Foam Fractionation Technology for Enrichment and Recovery of Cheese Whey Proteins

Venkateswarlu Sunkesula, Anil Kommineni, Chenchaiah Marella, K. Muthukumarappan[1], Lloyd E. Metzger

ABSTRACT

Background: Foam fractionation technology works on the adsorptive bubble separation principle. This technique involves adsorption of the surface-active substances on to a gas-liquid interface and separation of these components from the liquid along with bubbles as foam. The foam separation technology has been successfully utilized in the recovery of proteins from solutions containing either a single protein or binary mixtures. To develop a foam fractionation technology for selective enrichment and recovery of whey proteins, it is essential to investigate the effect of different feed and process variables that affect the foam fractionation process. The aim of the current study was to investigate the effect of two important feed variables, such as pH and initial protein concentration on recovery and enrichment of total whey proteins as well as α-lactalbumin and β-lactoglobulin.

Methods: All the experiments were conducted in Agriculture and biosystems engineering lab and Alfred Dairy Science lab at South Dakota State University, Brookings, South Dakota during 2011-2013. The experiments used four levels of initial protein concentration and five levels of feed pH. Yield and enrichment ratios were determined for total whey proteins, α-Lactalbumin (α-La) and β-Lactoglobulin (β-Lg).

Result: Whey protein yields ranged from 51.58 to 90.92%, while the enrichment ratios were between 1.2 to 5. The yield of α-La varied from 59 to 94% and the highest enrichment ratio of 8.45 was obtained with the treatment combination of initial protein concentration of 109 mg/L and pH of 5.1. Selective enrichment of α-La over β-Lg was observed at a pH of 4.65 with α-La to β-Lg ratio of 0.49. These findings will be helpful in selective enrichment and recovery of valuable proteins from Cheddar cheese whey using the foam fractionation process.

Key words: Cheese whey, Enrichment ratio, Foam fractionation, Whey proteins, α-Lactalbumin, β-Lactoglobulin.

INTRODUCTION

Cheddar cheese whey is a byproduct obtained after coagulation of milk caseins using starter cultures and enzyme (rennet). It contains about 6% total solids (TS), 0.7–0.8% protein (primarily β-Lactoglobulin and α-Lactalbumin), 0.25% fat, 0.52% ash and 5% lactose (Singh *et al.,* 2006; Johansen *et al.,* 2002). In general, ten kg of milk is required to produce one kg of cheese, thus generating 9 kg of cheese whey (Prazeres *et al.,* 2012). In the year of 2017, globally an estimated 208 million tons of cheese whey was produced (OECD/FAO, 2017), with the US having the largest whey produced (49.9 million tons) (USDA, 2017). The development of membrane separation technologies, especially ultrafiltration in the 1970s, made it possible to fractionate and concentrate valuable components of whey into whey protein concentrates and whey protein isolates.

The ongoing research is concentrating on finding novel applications for whey proteins that lead to revealing various functional, nutritional and therapeutic properties of individual whey protein fractions (Angela, 2004; Harper, 1999; Mate and Krochta, 1994; Strohmaier, 2004). Cheddar cheese whey contains glycomacropeptide (GMP), α-lactalbumin (α-La), β-lactoglobulin (β-Lg), Bovine serum albumin (BSA), immunoglobulins (IgGs), lactoferrin (LF) and Lactoperoxidase (LP) by 0.9-1.3, 0.7-1.5, 3.0-4.0, 0.3-0.6, 0.6-0.9, 0.05-0.35 and 0.006 g/L, respectively (Arunkumar and Etzel, 2018; Hammam, 2019). Fractionation of these proteins and the

Department of Dairy and Food Science, South Dakota State University, Brookings, SD 57007. South Dakota.

[1]Department of Agricultural and Biosystems Engineering, South Dakota State University, Brookings, SD 57007. South Dakota.

Corresponding Author: Venkateswarlu Sunkesula, Department of Dairy and Food Science, South Dakota State University, Brookings, SD 57007. South Dakota.
Email: sunkesula.venkat@gmail.com

development of pure or enriched protein products will further add value to the whey ingredients. As illustrated in Table 1, these protein fractions differ widely in their physical and chemical properties. The differences in their properties are the basis for selective fractionation of these protein portions using different fractionation processes such as selective fractionation with pH, salt and temperature, isoelectric focusing, solvent fractionation, adsorption chromatography, size exclusion chromatography, membrane fractionation, *etc.* The occurrence of biomolecules in dilute solutions, their sensitivity to extreme temperatures and pH make the conventional separation processes expensive

(Stevenson and Li, 2014) and similar challenges are posed to fractionation of cheese whey proteins. For production of low cost pure or enriched whey protein fractions, it is important to improve the existing methods and to develop alternative methods of recovery and fractionation of whey proteins. Foam fractionation is one of the techniques that can be further investigated for fractionation of whey proteins.

Foam fractionation is a process in which surface-active materials are removed by flotation to form foam. Enrichment of one component over another occurs both on the bubbles while in solution and the lamellae of the foam (Pinfold, 1970; Changade et al., 2009). When surface active components such as proteins move to the gas-liquid interface, they unfold at this interface and form a viscous film. The ability to unfold is influenced by the forces that contribute to maintaining the structure of the proteins. These factors include electrostatic, hydrophobic, hydrogen bonding and disulfide bonds (Phillips et al., 1995). Foam fractionation is known from the beginning of the century but there is renewed interest in this process during the last 30 years and brought out by the need for a relatively cheap, selective and easy to operate methods of protein recovery. Foam fractionation is a relatively inexpensive and easily scalable process (Prokop and Tanner, 1993), an appropriate process to fractionate very dilute solutions (Uraizee and Narsimhan, 1996) with high enrichment (Backleh-Sohrt et al., 2005) and is a promising method for separating the proteins (Chai et al., 1998; Liu et al., 2018). Except for the addition of air or other gases, such as carbon dioxide or nitrogen and utilize an acid or a base for adjusting pH, no additional substances or solvents are added in the process (Montero et al., 1993; Stowers et al., 2009; Chandrasekar et al., 2015).

So far most of the research work done on protein separation/fractionation using foam or bubble fractionation techniques used a single model protein (Brown et al., 1999a; Brown et al., 1990; Huang et al., 2016; Keller et al., 1997; Li et al., 2017; Li et al., 2016; Li et al., 2015; Montero et al., 1993; Tian et al., 2018; Uraizee and Narsimhan, 1996) or binary mixtures of proteins (Anand and Damodaran, 1995; Bhattacharya et al., 1991; Brown et al., 1999b; Hunter et al., 1991; Lockwood et al., 2000; Sarkar et al., 1987; Suzuki et al., 2002). In these studies, the effect of different feed variables (pH, temperature, initial protein concentration, ionic strength, etc.) and the presence of processing aids (such as sodium dodecyl sulfate, sodium citrate, etc.) and process parameter (bubble size, superficial gas velocity, foam column height, feed flow rate, foam drainage, etc.) have been studied. For this technique to become a viable protein recovery and enrichment tool, it is essential to demonstrate that this technique works equally well with multi-component mixtures, such as cheese whey. So far, few studies have been reported on fractionation of whey proteins using adsorptive bubble separation principles. Shea et al. (2009) studied foam fractionation of α-La and β-Lg from dilute whey protein solutions and reported the highest enrichment ratios for α-La (at 3.8 pH) and β-Lg (at 4.5 pH) as 3.4 and 2.3,

respectively. In this study, depending on the initial protein concentration and the gas flow rate used, α-La recoveries ranged from 50 to 95%, while β-Lg recoveries ranged from 35 to 70%. Mukhopadhyay et al. (2010) reported a maximum whey protein recovery of 96.4% from whey using batch foam fractionation process and sodium dodecyl sulfate (SDS) as a processing aid. In another study on enrichment of total and single whey proteins by pH-controlled foam fractionation, Ekici et al. (2005) used sodium dodecyl sulfate (SDS) as a surfactant to aid enrichment of the proteins. For single whey proteins (BSA, α-La and β-Lg) the enrichment ratios up to 30 and recoveries of 64.5 to 99.8% were reported. Matouq (2008) investigated the foam fractionation technique to extract whey proteins and reported very low enrichment ratios of less than 2 using undiluted cheese whey.

To develop a foam fractionation technology for selective enrichment and recovery of whey proteins, it is essential to investigate the effect of different feed and process variables that affect the foam fractionation process. This study aims to investigate the effect of two important feed variables, such as pH and initial protein concentration on yield (recovery) and enrichment of total whey proteins as well as α-La and β-Lg, experiments were conducted using four levels of initial protein concentration and at five different pH.

MATERIALS AND METHODS

Cheddar cheese whey

Cheddar cheese whey was collected from the Davis Dairy plant at Dairy and Food Science Department of South Dakota State University, Brookings, South Dakota. The whey was subjected to clarification using a centrifugal cream separator wherein residual lipids and casein fines were removed. The clarified whey contained 6.49% TS, 0.08% fat, 0.87% protein (Nitrogen x 6.38), 0.51% ash and 5.03% lactose. Whey was diluted with double distilled water to get whey feed with four different initial protein concentrations (870, 435, 218 and 109 mg/L). The ionic contents of all the four feed samples were measured in terms of equivalent Sodium chloride (NaCl) through conductivity measurements as described by Muller et al. (2003). The highest ionic content of 5.8 was observed for the feed sample with 870 mg/L protein concentration. The ionic content of the other three feed samples was adjusted to this level by adding appropriate quantities of NaCl. The conductivity of the feed samples was measured using Accumnet Excel XL 20 pH/ conductivity meter (Fisher Scientific, Hanover park, IL) and using a 2-cell epoxy body conductivity probe (cat # 13620 100) with automatic temperature compensation.

Experimental setup

The experimental foam fractionation setup is shown in Fig 1. The column is a chromatography column procured from Ace glass Inc., Vineland, NJ. It had an inside diameter of 37 mm and is 450 mm long. A porous polyethylene disc with 100 μ porosity sits at the bottom of the column. A glass elbow was

fitted at the end of the column to collect foamate into a beaker.

Operating variables

Initial Protein Concentration (PrConc)

Preliminary experiments were conducted using Cheddar cheese whey with the initial protein content of 870 mg/L. The yield of protein in the foamate was close to 100% and no appreciable protein enrichment was seen in the foamate. As foam fractionation works better at lower protein concentrations (Brown *et al.,* 1990; Uraizee and Narsimhan, 1996) it was decided to lower the initial protein content of the whey. Accordingly, four feeds with different initial protein concentrations were prepared as explained above.

pH

The ability of a specific protein to adsorb at an interface depends on physicochemical characteristics. The size, shape, charge and pH influence the degree of adsorption of a protein at the gas-liquid interface. At isoelectric point (pI), the net charge of the protein is zero and surface activity will be high. High surface activity (hydrophobicity) should promote greater adsorption of protein at the gas-liquid interface. Aggregation, denaturation and conformational changes are all influenced by the pH of the solution. Isoelectric pH of α- La and β- Lg, the two major whey proteins are 4.2 - 4.5 and 5.2, respectively. Therefore, for the present study, five levels of pH *viz* 3.0, 4.0, 4.65, 5.1 and 6.3 were selected. The pH of the samples was adjusted using 0.1N NaOH or HCl as required and was measured with Accumet pH /Conductivity meter (Fisher scientific, Hanover park, IL) using a combination pH probe.

Experimental procedure

Whey sample of 250 ml was adjusted to the required pH and warmed up to room temperature (25°C). The sample was equilibrated for 30 minutes before loading into the column. The sample when loaded in the column occupied a feed column height of 22.5 cm leaving a height of 22.5 cm for the foamate. After loading the sample, compressed air was passed through a flowmeter, a saturation chamber and then through the column (Fig 1). The air pressure was adjusted to 100 kPa with a combined pressure regulator and indicator (Milton industries, Chicago, IL) and the air flow rate was maintained at 3.92 cm³/s. This flow rate resulted in a linear air velocity of 1.29 cm/s through the column.

The air flow was measured using a flow meter equipped with a control valve and 150 mm flow tube containing a stainless-steel float (ColeParmer Instruments Co., Chicago, IL). Air flow rates below this flow rate (3.92 cm³/s) took a very long time for the foamate to collect into the beaker while higher air flow rates resulted into a very wet foam. All the experiments were conducted at room temperature (25°C) and the temperature was measured with a digital thermometer equipped with type 'T' thermocouple (Omega Engineering, Inc, Stamford, CT). The foamate collected in the beaker was collapsed naturally or mechanically using a glass rod (as needed). Foaming was continued for 30 minutes or terminated when no more foamate was coming into the collection beaker. The volume of the foamate and the retentate (the feed remained in the foaming column at the end of the experiment) were measured and samples were collected for quantification of total and individual protein fractions.

Sample analysis

Samples of feed, foamate and retentate were analyzed for individual protein fractions by RP-HPLC using a Jupitor5u C4 300 Å, 250 × 4.6 mm reverse phase column (Phenominex, CA) connected to an Agilent 1200 series LC unit equipped with Chemstation32-bit software for acquisition, processing and reporting the chromatographic data. Detection was by UV absorbance at 214 nm with a multi-wavelength detector and the total run time was 77 min. The feed and the retentate samples were filtered through
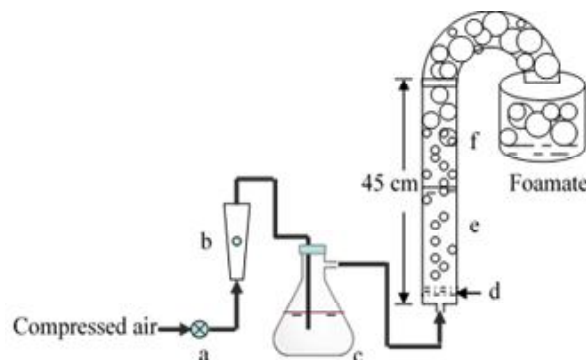
a- Pressure regulator cum indicator, b- flow meter, c- saturation chamber, d- porous polyethylene disc (100 μm), e- liquid column, f- foam column.

Fig 1: Schematic of foam fractionation experimental set up.

Table 1: Physicochemical characteristics of whey proteins (Morr and Ha, 1993; Muller *et al.,* 2003).

Property	α-La	β-Lg	BSA	IgGs	LF	LP
Mol. wt., kDa	14.2	36.6*	67.0	150.0	78	89
Stokes radius, nm	1.9	2.6	3.5	5.2	4-6	4-6
Isoelectric pH	4.2-4.5	5.2	4.8-5.1	5.5-8.3	8.0	9.5
Conc. in g/L	0.6-1.7	2-4	0.4	0.4-1.0	0.006-0.01	0.03-0.06
Conc. in % w/w	18-24	56-60	6-12	6-12	-	-

*in dimer form α-La- α-lactalbumin, β-Lg- β-lactoglobulin, BSA-blood serum albumin, LF-lactoferrin, LP-Lactoperoxidase, IgG-immunoglobulin G Mol. wt. is molecular weight, nm is nanometers, kDa is kilo Daltons, Conc. is concentration.

Table 2: Mean squares (probabilities) for different parameters analyzed for significant difference.

Source of variation	Parameter						
	Yield, %	PrER	α-La Yield, %	α-LaER	β-Lg Yield, %	β-LgER	α-La/β-Lg
PrConc	1188.5*(<0.0001)	43.34*(<0.0001)	43.64(0.32)	96.83*(<0.0001)	169.2(0.10)	83.78*(<0.0001)	4.16*(0.01)
pH	1117*(<0.0001)	0.65*(0.0004)	2350.58*(<0.0001)	3.08*(<0.0001)	1634.54*(<0.0001)	3.28*(0.0002)	31.43*(<0.0001)
PrConc x pH	71.53*(0.03)	0.19(0.07)	31.86(0.59)	1.15*(<0.0001)	232.75*(0.004)	0.57(0.29)	2.49*(0.02)
Error	32.79	1.0	36.43	0.23	76.32	0.46	0.00

*Statistically significant ($P < 0.05$). PrConc is protein concentration, PrER is protein enrichment ratio, α-La is alpha lactalbumin, β-Lg is betalactoglobuline, α-LaER and β-LgER are α-La and β-Lg enrichment ratios, respectively.

0.45 µm PVDF syringe filters before being injected on to the column. The injection volume of the samples was 100 µL.

Elution of the protein fractions was done using the procedure of Casal *et al.* (2006). Proximate analysis of the samples was done using official methods of analysis (Horwitz and Latimer Junior, 2005). TS were determined by oven drying at 100°C for 4 h. Total nitrogen was determined by the Kjeldahl method and nitrogen was converted to protein using 6.38 as a conversion factor. Ash content was determined by incinerating the samples at 550°C for 12 h. Lactose was determined by subtracting the fat, protein and ash contents from the TS content.

Calculations

The effect of initial protein concentration and pH of the feed on foam fraction was expressed in terms of yield (%) of protein, yield of α-La (α-La yield, %), yield of β-Lg (β-Lg yield, %), enrichment ratio of protein (PrER), α-La (α-LaER) and β-Lg (β-LgER) and the ratio of α-La to β-Lg in the foamate (α-La/β-Lg). Yield and enrichment ratios were calculated as

$$\text{Yield, \%} = \frac{\text{Mass of protein in the foamate}}{\text{Mass of protein in the feed}} \times 100$$

$$\text{Enrichment ratio} = \frac{\text{Concentration of protein in the foamate}}{\text{Concentration of protein in the feed}}$$

Statistical analysis

Experiments were conducted with four levels of initial protein concentration and five levels of pH. This resulted in a total of 20 treatment combinations (4 × 5 factorial design) for the study, which was implemented using a completely randomized design. Each treatment combination was replicated three times, giving a total of 60 experimental runs. Statistical analysis on the collected data was done using Proc GLM analysis of the SAS software (SAS institute, Cary, NC, USA) with a Type III error rate (α) of 0.05 to test for significant differences among the treatments.

RESULTS AND DISCUSSION
Yield and enrichment ratio of Whey proteins

The yield and the protein enrichment ratio are the two important parameters used to assess the effectiveness of

foam fractionation for recovering the protein fractions present in the feed. The mean squares for different parameters analyzed with SAS are presented in Table 2. The yield of protein from cheese whey was significantly ($P < 0.05$) affected by initial protein concentration in the feed (PrConc), pH of the feed and interaction of PrConc × pH ($P < 0.05$). The data for treatment effects presented in Fig 2 show the general trend in the yield of protein with respect to the effect of pH and initial PrConc. In general, the highest yields were obtained at the highest initial protein concentrations and the lowest pH levels studied. At higher initial PrConc, the foam flow rate was high and this increase in foam flow rate was a result of more stable foam at higher protein concentrations. Increased foam flow rate leads to an increased liquid holdup in the foam bubbles.

During the experiments, it was observed that smaller air bubbles were formed at lower pH levels and as pH increased, the size of the foam cells increased. Higher initial protein concentrations also resulted in smaller air bubbles. Small air bubbles provide a larger effective interfacial area and the bubbles were more uniform and were more resistant to collapse. All these factors contributed to the higher yield of protein in the foamate. The highest yield of 90.92% was obtained at a pH of 3.0 and initial PrConc of 870 mg/L. The lowest yield was obtained for pH 4.65 and 109 mg/L PrConc and this yield was not significantly different from the yields obtained at pH levels of 4.0, 5.1 and 6.3 for 109 mg/L initial PrConc.

Protein enrichment ratio (PrER) was significantly ($P < 0.05$) influenced by PrConc and pH. The interaction of
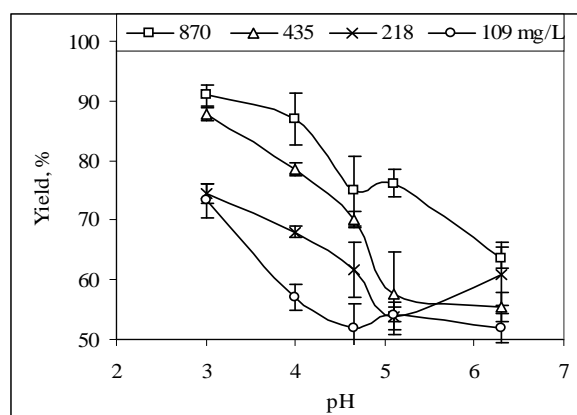

Fig 2: Effect of pH on yield of protein for different levels of initial protein concentrations (mg/L).

PrConc × pH was not significant ($P > 0.05$). PrER data for different levels of PrConc obtained at different pH levels studied are presented in Fig 3. As seen from these data, the highest PrER values were obtained at the lowest initial PrConc of 109 mg/L. As PrConc increased PrER values decreased. This trend was observed at all the pH levels studied in the experiments. As discussed in the earlier sections, low initial PrConc leads to lower foam flow rates and larger bubble sizes. Both these factors contribute to low liquid holdup at the gas-liquid interface and higher drainage of liquid through the bubbles. This leads to higher protein concentration in the foamate giving higher PrER. The highest PrER obtained in the present study was 5.68, obtained at 109 mg/L initial protein concentration. In a study to establish the optimum operating conditions for continuous foam separation of β-Casein, Brown *et al.* (1990) also reported higher PrER for low levels of initial feed PrConc.

In general, the feed pH has a strong effect on PrER. As seen from the data presented in Fig 3, as pH increased the PrER increased. Higher enrichment ratios are possible when the maximum amount of protein is retained in the foam. This is possible with low foam flow rates and larger bubble sizes. In the present experiments, these conditions were observed for higher pH values and lower initial PrConc. Theoretically at the isoelectric pH, the protein should have minimum solubility, highest hydrophobicity and the lowest surface tension (Mukhopadhyay *et al.,* 2010; Prokop and Tanner, 1993; Shea *et al.,* 2009). This will lead to the highest surface activity with the consequent increase in the adsorption of protein at the gas-liquid interface. However, in the present study Cheddar cheese whey was used and it contained GMP, BSA, α-La, β-Lg, etc. representing a multi-component mixture. These protein fractions have different pI values. Hence it is difficult to segregate the effect of pH on PrER. The lowest and the highest PrER values obtained for initial PrConc of 109 mg/ L are 4.3 and 5.68 at pH levels of 3.0 and 5.1, respectively.

Yield and enrichment ratio of α-La

The yield of α-La was significantly ($P < 0.05$) influenced by the pH of the feed. Initial PrConc and the interaction of pH × PrConc were not significant ($P > 0.05$). The insignificant effect of PrConc on yield was evident from the flatness of the curves as seen in Fig 4. The highest yield of 93.47% was obtained at a pH of 3.0 and this was not significantly different from the yield of 92.05% obtained at pH of 4.0. As the pH increased the yield of α-La decreased, with the lowest yield of 58.73% obtained at pH of 6.3. The yields obtained at a pH of 3.0 and 4.0 for different initial PrConc were not significantly different (data are not shown). The high yields obtained at these lower pH levels can be due to smaller bubble sizes observed in the experiments and increased surface activity of α-La (Dickinson and Matsumura, 1994). The enrichment ratio of α-La was significantly ($P < 0.05$) influenced by PrConc, pH and the interaction of PrConc × pH. The lowest α-LaER was obtained at a pH of 3.0 and the highest values were obtained at a pH of 5.1. As discussed
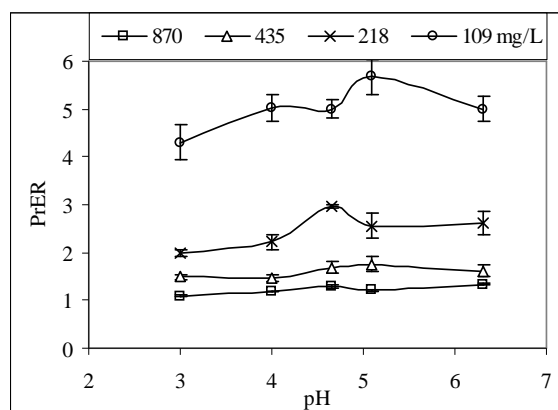

Fig 3: Protein enrichment data for different levels of initial protein concentrations. PrER is protein enrichment ratio.
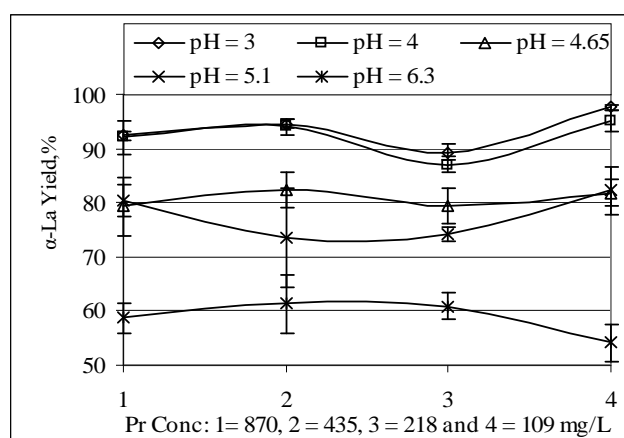

Fig 4: Effect of initial protein concentration on yield of α-La. α-La is alpha lactalbumin, PrConc is protein concentration.
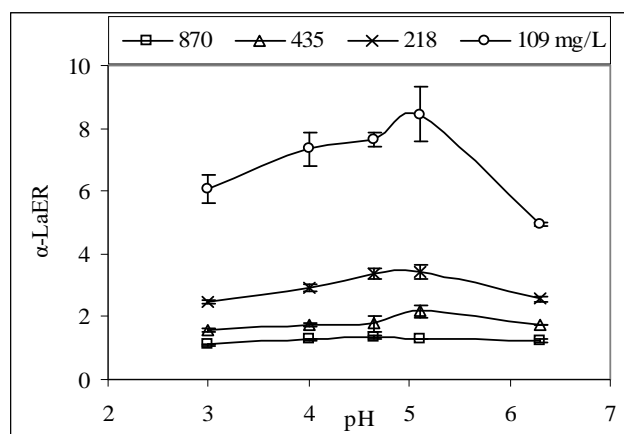

Fig 5: Enrichment ratios of α-La at different pH levels. α-LaER is alpha lactalbumin enrichment ratio.

in the earlier sections, at isoelectric pH, the protein should adsorb more easily onto the foam surface, thereby giving higher enrichment ratios. The isoelectric pH of α-La lies in the range of 4.2 to 4.5. However, as seen from Fig 5, the highest enrichment ratio was not obtained at this pH. The effect of pH might be masked by the smaller bubble size

obtained at lower pH levels (Brown *et al.,* 1990). In foam fractionation of BSA, Uraizee and Narasimhan (1996) also reported higher enrichment ratios at higher pH values, which were different from the pI of BSA. Shea *et al.* (2009) also reported a higher enrichment ratio for α-La at pH values different from pI. In the present study, the low enrichment
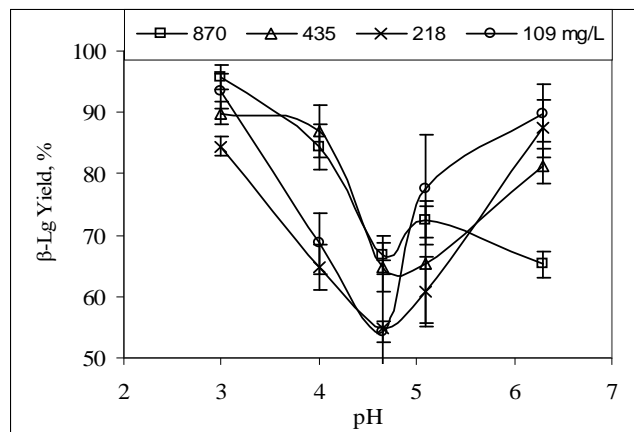

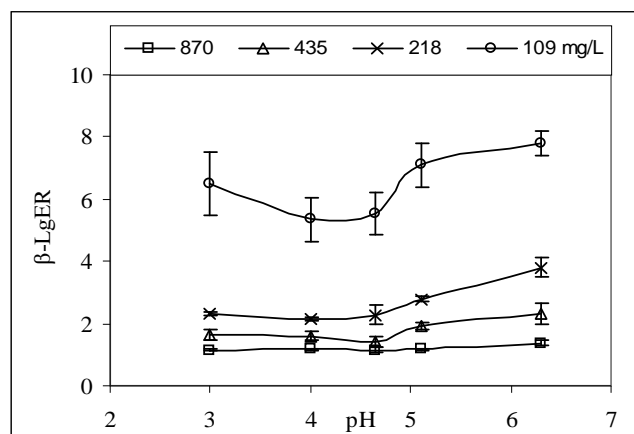**Fig 6:** Effect of pH on the yield of β- Lg. β-Lg is betalactoglobuline.

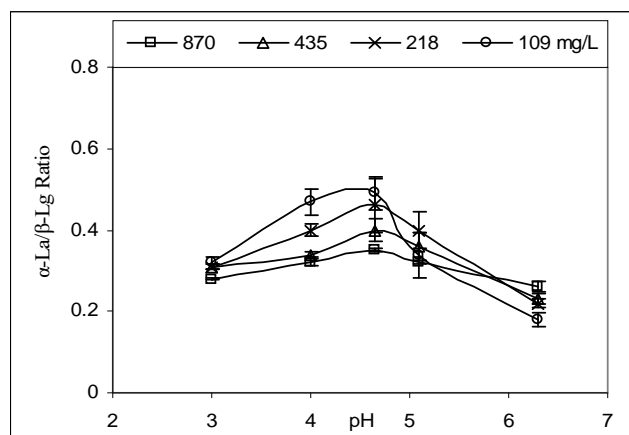**Fig 7:** Effect of pH on the enrichment of β- Lg. β-LgER is betalactoglobuline enrichment ratio.

Fig 8: Effect of pH on α-La to β-Lg ration in foamate. α-La and β-Lg are alpha lactalbumin and betalactoglobuline, respectively.

ratios obtained at pH of 3.0 and 4.0 may also be due to the fact, that α-La forms aggregates with β-Lg at pH < 4.0 (Harwalkar and Kalab, 1985). The highest α-LaER of 8.45 was obtained for a pH of 5.1 and initial PrConc of 109mg /L. The lowest enrichment ratios were obtained for initial PrConc of 870 mg/L and these values (for 870 mg/L) were significantly different from those of other pH levels studied.

Yield and enrichment ratio of β-Lg

The yield of β-Lg was significantly ($P < 0.05$) influenced by pH and the interaction of pH × PrConc. The initial PrConc had no significant ($P > 0.05$) effect on the enrichment ratio. From the data presented in Fig 6, it can be noticed that the highest yield for β-Lg was at a pH of 3.0 and the lowest yield was at pH of 4.65. β-Lg is known to form dimers at neutral pH (Phillips *et al.,* 1995; Shimizu *et al.,* 1985) and associates to form octamers at pH of 3.0-5.0 (Harwalkar and Kalab, 1985) and especially at pH of 4.7 (Verheul *et al.,* 1999). While the highest yield of β-Lg at pH of 3.0 may be due to smaller bubble size obtained at lower pH values, the lowest yield obtained at pH of 4.65 may be related to conformational changes and formation of larger octamer molecules. The effect of octamerization may be influencing the β-Lg enrichment ratios as well, resulting in the lowest β-LgER at this pH for all the initial protein concentrations studied (Fig 7).

The higher yields and the higher enrichment ratios (Fig 6 and 7) obtained at pH of 6.3 may be due to the fact, that β-Lg was preferentially adsorbing onto the gas-liquid interface at this pH. The highest yield (95.63%) obtained was at pH of 3.0 and an initial PrConc of 870 mg/L and this yield was not significantly different from the yields obtained for other levels of initial PrConc at this pH. The highest enrichment ratio of 7.79 was obtained for treatment combination of pH of 6.3 and PrConc of 109 mg/L and this was not significantly different from enrichment ratio obtained at pH of 5.1. The lowest enrichment ratios were obtained for initial PrConc of 870 mg/L and these values ranged from 1.15 to 1.37 and were not significantly different.

Ratio of α-La to β-Lg

The ratio of α-La/β-Lg is an indication of selective enrichment of one protein fraction over the other. This ratio was significantly ($P < 0.05$) influenced by both pH and initial PrConc. The interaction of pH x PrConc was also significant ($P < 0.05$). The data on α-La/β-Lg ratio obtained at different pH levels and initial PrConc are shown in Fig 8.

The highest ratios were obtained at a pH of 4.65 and the lowest ratios were obtained at a pH of 6.3. The highest ratios obtained at pH of 4.65 may be due to the fact, that β-Lg occurs in octamer form as discussed in the earlier sections and the lowest ratio obtained at pH of 6.3 may be due to preferential adsorption of β-Lg on to gas-liquid interfaces at this pH. This trend is in consistent with the enrichment -ratios observed for α-La and β-Lg at these pH levels. The highest α-La/ β-Lg ratio of 0.49 was obtained for treatment combination of 4.65 pH and 109 mg/L PrConc.

However, this value was not significantly different from the ratio of 0.47 obtained at pH of 4.65 and PrConc of 218 mg/L and pH of 4.0 and PrConc of 109mg /L.

CONCLUSION

Cheese whey is the unique source of proteins that have various nutritional, functional and therapeutic applications. For effective utilization of these protein fractions by producing value-added ingredients, there is a need for less complex and cost-effective separation processes, particularly when these protein fractions occur as a very dilute solution. Foam fractionation is one such a simple, less expensive and appropriate process for recovering solutes from low concentration solutions. Foam fractionation studies were conducted using a completely randomized 4×5 factorial experimental design with four levels of initial PrConc and five levels of pH. The separation efficiency of foam fractionation process was evaluated in terms of yield and enrichment ratio of total whey proteins, α-La and β-Lg and ratios of α-La to β-Lg.

PrConc did not show a significant ($P > 0.05$) effect on the yield of α-La and β-Lg, while PrConc × pH interactions had a significant ($P < 0.05$) effect on total protein yield, α-LaER, β-Lg yield and α-La /β-Lg ratio. The high initial protein content of 870 mg/L (like that of natural cheese whey) and low pH of 3.0 resulted in highest protein yield of 90.92%. These parameters appear to be the most suitable for efficient foam separation if yields were the focus of the process. On the contrary, highest PrER of 5.68 was obtained with the lowest PrConc of 109mg/L and high pH of 5.1. The preferential enrichment pH conditions for α-La and β-Lg were identified as 4.65 and 6.3, respectively. The highest α-La to β-Lg ratio of 0.49 was obtained for treatment combination of 4.65 pH and 109 mg/L PrConc. From these results, we can summarize that whey protein yield and enrichment ratio will have counter effective performance in foam separation process. This key finding makes it critical to predefine the process objective while using foam fractionation to recover proteins from cheese whey. From the results of the study, we can conclude that foam fraction process is technically feasible for fractionation of proteins from dilute solutions like cheese whey.

Nonetheless, the actual commercial feasibility of foam separation process either as a standalone process or an upstream process step integrated with conventional separation technologies such as precipitation and column chromatography, can only be established after industrial scale trails and further research on performance of foam separation process when integrated with other conventional separation processes.

REFERENCES

Anand, K. and Damodaran, S. (1995). Kinetics of adsorption of lysozyme and bovine serum albumin at the air-water interface from a binary mixture. Journal of Colloid and Interface Science. 176: 63-73.

Angela, G. (2004). Paper Presented at the 11th Annual Workshop for Dairy Economists and Policy Analysts, Washington, DC.

Arunkumar, A. and Etzel, M. (2018). Fractionation of glycomacropeptide from whey using positively charged ultrafiltration Membranes. Foods. 7: 166.

Backleh-Sohrt, M., Ekici, P., Leupold, G. and Parlar, H. (2005). Efficiency of foam fractionation for the enrichment of nonpolar compounds from aqueous extracts of plant materials. Journal of Natural Products. 68: 1386-1389.

Bhattacharya, P., Ghosal, S. and Sen, K. (1991). Effect of physico-chemical parameters on the separation of proteins from human placental extract by using a continuous foam fractionating column. Separation Science and Technology. 26: 1279-1293.

Brown, A., Kaul, A. and Varley, J. (1999a). Continuous foaming for protein recovery: Part I. Recovery of β casein. Biotechnology and Bioengineering. 62: 278-290.

Brown, A., Kaul, A. and Varley, J. (1999b). Continuous foaming for protein recovery: Part II. Selective recovery of proteins from binary mixtures. Biotechnology and Bioengineering. 62: 291-300.

Brown, L., Narsimhan, G. and Wankat, P. (1990). Foam fractionation of globular proteins. Biotechnology and Bioengineering. 36: 947-959.

Casal, E., Montilla, A., Moreno, F. J., Olano, A. and Corzo, N. (2006). Use of chitosan for selective removal of β-lactoglobulin from whey. Journal of Dairy Science. 89: 1384-1389.

Chai, J., Loha, V., Prokop, A. and Tanner, R.D. (1998). Effect of bubble velocity and pH step changes on the foam fractionation of sporamin. Journal of Agricultural and Food Chemistry. 46: 2868-2872.

Chandrasekar, V., Gabriela, J.S., Kannan, K. and Sangamithra, A. (2015). Effect of foaming agent concentration and drying temperature on physiochemical and antimicrobial properties of foam mat dried powder. Asian Journal of Dairy and Food Research. 34: 39-43.

Changade, S.P., Bhandari, P.N., Chapake, J.S. and Shinde, N.W. (2009). Foaming in food systems. Journal of Dairying, Foods and Home Sciences. 28: 26-30.

Dickinson, E. and Matsumura, Y. (1994). Proteins at liquid interfaces: role of the molten globule state. Colloids and Surfaces B: Biointerfaces. 3: 1-17.

Ekici, P., Backleh-Sohrt, M. and Parlar, H. (2005). High efficiency enrichment of total and single whey proteins by pH controlled foam fractionation. International Journal of Food Sciences and Nutrition. 56: 223-229.

Hammam, A.R. (2019). Technological, applications and characteristics of edible films and coatings: A review. SN Applied Sciences. 1: 632.

Harper, W.J. (1999). Biological Properties of Whey Components: A Review: Chicago IL: The American Dairy Products Institute 2001 with updates 2003. http://www.adpi.org/tabid/128/newsid545/52/Default.aspx. Accessed 20 January 2011.

Harwalkar, V. and Kalab, M. (1985). Thermal denaturation and aggregation of β-lactoglobulin in solution electron microscopic study. Milchwissenschaft. 40(2): 65-68.

Horwitz, W. and Latimer Junior, G. (2005). Official Methods of Analysis of the Association of Analytical Chemists International. Gaythersburg: AOAC International.

Huang, D., Wu, Z. L., Liu, W., Hu, N. and Li, H. Z. (2016). A novel

process intensification approach of recovering creatine from its wastewater by batch foam fractionation. Chemical Engineering and Processing: Process Intensification. 104: 13-21.

Hunter, J.R., Carbonell, R.G. and Kilpatrick, P.K. (1991). Coadsorption and exchange of lysozyme/β-casein mixtures at the air/water interface. Journal of Colloid and Interface Science. 143: 37-53.

Johansen, A.G., Vegarud, G.E. and Skeie, S. (2002). Seasonal and regional variation in the composition of whey from Norwegian Cheddar-type and Dutch-type cheeses. International Dairy Journal. 12: 621-629.

Keller, R., Orsel, R. and Hamer, R. (1997). Competitive adsorption behaviour of wheat flour components and emulsifiers at an air-water interface. Journal of Cereal Science. 25: 175-183.

Li, R., Chen, X. e., Chang, Y., Zhang, L., Zhang, Y., Zhu, Y. and Wang, T. (2017). Increase of bubble size playing a critical role in foam-induced protein aggregation: Aggregation of BSA in foam fractionation. Chemical Engineering Science. 174: 387-395.

Li, R., Fu, N., Wu, Z., Wang, Y. and Wang, Y. (2015). Protein aggregation in foam fractionation of bovine serum albumin: effect of protein concentration. Biochemical Engineering Journal. 103: 234-241.

Li, R., Fu, N., Wu, Z., Wang, Y., Liu, W. and Wang, Y. (2016). Enhancing protein self-association at the gas–liquid interface for foam fractionation of bovine serum albumin from its highly diluted solution. Chemical Engineering Research and Design. 109: 638-646.

Liu, L., Zhang, W., Yu, X., Lei, L. and Liu, H. (2018). Process optimization for foam separation of yak whey protein by response surface methodology. Separation Science and Technology. 53: 2327-2337.

Lockwood, C.E., Jay, M. and Bummer, P.M. (2000). Foam fractionation of binary mixtures of lysozyme and albumin. Journal of Pharmaceutical Sciences. 89: 693-704.

Mate, J. and Krochta, J.M. (1994). β Lactoglobulin separation from whey protein isolate on a large scale. Journal of Food Science. 59: 1111-1114.

Matouq, M. (2008). Investigation of the bubble foam separation technique to extract protein from whey. Am J Appl Sci. 5: 468-472.

Montero, G.A., Kirschner, T.F. and Tanner, R.D. (1993). Bubble and foam concentration of cellulase. Applied Biochemistry and Biotechnology. 39: 467-475.

Morr, C. and Ha, E. (1993). Whey protein concentrates and isolates: processing and functional properties. Critical Reviews in Food Science and Nutrition. 33: 431-476.

Mukhopadhyay, G., Khanam, J. and Nanda, A. (2010). Protein removal from whey waste by foam fractionation in a batch process. Separation Science and Technology. 45: 1331-1339.

Muller, A., Chaufer, B., Merin, U. and Daufin, G. (2003). Purification of α-lactalbumin from a prepurified acid whey: Ultrafiltration or precipitation. Le Lait. 83: 439-451.

OECD/FAO. (2017). OECD-FAO Agricultural Outlook2017-2026.

OECD Publishing. doi:https://doi.org/10.1787/agr_outlook-2017-en.

Phillips, L., Hawks, S. and German, J. (1995). Structural characteristics and foaming properties of β-lactoglobulin: effects of shear rate and temperature. Journal of Agricultural and Food Chemistry. 43: 613-619.

Pinfold, T. (1970). Adsorptive bubble separation methods. Separation Science. 5: 379-384.

Prazeres, A.R., Carvalho, F. and Rivas, J. (2012). Cheese whey management: A review. Journal of Environmental Management. 110: 48-68.

Prokop, A. and Tanner, R. D. (1993). Foam fractionation of proteins: Potential for separations from dilute starch suspensions. Starch Stärke. 45: 150-154.

Sarkar, P., Bhattacharya, P., Mukherjea, R. and Mukherjea, M. (1987). Isolation and purification of protease from human placenta by foam fractionation. Biotechnology and Bioengineering. 29: 934-940.

Shea, A., Crofcheck, C., Payne, F. and Xiong, Y. (2009). Foam fractionation of α-lactalbumin and β-lactoglobulin from a whey solution. Asia Pacific Journal of Chemical Engineering. 4: 191-203.

Shimizu, M., Saito, M. and Yamauchi, K. (1985). Emulsifying and structural properties of β-lactoglobulin at different pHs. Agricultural and Biological Chemistry. 49: 189-194.

Singh, A.K., Nath, N. and Arora, S. (2006). Composition and thermal behaviour of whey protein preparations under acidic conditions. Journal of Dairying, Foods and Home Sciences. 25: 8-14.

Stevenson, P. and Li, X. (2014). Foam Fractionation: Principles and Process Design: CRC press.

Stowers, C.C., Makarov, V., Walker, A., Edwards, R.A. and Tanner, R.D. (2009). Effect of air flow rate on the foam fractionation of a mixture of egg white and egg yolk. Asia Pacific Journal of Chemical Engineering. 4: 180-183.

Strohmaier, W. (2004). Chromatographic fractionation of whey proteins. Bulletin-International Dairy Federation. 389: 29-35.

Suzuki, A., Yasuhara, K., Seki, H. and Maruyama, H. (2002). Selective foam separation of binary protein solution by SDS comple--xation method. Journal of Colloid and Interface Science. 253: 402-408.

Tian, S., Wu, Z., Liu, W., Zhang, M., Lv, Y., Xu, Y. and Zhao, Y. (2018). Effective recovery of casein from its highly diluted solution by using a technology of foam fractionation coupled with isoelectric precipitation. Journal of Food Engineering. 216: 72-80.

Uraizee, F. and Narsimhan, G. (1996). Effects of kinetics of adsorption and coalescence on continuous foam concentration of proteins: comparison of experimental results with model predictions. Biotechnology and Bioengineering. 51: 384-398.

USDA. (2017). Dairy: world markets and trade. United States Department of Agriculture, Foreign Agricultural Service. doi:https://apps.fas.usda.gov/psdonline/circulars/dairy.pdf

Verheul, M., Pedersen, J. S., Roefs, S. P. and de Kruif, K. G. (1999). Association behavior of native β lactoglobulin. Biopolymers: Original Research on Biomolecules. 49: 11-20.