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Author(s)	Maruyama, Hideo; Seki, Hideshi
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## Recovery of milk whey proteins by foam separation

# Hideo MARUYAMA<sup>\*</sup> and Hideshi SEKI

Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido

University

Minato 3-1-1, Hakodate JAPAN, 041-8611 Telephone: +81-138-40-8813, Facsimile: +81-138-40-5048, E-mail: maruyama@fish.hokudai.ac.jp

\* corresponding to the author

### Highlights

1. Conducting recovery of proteins from milk whey solution by foam separation.

2. Langmuir's adsorption parameters can be determined by the proposed estimating method.

3. Estimated adsorption parameters verified with continuous mode operation.

4. Saturated adsorption density was largest at pH 5 in the range of pH 4-7.

5.  $\beta$ -lactoglobulin was considered most hydrophobic in the range pH 5-7.

#### Abstract

Milk whey proteins recovery was conducted with foam separation. Experiments were conducted by batch mode in pH 4-8. The separation rate was evaluated by the rate constant of the first-order kinetic equation. Both recovery efficiency and separation rate were highest at pH 7. Overall equilibrium adsorption constant, K, and saturated adsorption density,  $X_s$ , were determined from a proposed estimating method using data obtained in batch mode. At pH 7, K and  $X_s$  were determined using the data obtained in continuous mode. K and  $X_s$  determined by both operation modes were agreed well. Adsorption parameters of the major proteins in milk whey (bovine serum albumin (BSA),  $\alpha$ -lactalbumin (LA), and  $\beta$ -lactoglobulin (LG)) were estimated by the proposed estimating method in pH 4-7.  $X_s$  is largest at pH 5 and the K of LG over pH 5 was larger, which suggested that LG was the most hydrophobic species among them.

KEYWORDS: Foam separation; Milk whey protein; Adsorption; Water treatment; bio-resource recovery

#### 1. Introduction

Milk whey is a by-product of cheese production. In cheese production processes, about 90 % of raw material, cow milk became milk whey (Gonzdlez Siso, 1996; Illanes, 2011). In Japan, for example, the domestic amount of cheese production was 44,000 tons in 2019 and it is reported that the amount produced increased year by year. With an increase in the amount produced of cheeses, milk whey was produced over ca. 400,000 tons in 2020. Effective utilization of milk whey has been attempted, for example, food additive for dietary supplements, confectionery, ice cream, livestock foods, and so on. It has been reported that milk whey contains many useful substances, for example, lactose (40-50 g/L), some proteins;  $\alpha$ -lactalbumin;  $\beta$ -lactoglobulin (6-8 g/L), and minerals (80-100 g/L) (Walstra et al., 1984). It is important to recover these for variable utilization of potentially useful bio-resources.

However, to utilize whey effectively, a large amount of capital investment, management, and maintenance costs are required for storage facilities for quality control and various processing equipment. Therefore, the processing of whey is unprofitable for small and medium-scale factories, and the whey has often been disposed of as industrial waste, except for some manufacturers who use it for livestock feed (Japan livestock technology association, 2006). There are many small-scale cheese factories in Hokkaido prefecture, Japan and such factories should not be able to treat whey as well as large-scale companies. Therefore, a simple and inexpensive processing method is required for the effective utilization of whey.

Among many separation operations, the foam separation technique is one of the convenient methods to separate or enrich soluble substances which have hydrophobicity. From the viewpoint of efficient use of natural resources and industrial byproducts, it is

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important to recover useful substances, such as proteins from milk or cheese whey. The foam separation technique has some advantages, for example, fewer mechanical parts in apparatus, low operation cost, simpler operation, and smaller facility area. Judging from these advantages, the small-scale factories could maintain foam separation apparatus.

There have been several reports concerning the separation of milk or cheese whey protein by foam separation in two decades (Matouq, 2008; Shea et al., 2009; Mukhopadhyay et al., 2010; Mukhopadhyaya et al., 2011; Sunkesula et al., 2020). Most of the previously reported studies have been concerned with determining the optimal conditions for solving particular problems, and have not come close to proposing a general-purpose solution. From a chemical engineering aspect, the enrichment process by foam separation method is divided into two stages, (1) adsorption of objective substance onto bubble surface in dispersed bubble bed, and (2) enrichment in foam bed due to a coalescence of foam cells and gravitational liquid drainage (Maruyama et al., 2000). The former one is well characterized by the adsorptive physicochemical properties of objective substances. These properties affect values of adsorption rate constant and equilibrium adsorption constant, which could be determined experimentally. From a chemical engineering aspect, to develop and design a foam separation technique, it is important to determine the adsorption parameters of objective substances. In addition, the foam separation technique itself has been selected as a tool to determine adsorption parameters.

In this study, we proposed a method to determine the overall equilibrium adsorption constant and overall saturated adsorption density of milk whey proteins by batch foam separation operation. These values were also determined by continuous operation mode, which has been well employed for the determination of these parameters in literature. In addition, the influence of pH on recovery efficiency and these adsorption parameters were investigated in this study.

#### 2. Material and methods

#### 2.1. Materials

Cow milk used in this study was a marketed product containing over 8.4 wt.% nonfat milk sold and 3.7 wt.% milk fat and was purchased from a mass merchandise market in Hakodate city in Japan. Standard substances of  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, and bovine serum albumin were obtained from Sigma-Aldrich Co. Ltd. Trifluoroacetic acid (TFA) and acetonitrile were purchased from Kanto Chemical Co. Inc. as mobile phase solvents for HPLC. All the other reagents used in this study were also obtained from Kanto Chemical Co. Inc. and were used without further purification.

#### 2.2. Preparation of milk whey solution

The desired volume of cow milk was poured into a beaker, then, 1 M HCl aqueous solution was added to milk for adjusting the pH value of milk at pH 4.6, which corresponds to the isoelectric point of casein. After adjusting pH 4.6, the milk solution was centrifuged at 3,000 rpm for 15 minutes. The supernatant solution (whey solution) was filtered with filter paper (ADVANTEC No.2). Moreover, 1 M HCl or 1 M NaOH aqueous solutions were added to the obtained whey solution for adjusting desired pH value. After that, the whey solution was centrifuged at 3,000 rpm for 15 minutes. The supernatant solution for 15 minutes. The whey solution was filtered again with filter paper (ADVANTEC 5C). In most experiments in this study, the obtained whey solution was diluted to 5-fold (w/w) with distilled water, and the diluted whey solution was used for foam separation experiments. This operation was conducted at room temperature (22-28 °C).

#### 2.3. Experimental setup

A schematic drawing of the experimental setup of foam separation for batch mode is shown in Fig. 1. The column was constructed of a transparent acrylic resin tube of 4.4 cm in inside diameter. The main part of the column was 70 cm in height. A sintered glass filter with an average pore size of 10-15  $\mu$ m was installed as a gas distributor at the bottom of the column. Air was supplied to the column through the distributor. Pressure taps were installed along the wall at intervals of 25 cm for measuring gas holdup. The voltage signal from the transducer was recorded by a personal computer.

The foam separation column for continuous mode was almost the same as our previous studies (Maruyama et al., 2000). A feeding tap was installed into the wall at the center of the column height. Besides this, the column dimension and other equipment were mostly the same as the column for batch mode operation.

#### 2.4. Experimental procedure

Experimental procedures for batch and continuous modes in this study were mostly the same as those in the previous studies (Maruyama et al., 2000; Maruyama et al., 2007). The diluted whey solution prepared at desired concentration and pH value was charged into the column (liquid height 60 cm). After the column was filled with the solution, the air was supplied and was dispersed as bubbles by the distributor. In most experiments, the volumetric flow rate of air was 50 cm<sup>3</sup>/s. The solution within the column was sampled from a sampling tap installed at the bottom of the column. In the case of continuous experiments, the feed solutions were supplied from a feed tap by a

pump. In most cases, the volumetric flow rate of feed liquid was  $0.1 \text{ cm}^3/\text{s}$ . The liquid height (60 cm) was adjusted by control of the volumetric flow rate of the drained liquid using a rotameter.

The overall concentrations of the whey proteins were measured by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a standard protein. The protein concentrations including whey solution were determined by HPLC (Shimadzu). The pH of the bulk liquid in the column was measured with a pH meter (ORION Model 520 or 920). The gas holdup was determined from the difference in static pressure between the clear and aerated liquids using a differential pressure transducer. Voltage signals were recorded by a personal computer via digital multi-meter (Sanwa PC5000a). All foam separation experiments were conducted at room temperature (22-28 °C).

#### 3. Results and Discussion

#### 3.1. Influence of pH on separation rate and efficiency in batch foam separation

Typical results of the time course of overall whey protein concentration within the column are shown in Fig. 2a. The experiments were conducted at different pH (pH 4-8). The ordinate of Fig 2a corresponds to the residual fraction of whey protein. The initial concentrations have some scatters. We had to dilute the whey solution 5-fold to prevent it from becoming whole solutions into foam layers.

At first the pH of the undiluted (raw) whey solution was adjusted with HCl or NaOH aqueous solution, and then the raw whey solution was diluted to 5-fold with distilled water. Because the pH of the whey solution changed with the dilution, the pH of diluted whey solution was readjusted with HCl or NaOH solution. The volume of HCl or NaOH solution used for the pH adjustment was lesser than 5 mL. The volume percentage of added HCl or NaOH solution to the diluted whey solution (600-800 mL) was lesser than 1.0 %. Therefore, it is considered that the initial concentration of the diluted whey solution was not affected by the pH adjustment.

In order to verify that the scatter of the initial concentration in the data presented in Fig. 2 was not caused by the dilution, we employed the Q-test (Dean and Dixon, 1951) and Grubbs method (Grubbs, 1969) to the data.

In Q-test and Grubbs method, values of Q and  $g_0$  were calculated by the following equations, respectively.

$$Q = (x_{\rm d} - x_{\rm n})/r \tag{1}$$

$$g_0 = |x_{\rm d} - x_{\rm a}|/s$$

Where,  $x_d$ ,  $x_n$ , and  $\underline{x}_a$  are the doubtful value, the nearest neighbor value, and the average value. *r* and *s* are the range of data series and standard deviation, respectively. The Q-test and Grubbs method were applied to the experimental results conducted at the initial concentrations of 0.506, 0.461, 0.446, 0.303, and 0.305. The doubtful value was set to the largest value (0.506) or smallest value (0.303). The results were summarized in Table 1. The results of Q-test gave the 90% confidence limit for the five data series was less than the 5 %. The Grubbs method gave the same evaluation. The exact reason for the scatters of the initial concentration is not known. The following is speculation, we may consider that the pH adjustment may have caused protein aggregation, which in turn may have caused the scatters. Except for the result at pH 7, the decrease in whey protein finished within ca 50 min. At only pH 7, recovery of whey protein continued until ca. 150 min. At that time, the concentration within the column reached a constant value in the case of pH 7. The separation rate of whey proteins was estimated with the separation rate constant, *k*, defined by the following equation.

$$\ln(C/C_i) = -kt \tag{3}$$

Where, *C* and *C*<sub>i</sub> represent the concentration of whey protein at time *t* and the initial state, respectively. The fitting of the data shown in Fig. 2a to Eq. (3) is shown in Fig. 2b. The straight lines were obtained by a least-squares method with the fitting of the data to Eq. (3).  $R^2$  value in Fig. 2b represents a coefficient of determination in this least squares method. In the early stage of decrease in *C* (*t* < 40 min), time-course data agreed well with Eq. (3), thus, *k* could be determined from the slope values of the straight lines. The

relationship of pH values and the separation rate constant, k is shown in Fig. 3a. Fig. 3b also represents the relationship of pH and the residual fraction,  $C/C_i$ , of whey protein within the column. As seen in Figs. 3, at pH 7, it was found that the separation rate was fastest and the residual fraction is lowest in the experimental range. However, the scatter of k at pH 7 is considerably large, which corresponds to the standard deviation, 0.00161. This value is one order larger than that at the other pH. On the other hand, the  $C/C_i$ value at pH 7 (Fig. 3b) is considered to be significantly smaller than that at the other pH. Judging from the fact, at pH 7, the good separation efficiency was confirmed, however, the separation rate is considered to maybe not faster than that at the other pH. Shea et al. (2009) conducted semi-batch foam separation of proteins from spray-dried whey solution and reported that maximum recovery efficiency and enrichment were obtained at pH 4 and 8, respectively. This difference might be caused by the preparation method of each solution. Sunkesula et al. (2020) have carried out recovery of cheese whey proteins by foam separation in the range of pH 3-6.5 and have reported that the most effective pH value was pH 3 and the recovery efficiency decreased with increasing pH value. They also conducted experiments at a higher initial concentration (0.87 g/L) than our initial concentration. At the higher concentration, they showed high yields in all pH ranges in which they conducted their experiments. They defined the yield as "mass of protein in the foamate/ mass of protein in the feed", which is quite different from our definition since we estimated the efficiency from  $C/C_i$ .

The main proteins in milk whey solution in this study were estimated as  $\beta$ -lactoglobulin (LG),  $\alpha$ -lactalbumin (LA), and bovine serum albumin (BSA) from the result of HPLC analysis. The authors previously reported that the adsorption behavior of ovalbumin onto bubble surface was expressed as Langmuir type adsorption isotherm and the values of the adsorption equilibrium constant and the saturated adsorption

density were largest at pH 4.6, i.e., the isoelectric point of ovalbumin (Maruyama et al., 2000). The degree of hydrophobicity of proteins should become largest at their isoelectric point. The isoelectric points for three proteins described above, that is, LG, LA, and BSA are 5.2, 4.7-5.1, and 4.7-4.9, respectively. The isoelectric points for LG, LA, and BSA are not close to pH 7, however, each value of isoelectric point for three proteins are very similar values. During aeration in the experiments at pH 7, the generation of very stable foam at the liquid-foam interface was observed rather than at the other pHs. Judging from experimental observations, at pH 7, LG, LA, and BSA might be slightly coagulated with each other, although the reason is not clear yet. Judging from these results, the most effective pH should be regarded as pH 7 for whey protein.

#### 3.2. Determination of overall adsorption parameters of milk whey proteins.

Continuous foam separation has been conducted by many researchers. The purposes of these experiments have not been just only separation operation but have also been the determination method of adsorption density of an objective substance onto bubble surface and some adsorption parameters of an adsorption model (Maruyama et al., 2000; Brunner and Lemlich, 1963; Dick and Talbot, 1971; Shirotsuka and Ishiwata, 1973; Kato and Nakamori, 1976; Kubota and Hayashi, 1977; Kubota et al., 1979; Kato et al., 1992; Konduru, 1992a; Konduru, 1992b; Kou et al., 2015; Huan et al., 2019). In continuous mode, however, a huge amount of feed solution should be required than that amount in batch mode. Therefore, we propose a simple estimation method of adsorption parameters by batch mode foam separation experiment.

Mass balance of whey proteins in batch mode can be expressed by the following

equation,

$$\frac{d(VC_{\rm b})}{dt} = -S_{\rm b}X - qC_{\rm b} \tag{4}$$

Where V, q, and  $S_b$  represent the liquid volume within the column, the volumetric flow rate of the liquid in foam, and the production rate of bubble surface area within the column, respectively. q and  $S_b$  are defined as follows.

$$q = \frac{dV}{dt} \tag{5}$$

$$S_{\rm b} = q \, S_{\rm d} \tag{6}$$

Where  $S_d$  represents the specific bubble surface area based on column volume within the column. By substituting Eqs. (5) and (6) into Eq. (4), Eq (4) can be rewritten as follows,

$$V\frac{dC_{\rm b}}{dt} = \left(S_{\rm d}X\right)\frac{dV}{dt} \tag{7}$$

The adsorption equilibrium relationship between proteins and bubble surface can be expressed by Langmuir's adsorption equation (Maruyama et al., 2000).

$$X = X_{\rm s} \frac{KC_{\rm b}}{1 + KC_{\rm b}} \tag{8}$$

Where K, X, and  $X_s$  represent the overall equilibrium constant of whey proteins, the

equilibrium adsorption density, and the saturated adsorption density. By substituting this relation into Eq. (7), Eq (7) can be rewritten as,

$$V\frac{dC_{\rm b}}{dt} = \left(S_{\rm d}X_{\rm s}\frac{KC_{\rm b}}{1+KC_{\rm b}}\right)\frac{dV}{dt}$$
(9)

By solving the above equation with the initial condition as  $C_b = C_i$  and  $V = V_i$  at t = 0, the following equation is obtained as,

$$K(C_{\rm b} - C_{\rm i}) + \ln(C_{\rm b}/C_{\rm i}) = S_{\rm d}X_{\rm s}K\ln(V/V_{\rm i}).$$
(10)

Where  $C_i$  and  $V_i$  represent the initial concentration of milk whey protein and the initial volume of the whey solution within the column. By fitting the time course data to Eq. (10), the overall equilibrium adsorption constant, K, and the overall saturated adsorption density,  $X_s$ , can be determined with a least-squares method. q was estimated by measuring the time course of change in the liquid height (the distance between gas distributor and liquid-foam interface).  $S_b$  was determined by the following equation (Maruyama et al., 2000; Suzuki et al., 1995; Maruyama et al., 2007; Maruyama and Seki, 2020).

$$S_{\rm b} = 6A\varepsilon_{\rm G}(1-\varepsilon_{\rm G})^{4.65} [(4/225)(\rho_{\rm L}-\rho_{\rm G})^2 {\rm g}^2/(\mu_{\rm L}\rho_{\rm L})]^{1/3}, \tag{11}$$

where *A*,  $\varepsilon_{G}$ , *g*,  $\rho_{L}$ ,  $\rho_{G}$  and  $\mu_{L}$  represent a cross-sectional area of the column, the gas holdup, the gravitational acceleration, the density of liquid and gas, and the viscosity of the liquid, respectively. *S*<sub>d</sub> can be determined from Eq. (6) using *q* and *S*<sub>b</sub>.

Fig. 4 shows the fitting of the data to Eq. (10). The abscissa and the ordinate of Fig. 4 correspond to the right-hand side and the left-hand side of Eq. (10), respectively. The data shown in Fig. 4 are the same as the data at pH 4-7 shown in Fig. 2.  $R^2$  value in Fig. 4 represents a coefficient of determination in this least squares method. The plot points are generally placed on the diagonal line, indicating that these follow the proposed model, although some scatters are observed. The fitting to Eq. (10) could be applied to a region of rapid concentration change (0-50 min) of data at each pH. The values of K and  $X_{\rm s}$  estimated by fitting to Eq. (10) are summarized in Table 2. SD values in Table 2 correspond to a standard deviation. In addition, the influence of pH on K and  $X_s$  is shown in Fig. 5. The value of K becomes minimum at pH 5, on the other hand, the value of  $X_s$  becomes maximum at pH 5. The isoelectric point of the major three proteins, BSA, LA, and LG are ca. pH 4.7-4.9, pH 4.2-4.5, and pH 5.2, (Shea et al., 2009) respectively. Proteins are generally recognized to have the most hydrophobic state at the isoelectric point. Thus it can be considered that the stronger hydrophobicity of proteins, the stronger the adsorption intensity onto the bubble surface. This tendency is considered to reflect that the stronger hydrophobicity of proteins, the larger the equilibrium adsorption constant. In our previous study, however, we have reported that the equilibrium adsorption constant was not necessarily the largest value at the isoelectric point (Maruyama et al., 2000). In addition, protein molecules adsorbed onto bubble surface could have the closest packing structure in the saturated state at the isoelectric point (Maruyama et al., 2000) due to the weakest electrostatic interaction among the adsorbed protein molecules. Since the interaction among proteins in a multi-species system is so complicated, although the reason is not clear essentially, we consider it as follows. At pHs other than pH 5, proteins might make complex, At pH other than pH 5, the proteins form aggregates/complexes, which might have broken the tertiary structure of the

protein molecules and brought the inner hydrophobic functional groups inside the molecules to the surface of the molecules, making it more hydrophobic. Moreover, at pH 5, few aggregates/complexes might be generated, which suggested that the saturated adsorption density was larger than that at other pHs due to making a closed packing on the bubble surface in the saturated adsorbed state.

To verify the *K* and  $X_s$  values determined from the plotting of Eq. (10) proposed as a convenient estimation method, continuous foam separation was carried out. Judging from the result presented in the previous section, pH 7 is considered to be the optimum pH value. Experiments in the continuous mode were conducted with varying concentrations of the feed whey solution at pH 7. Each experiment was carried out until the whey milk protein concentration within the column reached a steady state. The experimental results are shown in Fig. 6. Fig 6a shows the adsorption isotherm of milk whey proteins at pH 7, and Fig. 6b shows Langmuir's plot of the data shown in Fig. 6a. The adsorption density, *X*, was calculated by the following equation (Maruyama et al., 2000).

$$X = W_{\rm i} \left( C_{\rm i} - C_{\rm be} \right) / S_{\rm b} \tag{12}$$

In addition, the following equation was used as Langmuir's plot (Maruyama et al., 2000).

$$\frac{S_{\rm b}}{W_{\rm i}(C_{\rm i} - C_{\rm be})} = \frac{1}{X_{\rm s}K} \frac{1}{C_{\rm be}} + \frac{1}{X_{\rm s}}$$
(13)

Where  $C_{be}$  and  $W_i$  represent milk whey proteins concentration at a steady-state within

the column and the volumetric flow rate of the feed solution. As seen in Fig. 6b, by plotting Eq. (13), a good linear relationship can be confirmed.  $R^2$  value in Fig. 6b represents a coefficient of determination in this least squares method. From values of the slope and intercept of the line, *K* and *X*<sub>s</sub> could be determined as  $2.26 \times 10^4$  cm<sup>3</sup>/g and  $0.96 \times 10^{-7}$  g/cm<sup>2</sup>, respectively. The solid line in Fig. 6a was drawn using the estimated two adsorption parameters. By comparing the *K* and *X*<sub>s</sub> values (Table 1) determined by the method proposed in the present study, these values determined in the continuous mode are considered to be mostly the same. Judging from the results of this comparison, it is verified that the proposed estimation method can estimate sufficiently reliable values.

#### 3.3. Determination of adsorption parameters of major species proteins.

In the previous section, we verified the proposed adsorption parameter estimation method using batch mode data. In addition, we attempted to estimate the adsorption parameters of major three proteins, BSA, LA, and LB.

Assuming that the equilibrium relationship between these proteins and bubble surface can be expressed by Langmuir's adsorption model even in the three-component system of proteins, a multi-component Langmuir adsorption isotherm equation can be applied as follows.

$$X_{\rm B} = X_{\rm s} \frac{K_{\rm B} C_{\rm bB}}{1 + K_{\rm B} C_{\rm bB} + K_{\rm LA} C_{\rm bLA} + K_{\rm LG} C_{\rm bLG}}$$
(14)

$$X_{\rm LA} = X_{\rm s} \frac{K_{\rm LA} C_{\rm bLA}}{1 + K_{\rm B} C_{\rm bB} + K_{\rm LA} C_{\rm bLA} + K_{\rm LG} C_{\rm b\ L\ C}}$$
(15)

$$X_{\rm LG} = X_{\rm s} \frac{K_{\rm LG} C_{\rm bLG}}{1 + K_{\rm B} C_{\rm bB} + K_{\rm LA} C_{\rm bLA} + K_{\rm LG} C_{\rm b \ L \ C}}$$
(16)

Where subscript B, LA, and LB represent bovine serum albumin,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin. As for BSA, the adsorption density,  $X_{\rm B}$ , of BSA in Eq. (16) substitute into Eq. (7), the next equation can be obtained as.

$$\left[K_{\rm B} + \left(1 + K_{\rm LA}C_{\rm bLA} + K_{\rm LG}C_{\rm bLG}\right)\frac{1}{C_{\rm bB}}\right]\frac{dC_{\rm b}}{dt} = \left(S_{\rm d}X_{\rm s}X_{\rm B}\frac{1}{V}\right)\frac{dV}{dt}$$
(17)

By solving Eq. (17) with the initial condition as  $C_{bB} = C_{iB}$  and  $V = V_i$  at t = 0, the following equation is obtained as.

$$X_{\rm B} \left( C_{\rm bB} - C_{\rm iB} \right) + \left( 1 + K_{\rm LA} C_{\rm bLA} + K_{\rm LG} C_{\rm bLG} \right) \ln \left( \frac{C_{\rm bB}}{C_{\rm iB}} \right) = S_{\rm d} X_{\rm s} X_{\rm B} \ln \left( \frac{V}{V_{\rm i}} \right)$$
(18)

By the same derivation, a similar relationship for LA and LG can be obtained as follows.

$$X_{\rm LA} \left( C_{\rm bLA} - C_{\rm iLA} \right) + \left( 1 + K_{\rm B} C_{\rm bB} + K_{\rm LG} C_{\rm bLG} \right) \ln \left( \frac{C_{\rm bLA}}{C_{\rm iLA}} \right) = S_{\rm d} X_{\rm s} X_{\rm LA} \ln \left( \frac{V}{V_{\rm i}} \right)$$
(19)

$$X_{\rm LG} \left( C_{\rm bLG} - C_{\rm iLG} \right) + \left( 1 + K_{\rm B} C_{\rm bB} + K_{\rm LA} C_{\rm bLA} \right) \ln \left( \frac{C_{\rm bLG}}{C_{\rm iLG}} \right) = S_{\rm d} X_{\rm s} X_{\rm LG} \ln \left( \frac{V}{V_{\rm i}} \right)$$
(20)

As same as in the case of Eq. (10), by the fitting of the data to Eqs. (18)-(20), K for each

protein and  $X_s$  can be determined by a least-squares method.

The time course of each protein concentration profile at pH 7 is shown in Fig. 7a. The ordinate corresponds to the dimensionless concentration. The each initial concentration for BSA, LA, and LG were 0.0178 g/L ( $2.70 \times 10^{-7}$  mol/L), 0.0481 g/L ( $3.40 \times 10^{-6}$  mol/L), and 0.0473 g/L ( $2.59 \times 10^{-6}$  mol/L), respectively. It can be observed that the separation rate from the liquid phase is faster for LG, BSA, and LA in that order. LG has disappeared from the liquid phase within *t* = 180 min. LG might have the most hydrophobic property among BSA, LA, and LB.

By fitting the data shown in Fig. 7a to Eqs. (18)-(20), the proposed plot for estimating adsorption parameters is shown in Fig. 7b.  $R^2$  value in Fig. 7b represents a coefficient of determination in this least squares method. The data essentially agree well with Eqs. (18)-(20) for each protein, although some scatter were observed. The estimated parameter, *K* and  $X_s$  are shown in Fig. 8 as a function of pH and are summarized in Table 3. SD values in Table 3 correspond to a standard deviation. The equilibrium adsorption constants for BSA, LA, and LG were largest at pH 7. This tendency is mostly the same as in the case of the overall equilibrium adsorption constant. We considered the reason for this similarity is as follows. LG exists as a dimer or trimer at neutral pH while it dissociates to a native monomer below pH 3 (Sakurai and Goto, 2002; Ramamirtham et al., 2021). In addition, it has been reported that LG dissolved form was the influence of coexisting salts and other proteins (Sakurai et al., 2001; Seychell and Beck, 2021). Thus, interactions between dissolved proteins are so complicated that further discussion would be difficult.

In a practical case, it is reported that the pH of milk whey solution generated from the practical cheese processing process is ca. pH 6.2 (Tsakali et al., 2010). It should be desired to recovery more amount of whey proteins without controlling pH from an empirical aspect. In a further study, we will have to focus on the aspect to improve and develop the practical application of foam separation for the recovery of milk whey proteins.

A comparison of major whey protein recovery with chromatography and membrane filtration is summarized in Table 4. The two separation techniques have been well established to recovery whey proteins. This comparison shows that ion exchange chromatography is considered to be a good separation technique. Foam separation used in this study also recovered LG very well, but the purity was not high.

#### 4. Conclusions

Recovery of proteins in milk whey solution was conducted with foam separation technique.

As for overall milk whey protein, the separation rate was estimated by the first-order rate constant in the batch mode at pH 4-8. At pH 7, the separation efficiency and the separation rate were higher than those at other pHs.

The proposed estimating method was applied to the data in batch mode. The overall equilibrium adsorption constant, *K*, and the overall saturated adsorption density,  $X_s$ , for milk whey protein were determined as  $2.21 \times 10^4$  cm<sup>3</sup>/g and  $1.15 \times 10^{-7}$  g/cm<sup>2</sup>, respectively. For verifying these estimated values, continuous foam separation was carried out. The estimated *K* and  $X_s$  values from continuous mode were in good agreement with these estimated from batch mode. The overall values of *K* and  $X_s$  were largest at pH 7 and 5, respectively.

The proposed estimating method was also applied to the batch mode foam separation data of major three species, bovine serum albumin (BSA),  $\alpha$ -lactalbumin (LA), and  $\beta$ -lactoglobulin (LG) at pH 7. The *K* and *X*<sub>s</sub> for each protein were also successfully determined by the proposed method. Comparison of the *K* for each protein suggested that LG was considered to be the most hydrophobic protein. the *K* for BSA, LA, and LG were largest at pH 7. The *X*<sub>s</sub> values for BSA, LA, and LG were also largest at pH 5. These tendencies were the same as the *K* for overall milk whey proteins.

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## Notation

Α	= a cross sectional area of bubble column	[m <sup>2</sup> ]
$C_{\mathrm{b}}$	= concentration of milk whey proteins	
	within the column in batch mode	[kg/m <sup>3</sup> ]
$C_{\mathrm{bB}}$	= concentration of bovine serum albumin	
	within the column in batch mode	[kg/m <sup>3</sup> ]
$C_{\rm be}$	= concentration of milk whey proteins at steady state	
	within the column in continuous mode	$[kg/m^3]$
$C_{\rm bLA}$	= concentration of $\alpha$ -lactalbumin within the column in batch mode	$[kg/m^3]$
$C_{\rm bLG}$	= concentration of $\beta$ -lactoglobulin within the column in batch mode	$[kg/m^3]$
Ci	= initial concentration of proteins within the column	[kg/m <sup>3</sup> ]
$C_{\mathrm{iB}}$	= initial concentration of bovine serum albumin within the column	[kg/m <sup>3</sup> ]
C <sub>iLA</sub>	= initial concentration of $\alpha$ -lactalbumin within the column	[kg/m <sup>3</sup> ]
$C_{\mathrm{iLG}}$	= initial concentration of $\beta$ -lactoglobulin within the column	[kg/m <sup>3</sup> ]
<i>E</i> G	= gas holdup	[-]
g	= gravitational acceleration	$[m/s^2]$
k	= separation rate constant of milk whey proteins in batch mode	$[\min^{-1}]$
Κ	= overall equilibrium adsorption constant for milk whey proteins	[m <sup>3</sup> /kg]
$\mu_{ m L}$	= liquid viscosity	[kg/(m s)]
q	= volumetric flow rate of liquid in foam in batch mode	$[m^3/s]$
$ ho_{ m L}$	= liquid density	$[kg/m^3]$
$ ho_{ m G}$	= gas density	$[kg/m^3]$
$S_{\mathrm{b}}$	= bubble surface area production rate within the column	$[m^2/s]$
Sd	= specific bubble surface area based on column volume within colu	mn [m <sup>2</sup> /m <sup>3</sup> ]

t	= time	[ s ]
V	= liquid volume within column	[m <sup>3</sup> ]
$V_{\mathrm{i}}$	= initial liquid volume within column	[m <sup>3</sup> ]
W <sub>i</sub>	= volumetric flow rate of feed solution in continuous mode	$[m^3/s]$
Χ	= adsorption density of milk whey proteins onto bubble surface	$[kg/m^2]$
$X_{\mathrm{B}}$	= adsorption density of bovine serum albumin onto bubble surface	$[kg/m^2]$
$X_{ m LA}$	= adsorption density of $\alpha$ -lactalbumin onto bubble surface	$[kg/m^2]$
$X_{ m LG}$	= adsorption density of $\beta$ -lactoglobulin onto bubble surface	$[kg/m^2]$
$X_{\rm s}$	= saturated adsorption density of oil droplet or floc onto bubble surfa	ce [kg/m <sup>2</sup> ]

#### References

- Brunner, C.A., Lemlich, R., 1963. Foam fractionation standard separator and refluxing columns. Ind. Eng. Chem. Fund. 2(4), 297-300. https://doi.org/10.1021/i160008a010
- Cheang, B., Zydney, A.L., 2003. Separation of a-lactalbumin and b-lactoglobulin using membrane filtration. Biotechnology and Bioengineering 83, 201-209. https://doi.org/10.1002/bit.10659
- Dean, R.B., Dixon, W.J., 1951. Simplified statistics for small numbers of observations, Anal. Chem. 23(4), 636-638. https://doi.org/10.1021/ac60052a025
- Dick, W.L., Talbot, F.D., 1971. The use of an auxiliary ligand in foam fractionation of copper. Ind. Eng. Chem. Fund. 10(2), 309-312. https://doi.org/10.1021/i160038a021
- El-Sayed, M.M.H., Chase, H.A, 2009. Single adn two-component cation-exchange adsorption of the two pure major proteins. Journal of Chromatography A 1216, 8705-8711. https://doi.org/10.1016/j.chroma.2009.02.080
- El-Sayed, M.M.H. Chase, H.A., 2010. Purification of the two major proteins from whey concentrated using a cation-exchange selective adsorption process. AIChE J. 26, 192-199. https://doi.org/10.1002/btpr.316
- Gerberding, S.J., Byers, C.H., 1998. Preparative ion-exchange chromatography of proteins from dairy whey. Journal of Chromatography A 808. 141-151. https://doi.org/10.1016/S0021-9673(98)00103-4
- Gonzdlez Siso, M.I., 1996. The biotechnological utilization of cheese whey: a review. Bioresour. Technol. 57, 1-11. https://doi.org/10.1016/0960-8524(96)00036-3

Grubbs, F.E., 1969. Procedures for detection outlying observations in samples,

Technometrics 11(1), 1-21. https://doi.org/10.1080/00401706.1969.10490657

- Huan, D., Wang, C., Yin, H., Zhang, S., Qin, S., Liu, W., Wu, Z., Li, Z., Yang, C., 2019. Enhancement of the quinoline separation from pyridine: Study on competitive adsorption kinetics in foam fractionation with salt. Chem. Eng. J. 359, 1150-1158. https://doi.org/10.1016/j.cej.2018.11.066
- Illanes, A., 2011. Whey upgrading by enzyme biocatalysis. Electron. J. Biotechnol. 14, 1-28. https://doi.org/10.2225/vol14-issue6-fulltext-11
- Japan livestock technology association, To promote the utilization of whey as feed", Report on the Project to Promote the Production of Pork Using By-products. (2006)
- Kato, K., Nakamori, I., 1976. Adsorptive characteristics of ion in foam separation techniques. Kagaku Kogaku Ronbunshu 2, 272-276. https://doi.org/10.1252/kakoronbunshu.2.272
- Kato, K., Ishikawa, T., Kasahara, Y., Miyaki, S., 1992. Method of estimating surface excess of metal ion in foam separation. J. Chem. Eng. Japan 25, 561-569. https://doi.org/10.1252/jcej.25.561
- Konduru, R., 1992a. Operating a foam fractionating column in simple mode. J. Chem. Eng. Japan. 25(5), 548-554. https://doi.org/10.1252/jcej.25.548
- Konduru, R., 1992b. Absorptive studies of aqueous zinic ions by foam fractionation in simple mode. J. Chem. Eng. Jpn. 25(5), 555-560. https://doi.org/10.1252/jcej.25.555
- Konrad, G., Kleinschmidt, T., 2008. A new method isolation of native a-lactalbimin from sweet whey. International Dairy Journal 18, 47-54. https://doi.org/10.1016/j.idairyj.2007.06.004
- Kou, Q., Wu, Z., Hu, N., 2015. Thermodynamic adsorption properties of bovine serum albumin and lysozyme on the bubble surface from the binary solution. Chem. Eng.

Res. Des. 102, 26-33. http://dx.doi.org/10.1016/j.cherd.2015.06.013

- Kubota, K., Hayashi, S., 1977. The removal of sodium, cadmium and chromium ions from dilute aqueous solutions using foam fractionation. Can. J. Chem. Eng. 55(3), 286-92. https://doi.org/10.1002/cjce.5450550308
- Kubota, K., Hayashi, S., Takubo, Y., 1979. Fundamental studies of foam fractionation of cadmium: effect of the addition of monovalent ions on surface excess of cadmium.
  Can. J. Chem. Eng. 57, 591-598. https://doi.org/10.1002/cjce.5450570507
- Lowry, O.H., Rowebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6
- Maruyama, H., Suzuki, A., Seki, H., 2000. Adsorption of water-soluble proteins onto bubbles in continuous foam separation. J. Colloid Interface Sci. 224, 76-83. https://doi.org/10.1006/jcis.1999.6673
- Maruyama, H., Seki, H., Suzuki, A., Inoue, N., 2007. Batch foam separation of a soluble protein. Water Res. 41, 710-718. https://doi.org/10.1016/j.watres.2006.07.017
- Maruyama, H., Seki, H., 2020. Influence of methylated milk casein flocculant dosage on removal rate on oil droplet removal from o/w in flotation. Biochem. Eng. J. 159, 107584. https://doi.org/10.1016/j.bej.2020.107584
- Matouq, M., 2008. Investigation of the bubble foam separation technique to extract protein from whey. Am. J. Appl. Sci. 5(5), 468-472. https://doi.org/10.3844/ajassp.2008.468.472
- Metsämuuroen, S., Nyström, M., 2009. Enrichment of a-lactalbumin from dilute whey with polymeric ultrafiltration membranes. Journal of Membrane Science 337, 248-256. https://doi.org/10.1016/j.memsci.2009.03.052

Mukhopadhyay, G., Khanam, J., Nanda, A., 2010. Protein Removal from whey waste by

foam fractionation in a batch process. Sep. Sci. Technol. 45, 1331-1339. https://doi.org/10.1080/01496391003697382

- Mukhopadhyaya, G., Khanamb, J., Nandab, A., Nath Balaa, N., Chun Ohc, W., 2011.
  Statistical analysis in enrichment of total whey protein by continuous foam fractionation method. Eur. J. Chem. 2, 519-523.
  https://doi.org/10.5155/eurjchem.2.4.519-523.483
- Qutinen, M., Tossavainen, O., Syväoja, E.L., 1996. Chromatographic fractionation of α-lactalbimin and β-lactoglobulin with polystyrenic strongly basic anion exchange resins. Lebensm.-Wiss. u.-Technol. 29, 340-343. https://doi.org/10.1006/fstl.1996.0051
- Ramamirtham, S., Whitby, C.P., Zare, D., Weeks, M., Williams, M.A.K., 2021. The rheological properties of bovine β-Lactoglobulin stabilized oil/water interfaces depend on the protein's quaternary structure. Food Hydrocoll. 120, 106834. https://doi.org/10.1016/j.foodhyd.2021.106834
- Sakurai, K., Goto, Y., 2002. Manipulationg monomer-dimer equilibrium of bovine betalactoglobulin by amino acid substitution. J. Biol. Chem. 277, 25735-25740. https://doi.org/10.1074/jbc.M203659200
- Sakurai, K., Oobatake, M., Goto, Y., 2001. Salt-dependent monomer-dimer equilibrium of bovine β-lactoglobulin at pH 3. Protein Sci. 10(11), 2325-2335. https://doi.org/10.1110/ps.17001
- Santos, M.J., Teixeira, J.A., Rodrigues, L.R., 2012. Fractionation of the majjor whey proteins and isolation of β-lactoglobulin variants by anion exchange chromatography. Separation and Purification Technology 90, 133-139. https://doi.org/10.1016/j.seppur.2012.02.030

Saufi, S.M., Fee, C.J., 2009. Fractionation of β-lactoglobulin from whey by mixed

matrix membrane ion exchange chromatography. Biotechnology and Bioengineering, 103, 138-147. https://doi.org/10.1002/bit.22239

- Seychell, B.C., Beck, T., 2021. Molecular basis for protein-protein interactions. Beilstein J. Org. Chem. 17, 1-10. https://doi.org/10.3762/bjoc.17.1
- Shea, A.P., Crofcheck, C.L., Payne, F.A., Xiong, Y.L., 2009. Foam fractionation of α-lactalbumin and β-lactoglobulin from a whey solution. Asia Pac. J. Chem. Eng. 4, 191-203. https://doi.org/10.1002/apj.221
- Shirotsuka, T., Ishiwata, M., 1973. Studies on bubble fractionation: Adsorption rate andelimination of D.B.S. Kagaku Kougaku 37, 397-402. https://doi.org/10.1252/kakoronbunshu1953.37.397
- Sunkesula, V., Kommineni, A., Marella, C., 2020. Foam fractionation technology for enrichment and recovery of cheese whey proteins. Asian J. Dairy and Food Res. 39(3), 187-194. https://doi.org/10.18805/ajdfr.DR-182
- Suzuki, A., Maruyama, H., Seki, H., Hayashi, T., 1995. Application of nonfoaming bubble separation to enrichment of dilute dye solution. J. Chem. Eng. Japan. 28(1), 115-117. https://doi.org/10.1252/jcej.28.115
- Tsakali, E., Petrotos, K., Allessandro, A.D., Goulas, P., 2010. A review on whey composition and the methods used for its utilization for food and pharmaceutical products, 6th International Conference on Simulation and Modelling in the Food and Bio-Industry FOODSIM 2010.
- Walstra, P., Jenness, R., Dairy Chenistry and Physics., p.365, John Wiley & Sons, NewYork, (1984)

#### **Figure and table captions**

**Fig. 1.** Schematic diagram of the experimental setup for batch foam separation. 1. bubble column; 2. gas distributor; 3. pressure measuring tap; 4. gas flow meter; 5. flow control valve; 6. nitrogen gas cylinder; 7. pressure/voltage transducer; 8. amplifier; 9. voltmeter; 10. personal computer, 11. sampling tap, 12. feeding tap (continuous mode).

**Fig. 2.** (a) Typical time course of the concentration of milk whey proteins within the column in batch foam separation. The experiments were conducted at pH 4-8. The volumetric airflow rate was 50 cm<sup>3</sup>/min. (b) Fitting of the data for Fig. 2a to Eq. (3). The slope value of the solid line corresponds to the foam separation rate constant, *k*.

**Fig. 3.** Influence of pH of the whey protein solution on (a) the separation rate constant, k, and (b) the separation efficiency.

**Fig. 4.** Fitting of the data obtained by batch foam separation to Eq. (10) for determination of the overall equilibrium adsorption constant, K, and the saturated adsorption density,  $X_{s}$ .

**Fig. 5.** Influence of pH on (a) the overall equilibrium adsorption constant, K, and (b) the saturated adsorption density,  $X_s$ .

**Fig. 6.** (a) The adsorption isotherm of the milk whey proteins at pH 7. (b) The Langmuir plot of the data of Fig. 4(a) for determination of the overall equilibrium adsorption constant, K, and the saturated adsorption density,  $X_s$ .

**Fig. 7.** (a) Typical time course of the concentration of the main component proteins ( $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, bovine serum albumin) of milk whey within the column. (b) Fitting of the data of fig. 6a to Eqs. (18)-(20) for determination of the overall equilibrium adsorption constant, *K*, and, the saturated adsorption density, *X*<sub>s</sub>. The experiment was conducted at pH 7.

**Fig. 8.** Influence of pH on the equilibrium adsorption constant, *K*, of (a)  $\beta$ -lactoglobulin, (b)  $\alpha$ -lactalbumin and (c) bovine serum albumin, and (d) the saturated adsorption density, *X*<sub>s</sub>.

**Table 1**Summary of the results of Q-test and Grubbs method for scatters in theinitial concentrations

**Table 2** The overall adsorption equilibrium constant, K, and the overall saturated adsorption density,  $X_s$ , of milk whey proteins.

**Table 3** The adsorption equilibrium constant, *K*, and the saturated adsorption density,  $X_s$ , of  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, and bovine serum albumin in milk whey.

**Table 4**Comparison of major whey protein recovery with chromatography andmembrane filtration.



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## Table 1

Summary of the results of Q-test and Grubbs method for scatters in the initial concentrations

method	x <sub>d</sub>	x <sub>n</sub>	x <sub>a</sub>	r	S	Q	$g_0$	
Q-test	0.506 0.303	0.461 0.305		0.203 0.203		0.221 0.00985		< 0.64 <sup>a</sup> < 0.64 <sup>a</sup>
Grubbs	0.506 0.303		0.404 0.404		0.0940 0.0940		1.082 1.075	< 1.71 <sup>b</sup> < 1.71 <sup>b</sup>

a: 90% confidence limits for n = 5

b: 5% significance level for n = 5

## Table 2

The overall adsorption equilibrium constant, K, and the overall saturated adsorption density,  $X_s$ , of milk whey proteins.

рН	$K [cm^3/g]$	SD	$X_{\rm s}$ [g/cm <sup>2</sup> ]	SD
4	5.13 ×10 <sup>2</sup>	5.95 ×10 <sup>1</sup>	3.55 ×10-7	1.01 ×10 <sup>-7</sup>
5	$3.07 \times 10^{0}$	$1.14 \times 10^{0}$	7.88 ×10 <sup>-5</sup>	2.06 ×10 <sup>-5</sup>
6	$1.72 \times 10^{2}$	$7.34 \times 10^{0}$	2.08 ×10 <sup>-6</sup>	6.27 ×10 <sup>-7</sup>
7	$2.21 \times 10^{4}$	$1.65 \times 10^{4}$	1.15 ×10 <sup>-7</sup>	5.37 ×10 <sup>-8</sup>

The ads bovine	sorption equili serum albumir	brium const 1 in milk wh	ant, $K$ , and the sey.	saturated ad	sorption density, $X_{\rm s}$ , of	α-lactalbu	min, β-lactog	lobulin, and
			$K [\mathrm{cm}^{3}/\mathrm{g}]$				$X_{\rm s}$ [g/cm <sup>2</sup> ]	
рН	α-lactalbumi	n SD	β-lactoglobulin	SD	bovine serum albumin	SD		SD
4	$3.02 \times 10^{3}$	$1.78 \times 10^{2}$	$7.10 \times 10^{2}$	$4.94 \times 10^{2}$	$1.56 \times 10^3$ 7	$.00 \times 10^{2}$	5.39×10 <sup>-8</sup>	1.14×10 <sup>-8</sup>
S	$7.16 \times 10^{2}$	$4.24 \times 10^{2}$	$1.89 \times 10^{3}$	$5.46 \times 10^{2}$	$4.39 \times 10^2$ 2	$.34 \times 10^{2}$	2.54 ×10 <sup>-7</sup>	4.08 ×10 <sup>-8</sup>
6	$7.58 \times 10^{3}$	$2.66 \times 10^{3}$	$2.09 \times 10^4$	$3.45 \times 10^{3}$	$6.02 \times 10^3$ 1	$.51 \times 10^{2}$	$4.14 \times 10^{-8}$	$1.40 \times 10^{-8}$
7	$4.21 \times 10^{4}$	$1.49 \times 10^{4}$	$1.19 \times 10^{5}$	$6.95 \times 10^{4}$	$3.85 \times 10^4$ 2	$.07 \times 10^{4}$	$6.40 \times 10^{-9}$	$2.04 \times 10^{-9}$

# Table 4

Comparison of major whey protein recovery with chromatography and membrane filtration.

Technique	LA(%)	LG (%)	Ref.
Ultrafiltration	15	-	Konrad and Kleinschmidt 2008
Ultrafiltration	48.7	65.2	Metsämuuroen and Nyström 2009
Ultrafiltration	70	90	Cheang and Zydney 2009
Cation exchange chromatograpy	91	91	El-Sayed and Chase 2009
Cation exchange chromatograpy	67	78	El-Sayed and Chase 2010
Anion exchange chromatograpy	-	61	Santos et al., 2012
Mixed matrix membrane ion exchange chromatography	-	81	Saufi and Fee 2009
Foam separation	67	99	This study