension curve is relatively higher at p*I*; this suggests a greater accumulation of proteins at the interface (23) and with the same rate of foam flow should give a higher enrichment.

For β-lactoglobulin, enrichment increased (almost double the value at higher pH) sharply from pH 6 to 7.8 and rapidly decreased to low values beyond this pH for foaming at a feed concentration of 34 mg/L and a superficial velocity of 0.079 cm/s. It did not produce sufficient stable foam at the isoelectric point pH 5.2. This can be explained by the fact that at this pH the surface tension value is very small and the rate of change is apparently zero (Fig. 2d, dotted line).

α-Lactalbumin foamed sufficiently at the isoelectric point (pH 5.1) and could be enriched over the pH range 5.2–10 for a feed concentration of 46 mg/L. A rather high superficial air velocity of 0.39 cm/s, compared to the that for foaming of BSA, was required to produce stable foam. It is noted that enrichment values are insensitive to the solution pH although there is a significant difference in the rate of change of surface tension (Fig. 2e).

Enrichment for chymotrypsinogen A was low at pH 7.6 and gradually increased toward the isoelectric point pH 9.2 for foaming at a feed concentration of 16.6 mg/L and at a higher superficial velocity of 0.92 cm/s. At this concentration the surface tension is smaller and its rate of change is greater at the lower pH (i.e., pH 7.2 in Fig. 2f) and under this condition enrichment should have been maximum (Gibbs' theory). On the contrary, enrichment increased toward higher pH where the slope of the surface tension curve was smaller.

Since BSA and β-lactoglobulin showed very sharp profiles over a narrow range of pH, the effect of feed pH was further studied by varying the superficial velocity through the foam column. The enrichment factors of BSA at superficial velocities of 0.079, 0.15, and 0.26 cm/s are compared in Fig. 4. By increasing the superficial velocity it was possible to expand the operating pH range (up to 6.5) with little drop in protein enrichment.

The effect of varying feed pH at superficial velocities of 0.079, 0.16, and 0.26 cm/s for β-lactoglobulin are shown in Fig. 5. The patterns of the enrichment–pH profiles are similar with increased values for maximum enrichment at lower superficial velocities. The pH for maximum enrichment was also shifted by about a unit when the superficial velocity was changed. The results could be explained by two factors involved in foaming: 1) an increase in interfacial area for an increase in superficial velocity, allowing more proteins to be transported, and 2) a decrease in drainage at higher velocity, indicating a dilute foam (i.e., smaller enrichment).

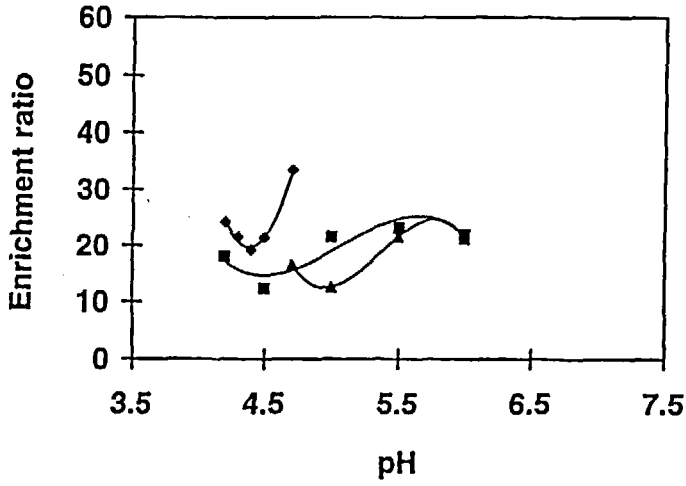

FIG. 4 Effect of solution pH on enrichment of BSA at superficial air velocities of 0.079 cm/s (◆), 0.16 cm/s (■), and 0.26 cm/s (▲).

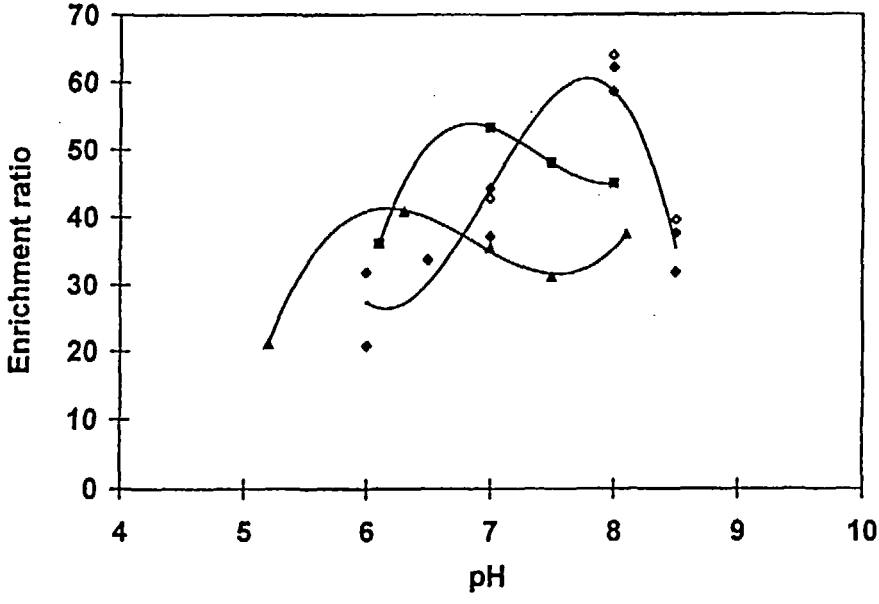

FIG. 5 Effect of solution pH on enrichment of β-lactoglobulin at superficial air velocities of 0.079 cm/s (◆, ◇), 0.16 cm/s (■), and 0.26 cm/s (▲).

Effect of Initial Protein Concentration

Figures 6(a–f) show the effect of initial protein concentration on enrichment for: (a) sodium caseinate at pH 8 and superficial velocity 0.52 cm/s, (b) β-casein at pH 7.1 and superficial velocity 0.52 cm/s, (c) BSA at pH 4.8 and superficial velocity 0.079 cm/s, (d) β-lactoglobulin at at pH 7 and superficial velocity 0.079 cm/s, (e) α-lactalbumin at pH 5.1 and superficial velocity 0.39 cm/s, and (f) chymotrypsinogen A at pH 9.2 and superficial velocity 0.92 cm/s. β-Lactoglobulin was concentrated to a very high degree whereas α-lactalbumin was enriched to a low degree. For all the proteins, enrichment values decreased monotonically with an increase in bulk concentration. This is expected because at high bulk concentrations the equilibrium adsorption at the gas–liquid interface is higher and hence the surface tension is lower. Also, coalescence of foam bubbles is less (as the surface-active proteins tend to stabilize the film), and as a result the chance of drainage is decreased. At a given superficial velocity, high bulk concentrations lead to greater liquid entrainment at a concentration of residual solution, and without sufficient drainage its concentration remains small. This entrained liquid, being large, would decrease the overall protein concentration in the foam. Thus, enrichment of proteins at higher initial concentrations is lower.

There could be other physical or chemical interactions occurring at high protein concentration, e.g., BSA forming micelles at high concentration (24) and β-lactoglobulin undergoing self-aggregation (25). This would interfere with the foaming process and adversely affect the protein concentration in the foam. Thus it is shown that foaming separation works better for extraction from dilute feed solution, i.e., concentrations less than 50 mg/L.

The effect of feed concentration (C_F) on protein enrichment (Ef) can be predicted from a power law equation:

$$\mathrm{Ef} = M(C_F)^{-N} \tag{5}$$

where M and N are adjustable parameters. The values of M and N for all the proteins are listed in Table 4. This equation suggests that in order to have high enrichment, smaller initial concentrations should be used.

Effect of Air Flow Rate

The effect of air flow rate is shown in Fig. 7 for: (a) sodium caseinate solution at pH 8 and 50 mg/L, (b) β-casein solution at pH 7.1 and 50 mg/L, (c) BSA solution at pH 4.7 and 26 mg/L, (d) β-lactoglobulin solution at pH 7 and 50 mg/L, (e) α-lactalbumin solution at pH 5.1 and 65 mg/L, and (f) chymotrypsinogen A solution at pH 9.2 and 60 mg/L. The enrichment decreased for sodium caseinate, β-casein, and β-lactoglobulin, and remained fairly constant for α-lactalbumin and chymotrypsinogen A.

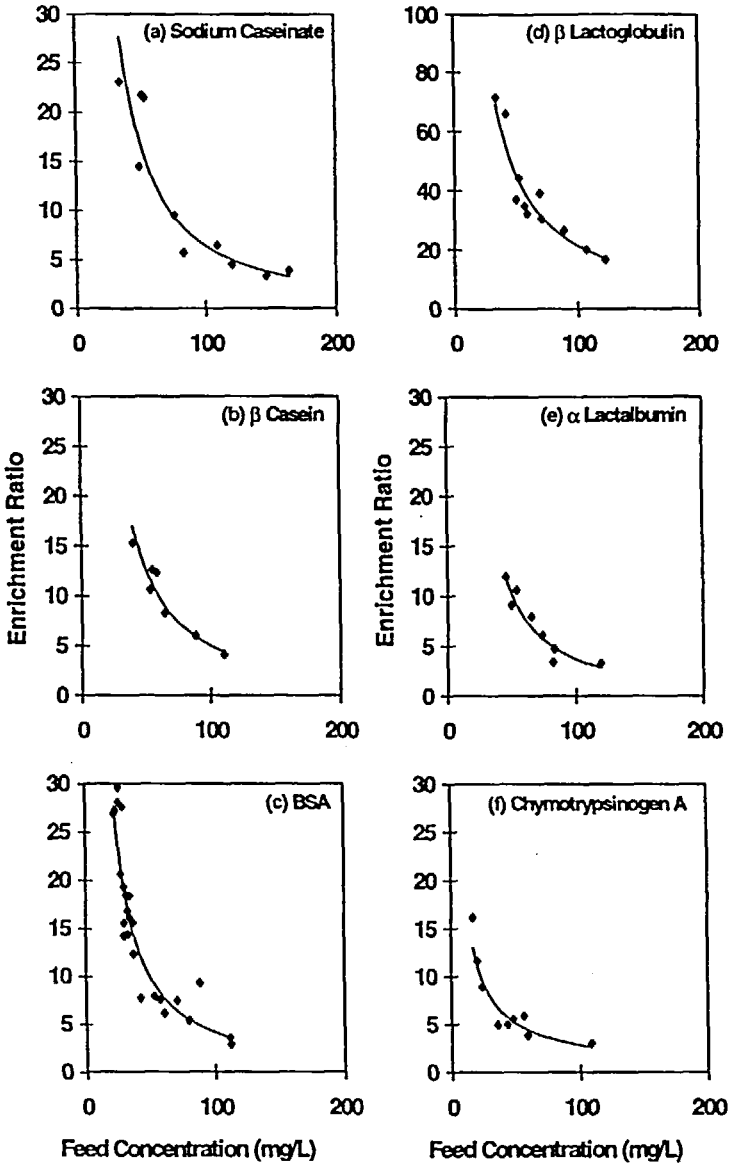

FIG. 6 Effect of initial protein concentration on enrichment of the proteins mentioned in Fig. 2.

TABLE 4
Parameter Values for Equation of Enrichment Factor (Ef) for the Proteins Mentioned in Table 3

Parameter	Sodium caseinate	β-Casein	BSA	β-Lactoglobulin	α-Lactalbumin	Chymotrypsinogen A
M	3873	2476	1424	3274	3274	141
N	1.4	1.4	1.3	1.1	1.5	0.9

For BSA, higher enrichment was obtained at a very low superficial velocity of 0.079 cm/s, it decreased considerably up to a superficial velocity of 0.26 cm/s, and beyond this velocity enrichment attained a constant value. An increase in superficial velocity changes the process conditions: 1) contact time of air in liquid, 2) average bubble size, and 3) liquid entrainment.

At higher superficial velocity the contact time is decreased for bubble coalescence, foam breakage, and liquid drainage (12). The average size of the bubbles in liquid and foam sections is increased at higher superficial velocities, and larger bubbles have a smaller capacity for protein adsorption. At a much higher superficial velocity a larger volume of liquid is entrained in the foam and forced out at a faster speed. As a result of these factors, protein concentration in foam decreased as the superficial velocity was increased, giving smaller enrichment values. Furthermore, loss of protein solution at higher superficial velocities may not be desirable for many process streams. Therefore, lower air superficial velocities should be used to achieve higher enrichment and to prevent loss of liquid from process solution.

This foam separation technique produced high protein enrichment from aqueous solutions of the six single component systems. The values for enrichment, separation factor, and percentage recovery at the best conditions are presented in Table 5a. It shows that enrichment, separation, and recovery can be achieved to a very high degree for β-lactoglobulin, moderate for BSA, and very low for α-lactalbumin. The proteins chymotrypsinogen A, sodium caseinate, and β-casein can be enriched to a reasonable degree; the percentage recovery of these proteins is small under these conditions. It is noted that the percentage recovery can be changed by varying the foaming conditions, e.g., operating at higher superficial gas velocity, which will diminish protein enrichment. The best operating conditions (solution pH, initial protein concentration, and superficial air velocity) to achieve the above performance are listed in Table 5b.

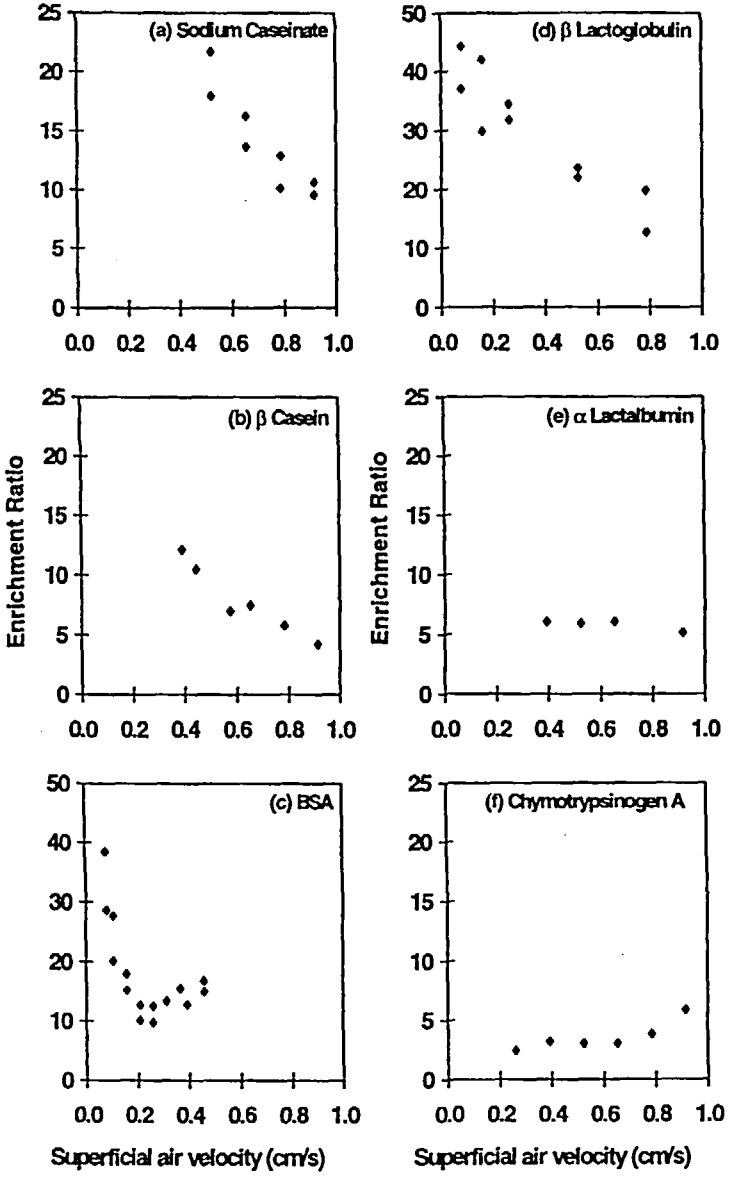

FIG. 7 Effect of superficial air velocity on enrichment of the proteins mentioned in Fig. 2.

TABLE 5a
Values of the Performance Parameters for the Proteins Mentioned in Table 3

Operation variable	Sodium caseinate	β-Casein	BSA	β-Lactoglobulin	α-Lactalbumin	Chymotrypsinogen A
Enrichment factor Ef (—)	23	15	33	72	12	16
Separation factor, Sf (—)	30	20	51	551	13	23
Percentage recovery (% RP)	23	36	83	96	12	51

TABLE 5b
Operating Conditions to Achieve the Performance Parameters in Table 5a

Performance parameter	Sodium caseinate	β-Casein	BSA	β-Lactoglobulin	α-Lactalbumin	Chymotrypsinogen A
pH	8	7.1	4.8	7	5.1	9.2
Feed concentration (mg/L)	35	40.5	26	34	46	16.6
Flow rate (L/h)	0.52	0.52	0.079	0.079	0.39	0.92

CONCLUSION

Application of this foam separation technique produced protein enrichment of a high value for β-lactoglobulin; a moderate value for BSA, sodium caseinate, and β-casein, and a low value for chymotrypsinogen A and α-lactalbumin. Recovery was large for β-lactoglobulin and BSA; the other proteins could not be recovered in significant percentages.

In general, enrichment depended strongly on solution pH. Maximum values obtained for the proteins were: (a) sodium caseinate at pH 8, (b) β-casein at pH 7, (c) BSA at pH 4.8, (d) β-lactoglobulin at pH 7.8, (e) α-lactalbumin at 10.2 and (f) chymotrypsinogen A at 9.2. For BSA and chymotrypsinogen A only, maximum enrichment was achieved at their respective isoelectric points.

Higher enrichment values were obtained by lowering the feed concentration for all proteins. This suggests that foam fractionation is only practical for recovering proteins from dilute process streams.

At a smaller superficial air velocity, better protein enrichment was achieved (except for α-lactalbumin and chymotrypsinogen A whose foam concentrations were insensitive to changes in superficial air velocity).

SYMBOLS

A, B	parameters defined in Eq. (4)
C	protein concentration ($mg \cdot L^{-1}$)
m	mass of protein (mg)
Ef	enrichment factor (dimensionless)
M, N	parameters as defined in Eq. (5)
RP	percentage recovery (dimensionless)
Sf	separation factor (dimensionless)
γ	surface tension ($dyn \cdot cm^{-1}$)
Γ	surface excess at the interface ($mol \cdot m^{-2}$)

Subscripts

F	feed solution
P	foam
R	residual solution
s	solute (protein) solution
w	water

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