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Research Article

QUANTIFICATION OF LACTOFERRIN IN MEDICINAL DAIRY WASTE ENRICHED BY BUBBLE SEPARATION

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ABSTRACT

The aim of this study is to enrich therapeutic dairy proteins from 1L of dilute dairy waste by foam fractionation method using anionic surfactant, sodium dodecyl sulphate (SDS) as well as to determine operating parameters of the method like mean bubble diameter, % gas hold up, interfacial area for adsorption, heat of desorption, mass transfer coefficient of adsorption, enrichment ratio (E_R) and percentage recovery (%Rp) of proteins to foam phase. The process parameters were optimised by carrying out several experiments and one antineoplastic component namely lactoferrin was quantified in the isolated extract (foamate) by RP-HPLC. The method used 100cm long glass column of internal diameter 8cm and thickness 0.5cm attached with G3 sintered glass sparger(15-40µm) as bubble distributor to feed, rotameter for measurement of gas flow rate (GFR) and N_2 gas cylinder as gas supplier. Process parameters like pH and ionic concentration of feed, GFR, initial feed concentration was varied to examine the optimum performance criteria. The result gives maximum enrichment ratio(49.09), %Rp(98.18) of total proteins and 0.98%(w/w) of lactoferrin in foamate at pH (5.5), GFR (350mL/min), initial feed concentration(500µg/mL) , ionic concentration of feed 0.1(M) of NaCL and waste-SDS mass ratio(1.5:1).The heat of desorption(λ) and mass transfer coefficient(K) were estimated at the value of 3140 cal/mol and12.686 * 10^{-9}mol $cal^{-1}cm^{-2}s^{-1}$ respectively for a specific experiment. It can be concluded that method may be a useful unit operation for enrichment and purification of thermo labile and removal of pollutant proteins from industrial waste water for coming days.

Key Words: foam fractionation, sodium dodecyl sulphate, enrichment, lactoferrin, proteins, RP-HPLC.

INTRODUCTION

Worldwide milk production mounting every year by more than 1% reached approximately 800 tons in the year of 2017.India will become the leading milk production country for the coming year 2026.Under this circumstances, huge amount of dairy wastes will be generating from various dairy products such as milk, yoghurt, desserts and custards, cheese, butter, milk powders etc. Dairy waste water contains a variety of therapeutic wastes along with other compounds .[1, 2, 3]

In this context, application of lucrative technique for co-product recovery from dairy waste water is very important to serve the dual purpose for controlling environmental pollution as well as recovery of therapeutic and nutritional dairy waste such as variety of proteins and other molecules for the benefit of man and animal kingdom. Now a day's some current applied techniques like ultra-filtration, nano-filtration, electro-dialysis, ion-exchange, gel-filtration, precipitation and coagulation are costly. Therefore, it needs to search alternative gainful techniques for the benefit of coming days. Foam fractionation provides various benefits like easy scale up, continuous operation, suitable for purification of thermo labile molecules in the biotechnological process pathway without application of heat, limited space, low power consumption and no extra cost of solvent and high output for dilute feed. It is under the foam branch of "Adsorptive Bubble Separation Method" projected by Robert Lemlich in his edited book[4]. The principle of separation of the technique is based on physical or chemical adsorption of surface-active molecules on the gas-liquid interface (bubble's surface). The amount of surface-active species adsorbed can be quantitatively articulated by Gibb's Equation of Adsorption Isotherm.[4, 5]

Several researchers applied this technique in the field of pharmaceutical biotechnology for enrichment, purification and extraction of thermo labile medicinal proteins and a variety of natural pharmaceuticals from plant extracts and bio sources.[6,7,8] This technique has also been applied for removal of toxic metals and chemicals from industrial waste streams .[9,10,11]

The dairy waste water chiefly contains a multicomponent mixture of medicinal proteins namely bovine serum albumin(BSA), alpha lactalbumin (α-LA),beta lactoglobulin (β-LG), immunoglobulin (IG)[major proteins] as well as bovine lactoferrin (BLF) and bovine lactoperoxidase(BLP) [minor proteins].Lactoferrin is recently identified as remarkable anti-neoplastic agent for various organs of human body.[12,13,14,15]Dairy waste proteins(major and minor) have iso electric pH (pI) at approximately 5.5 and 9.0 respectively.[16,17]

In our study, we evaluated the batch process of foam fractionation to enrich multi-component proteins from dilute dairy waste. Additionally, RP-HPLC analysis has been performed to find out the quantity of single protein (BLF) present in enriched extract (foamate) by foam fractionation.

MATERIALS AND METHOD

Chemicals, instrument and apparatus

Dairy waste water was collected from local dairy industry (Kolkata).AR grade Sodium dodecyl sulphate and Sodium chloride purchased from E. Merk Ltd. Standard bovine lactoferrin (BLF)was the gift sample from Nifty Lab Pvt.Ltd (India). Acetonitrile, methanol, trifluoroacetic acid (HPLC grade), concentrated hydrochloric acid and sodium hydroxide were procured from Merk Ltd (Mumbai, India). All other solvents used were of analytical grade, procured from Merk Ltd.

The foam fractionation apparatus [Figure1] was purchased from local manufacturer (Kolkata). The acrylic rotameter (50-500 cm^3/min) from Rivotek instruments, digital pH meter from Toshniwal instruments, centrifuge and foam breaker by Remi, Eyela Rotary Evaporator from Indosathi scientific lab and tensiometer by Deeksha instrument corporation (India), the spectrophotometer Shimadzu UV-1800 from Shimadzu corporation (Japan).

Initial processing of dairy waste water

Dairy waste water was filtered through muslin cloth and centrifuged several times for removal of fat from the protein till constant absorbance was recorded and 250 mL of such processed dairy protein solution was evaporated at 45˚C for 5 hrs by using Eyela Rotary Evaporator. The obtained dry protein mass (190 g) was preserved in a refrigerator at (-18°C) until use.

Preparation of standard curve for total protein quantification

The protein mass was diluted in the concentration range of 50-900 µg/ml in double distilled water and absorbance of each concentration was determined in a spectrophotometer at 280 nm to draw the standard curve of protein waste powder.

Determination of critical micelle concentration and isoelectric pH of total and target protein (lactoferrin) by surface tension (γ) method at operating temperature 25±2˚C

Surface tensions of dairy protein waste and lactoferrin were determined in the concentration ranges (50-900 µg/mL) and (5-150µg/mL) respectively in double distilled water in a tensiometer to find out critical micelle concentration (CMC). The isoelectric pH of were determined at different pH by using 0.1(N) HCL and NaOH below CMC through tensiometer and data represented by Figure1.[18]

Foam fractionation

The experimental set up (Figure 2) consists of a glass column (100 cm long), internal diameter (8cm) and thickness (0.5cm) attached with nitrogen cylinder as the source of gas supply through a sintered glass porous frit no. G_3 (15-40 µm porosity) fused at the base of the column. Gas bubbles generated by the sparger ascend through the column and deposit as foam over the dilute feed. Foam moves through the column by gas pressure and finally deposits as extract(foamate) at the top outlet of the column. The dilute feed (1L) was prepared by adding SDS with mass ratio 1.5(waste):1(SDS) at different concentrations. 9 lots of experiments with each lot consisting of 3 experiments (total 27 experiments) were carried out at different pH of feed (2.5, 5.5 and 8.5), GFR (250, 300 and 350mL/min) and feed concentration (C_I=400,500and 600µg/mL) at ionic concentration (I_C) by adding 0.1-gram mol of NaCL per litre of feed. The pH of dilute feed was adjusted with 0.1(M) of hydrochloric acid or sodium hydroxide solution. GFR was kept under observation by rotameter for every experiment. The gas flow rate, pH and initial concentration of feed were varied to find the impact on total protein separation efficiency of the method in foamate by several experiments. The weight and volume of foamate and foam breaking time were accurately calculated. Column was run for 1hr and foamate samples were collected from sample port at different time intervals (5, 10, 15, 25, 35, 45 and 55 min respectively) for spectrophotometric analysis of total protein in foamate and RP-HPLC analysis of BLF. Operating temperature was kept at 25±2^{0}C.

Evaluation of Performance

Efficiency of foam fractionation are governed by three parameters namely (i) enrichment ratio (E_R) equal to the ratio of C_S/ C_I, where C_S is the concentration of protein in foamate and C_I is the initial concentration in feed, (ii) percent recovery (%R_p) calculated by [(A_{FM}/A_{FD}) *100], where A_{FM} and A_{FD} are the total amount of protein in foamate and feed respectively. (iii) Separation ratio (S_R) is equal to the ratio of C_S/C_R, where C_R is expressed as concentration in residual solution. Highest values indicate the optimum efficiency of the method for separation.

Quantification of total protein in foamate extract by UV absorbance

The total protein in foamate, feed and residue in foam fractionation were quantified at 280 nm by using UV-1800 spectrophotometer .[19]

Quantification of lactoferrin in foamate by HPLC analysis

The HPLC system (Waters, MA, USA) was consisted of Symmetry 300 C_4 protein analysis column (50 mm × 4.6 mm); particle size 5 µm; pore size 300 Å and equipped with a guard column. The temperature of the column was kept at 25°C. The analysis was consisted of a 600-controller pump, a multiple-wavelength ultraviolet-visible (UV-VIS) detector equipped with 50 µL loop injector (Rheodyne±, Cotati, CA, USA). The outputs were processed and recorded in a compatible integrator (model 486, Waters, MA, USA).
HPLC assays were performed by using an isocratic system of 0.1% trifluoroacetic acid (TFA) in water (A) and acetonitrile (B) with the ratio of 95:5 (v/v). The run time was set at 20 min. The Flow rate was set at 1.0 mL/min and the absorbance was detected at 210 nm (represented by figure 8 and 9).

Studies on interfacial area of adsorption

Determination of surface area of adsorption is important to determine the mass transfer coefficient (K) of protein molecules to foam phase [19]. The interfacial area is calculated by the following in equation 1.

$$As = \frac{6A_C H\varepsilon}{d_{32}} \text{----------(1)}$$

Where H is the height of liquid feed in the column, A_c is the column cross sectional area (in this case50.25cm^2), A_s is the interfacial area of foam phase for adsorption, ε is the void fraction determined from % gas hold up and d_{32} is bubble sauter mean diameter for individual location determined by the equation2.

$$\overline{d_{32}} = \frac{\sum d^3}{\sum d^2} \text{----------(2)}$$

d_{32} is the bubble mean sauter diameter given by equation3, where (k) is the number of locations (in this case 4) of the column where bubbles photograph taken.

$$d_{32} = \frac{k}{\sum \frac{1}{d_{32}}} \text{----------(3)}$$

Gas was passed at varying flow rates (250,300and350mL/min respectively) through 1L feed solution and bubbles were photographed at 4 different locations namely 5, 15, 25 and 35 cm vertical distances from the sparger. The photograph was developed in the computer and bubbles diameter was determined manually by mega pixel scale after enlargement. 160-165 such bubbles were measured per plate and mean sauter bubble diameter (d_{32}) was calculated for respective flow rates. Data were recorded in Table2.[19]

The percentage gas hold-up(ε*) was also calculated for different superficial gas velocities through the liquid feed from the maximum drop in liquid level (ΔL) from initial level(L) by sudden stoppage of the gas supply by equation (4). Data were tabulated in Table 2.

$(\varepsilon^*) = \frac{\Delta L}{L} * 100$---------- (4)

where, $\frac{\Delta L}{L} = \varepsilon$ = gas hold-up fraction.

Studies of effect of ionic concentration on gas hold up

The effect of ionic concentration in feed on % gas hold up, enrichment ratio(E_R) and percent recovery(%Rp) was studied at a fixed GFR(250ml/min),C_I(500µg/mL), pH(5.5) and waste surfactant ratio 1.5:1. Four different concentrations namely 0.0125,0.05 0.10and 0.15g- mol of NaCL/L of feed were chosen for the study and data recorded in Table3.[20]

Theory of molar mass transfer to the foam phase

Molar adsorption of a surface-active protein on bubbles surface from dilute feed is quantitatively expressed by Gibb's equation of adsorption isotherm, τ=1/RT [-dγ/dc] *C

Where, τ = quantity of surface-active molecule adsorbed per unit area of bubble's surface [gm mol/cm^2], T= operating temperature in Kelvin, C= Concentration of molecule in feed (gm/cc) and distributing factor for adsorption. γ = surface tension of experimental molecule (dyne/cm), R= Gas molar constant (8.317 * 10^{7}ergs/°C/mol or 1.987 cal/°C/mol).

The negative slope [-dγ/dc] of γ vs c curve indicates the surface tension is inversely proportional to bulk protein concentration in feed. The value of τ will be zero, when [dγ/dc] =0 and the curve is parallel to concentration-axis. The concentration of least surface tension is called critical micelle concentration (CMC)at which molecules form micelle and do not adsorb on the bubbles surface. So, concentration of feed must be kept less than CMC for adsorptive bubble separation .[4]

Determination of heat of desorption(λ) and mass transfer coefficient (K)

By application of material balance for surface active proteins in the two-phase system (liquid and foam), we can determine experimentally the latent heat of desorption(λ) by equation (5) and mass transfer coefficient(K) by equation (6) after finding λ value These two parameters are the indicators for measuring the separation efficiency the method to foam phase.

$$\ln\left[\frac{V_O}{V_B}\right] = \frac{1}{\left(e\lambda^{/RT} - 1\right)} \ln\left[\frac{C_O}{C_B}\right] \text{----------(5)}$$

$$\int_{C_{S_0}}^{C_S} \frac{d(Cs)}{\left[\lambda - RT \ln \frac{C_S}{C_B}\right]} = K\left(\frac{A_S}{V_S}\right)\int_0^{\theta} d\theta = K\, As\theta/Vs \text{----------(6)}$$

Where, V_0= initial bulk volume of liquid at zero time (θ=0) ,V_B= bulk volume of liquid after any time(θ),V_S= volume of liquid of foam phase at any time (θ),C_0 =concentration in bulk at time(θ=0),C_B= protein concentration in bulk after any time (θ),C_S=protein concentration in foam phase after any time (θ),T(K) = absolute operating temperature, A_S= interfacial area in cm^2 , θ= residence time of foam phase obtained from the volumetric flow rate of bubbles and the height of the column. λ, the latent heat of desorption in cal/mol can be determined from the slope [1/ (e $^{\lambda/RT}$-1)] of ln [V_0/V_B] vs. [ln C_0/C_B] plot [equation5, figure6].[4]

For determination of K, the left hand side integral of equation (6)was determined by graphical integration of [λ-RT ln (C_S/ C_B)]$^{-1}$vs.[C_S]plot initiating from C_{S0} to C_S by determining different values of area under curve those are integrals of [λ-RT ln (C_S/ C_B)]$^{-1}$ at different intervals of foamate collection i.e.(0-5),(5-15),(15-25),(25-35) minutes etc. The different values of integrals i.e. C_S*[λ-RT ln (C_S/ C_B)]$^{-1}$ * 10^7 were plotted against different collection times(θ=t)and the slope(m) of the line was equal to the value of , m=[K(A_S/V_S)](figure no7and equation6).The foam thickness [t]=[Volume of foam(V_S)/area of foam(A_S)]was determined by Gibb's equation :(e $^{\lambda/RT}$-1) =(1/t RT) *(-dγ/dC) and "t" value can be calculated from R(=8.317*10^{7}ergs /°C/mol),T(=298K), (-dγ/dC)= 0.329 (fromTable1), λ and average mol. wt.(25,600)of dairy proteins waste. The mass transfer coefficient (K) was determined from measured value of t and value of slope (m) ,K=[t*m].[4] In this study, the mass transfer co-efficient was determined on the basis of average molecular weight calculated from the respective molar mass fraction of individual proteins (Bovine serum albumin- 5%,β-lactoglobulin-50%,α-Lactalbumin-12%,Immnoglobulin-10%,Bovine lactoferrin-1%, Bovine lactoperoxidase- 0.5%.).

RESULTS

Determination of λ and K values

From fig.6, [1/ (e$^{\lambda RT}$-1)] =0.0059(slope) and λ=3140cal/mol approximately.

From Fig.7, K [A_S/V_S] = (K/t) =0.021*10^{-7}(slope). So, K= [t]*[0.021*10^{-7}] = 6.041*0.0218*10^{-7}=12.68*10^{-9}mol/cal/cm^2/s

Foam thickness (t) = (V_S/A_S) was calculated by the equation: [1/ (e$^{\lambda RT}$-1)] = (1/tRT) (-dγ/dc).

Effect of pH at a fixed GFR of 350mL/min

Effect of pH of feed solution at a fixed GFR was recorded in Table 4 and represented in Figure 8. Maximum recorded values of enrichment ratio (E_R=49.09) and percent recovery(%Rp=98.18) for total protein as well as lactoferrin of 0.98%(w/w) in enriched protein extract of foamate at pH 5.5. All data were found of the order of pH5.5>2.5>8.5 respectively.

Effect of GFR at a fixed pH 5.5 of feed

From Table 5, Table 6 along with figure 9 and 10, it was found that enrichment ratio (E_R) and percent recovery (%Rp) of total protein as well as the component lactoferrin of 0.98%(w/w) in foamate enhanced with the increase of GFR at fixed pH 5.5.From the experimental results, it was observed that the enrichment ratio(E_R) and percent recovery (%Rp) increased when GFR changed gradually from the values of 250,300 and 350 mL/min respectively.

Effect of superficial gas velocity (SGV) on% gas hold up

The effect of superficial gas velocity on % gas hold & % Rp were represented in Figure 4. Gas hold up enhances linearly up to the superficial gas velocity (SGV) of 0.199 cm/s. In the present study, SGV was maintained in the range of 0.0829 – 0.116 cm/s as

shown in Table 2. Percent recovery (% Rp) is enhanced with the increase of interfacial area.

Effect of ionic concentration (I_C) on % gas hold up at a fixed GFR 250mL/min

From Table 3 and Figure 5, it was observed values of E_R and %Rp increased from 0.0125(M) to 0.1(M) and after reduced at 0.15(M). The maximum % gas hold up was noted 0.905 at 0.1(M) of NaCL concentration in feed.[20]

Quantification of lactoferrin by HPLC method

The mean R_t was observed for BLF at 11.62 ± 0.06 min by comparing between standard [Figure11(a)] and dairy waste protein chromatogram [Figure11(b)]. The calibration range of BLF was found to be 100-800 µg/ml, with the linear equation Y= 225937.33X + 36216, with coefficient of determinants (r^2) of 0.993. The optimum amount of lactoferrin was found0.98% (w/w) of enriched foamate extract from foam fractionation experiment which was recorded in Table4 and represented by figure 11 (a and b).

Table 1: Characteristics of medicinal proteins in dairy waste

Medicinal protein in dairy waste	Mol. wt. ($Da*10^3$)	Isoelectric pH(pI)	[dγ/dc] (dyne cm2/µg)	Range of conc. (µg/ml) of constant slope	CMC (µg/ml)
BSA(major)	69	5.1	-----	------	-----
BLF(minor)	84	9.0	0.082	5-150	150
BLP(minor)	89	9.6	------	------	------
α-LA(major)	14	5.3	------	------	------
β-LG(major)	18.30	4.8	------	------	------
IG(major)	100	5.5	------	------	------
Dil. protein waste	25.60	5.2	0.329	50- 750	750
Dil.(Protein waste + SDS [1.5:1(w/w)]	-------	-------	0.301	85-800	800

Table 2: Effect of superficial gas velocity on interfacial area

SGV (cm/s)	Gas flow Rate (mL/min)	Sauter mean diameter d_{32} (cm)	%gas hold up (ε*100)	Interfacial area (cm^2)	Percent Recovery (%Rp)	Feed density (g/cc)	Feed Viscosity (Poise)
0.083	250	0.0621	0.90	845.09	90.98	1.235	0.0095
0.099	300	0.0705	1.19	1012.85	93.99		
0.116	350	0.0821	1.38	1117.29	98.18		

Table 3: Effect of ionic concentration on (E_R) and (%Rp)

Molar Ionic concentration	Percent gas hold up (ε*100)	Enrichment ratio (E_R)	Percent Recovery (%Rp)
0.0125	0.70	32.80	70.40
0.05	0.785	33.39	78.80
0.1	0.905	40.45	90.99
0.15	0.810	36.52	80.35

Table 4: Experimental results showing the effect of changing pH at GFR 350ml/min

Lot No.	Exp No.	pH	Feed conc. (µg/mL) (C_I)	Gas Flow Rate (mL/min)	Concentration in foamate(CS) (µg/ml)	Enrichment ratio(E_R)	%Rp (Total protein)	(BLF) %(w/w)	Heat of desorption λ (cal/mol)
8	1	2.5	500	350	20440	40.90	79.91	0.79	3330
8	2	5.5	500	350	24545	49.09	98.18	0.97	3360
8	3	8.5	500	350	19950	39.90	77.85	0.77	3270

Table 5: Effect of GFR on mass %(w/w) of Lactoferrin at pH 5.5 and feed concentration

GFR (mL/min)	Feed concentration(µg/mL)	Lactoferrin %(w/w) in foamate
250	500	0.89
300	500	0.91
350	500	0.98

Table 6: Experimental results showing the effect of changing GFR at pH 5.5

Lot No.	Exp No.	pH	Feed conc. (mcg/ml) (C_I)	Gas Flow Rate (mL/min)	Concentration in foamate(CS) (µg/mL)	Enrichment ratio (E_R)	%Rp (Total protein)	(BLF) %(w/w)	Heat of desorption λ (cal/mol)
1	2	5.5	400	250	13478	33.70	77.50	0.76	2907
2	2	5.5	500	250	20898	34.84	91.95	0.89	2833
3	2	5.5	600	250	22295	37.16	81.75	0.80	2991
4	2	5.5	400	300	15087	37.72	86.75	0.84	3420
5	2	5.5	500	300	20207	40.41	92.95	0.91	2878
6	2	5.5	600	300	22595	37.65	82.85	0.82	3204
7	2	5.5	400	350	16718	41.80	91.85	0.90	3127
8	2	5.5	500	350	24545	49.09	98.18	0.98	3360
9	2	5.5	600	350	24585	40.98	83.75	0.84	3140

* Feed Volume=1L; foamate collection time =55 min

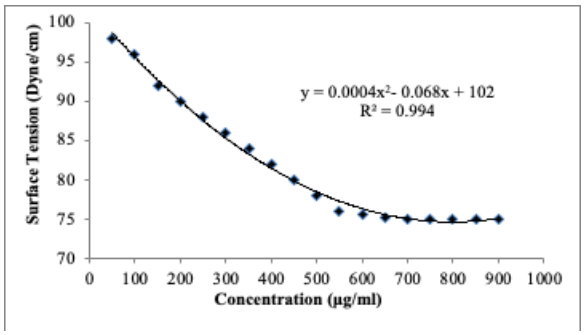

Figure 1: Plot of Surface Tension vs. Concentration of dairy protein waste Solution

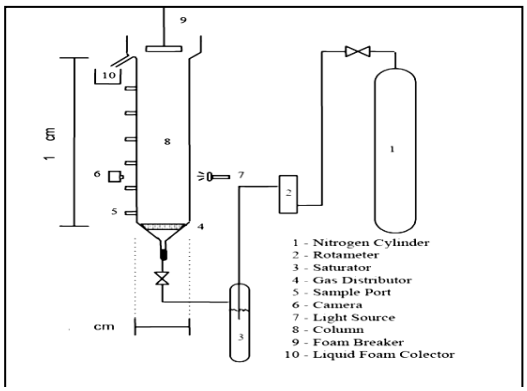

Figure 2: Experimental set up for foam fractionation

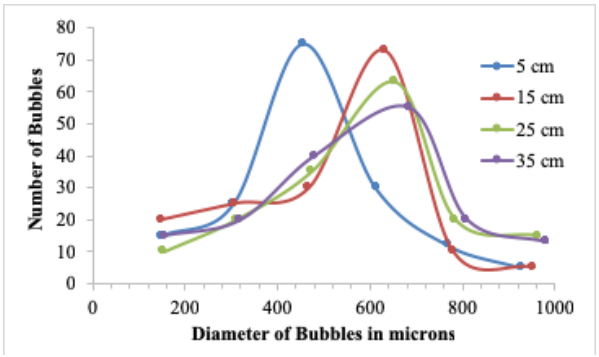

Figure 3: Bubble distribution at SGV 0.083 cm/s

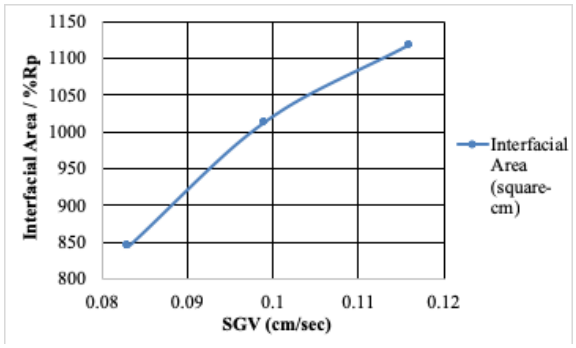

Figure 4: Effect of superficial gas velocity on Interfacial Area

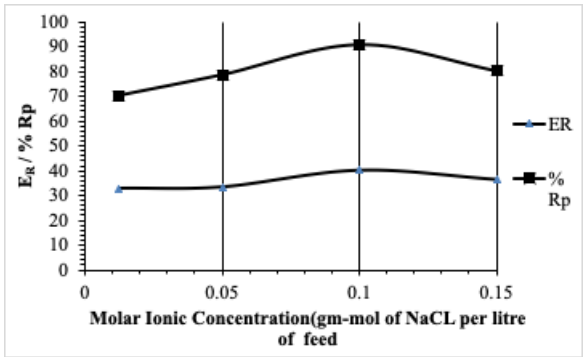

Figure 5: Effect of ionic concentration on E_R and % Rp

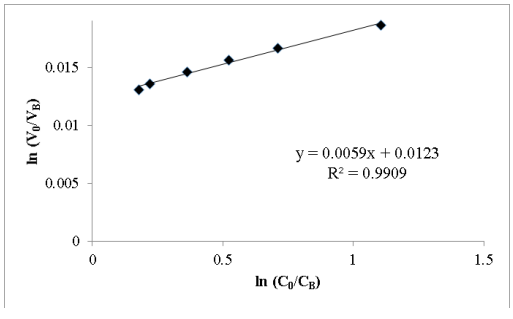

Figure 6: ln (V_0/V_B) vs. ln (C_0/C_B) Curve for (λ) Determination

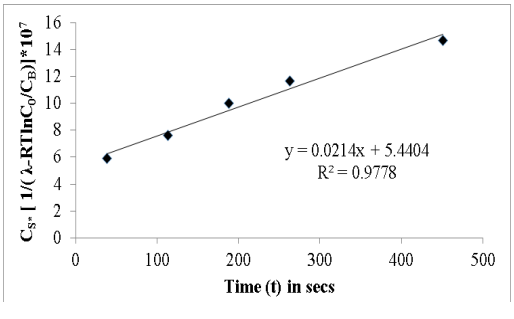

Figure 7: C_{S^*} [1/ (λ-RTlnC_0/C_B)]*10^7 vs. time (t) plot for K determination

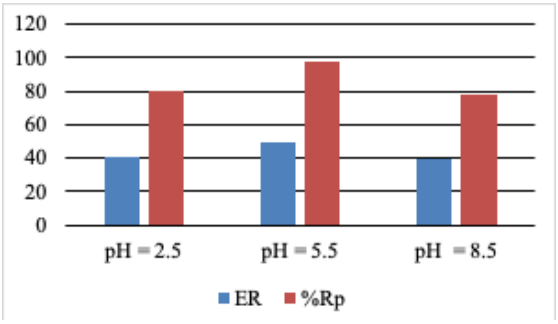

Figure 8: Effect of varying pH (2.5; 5.5 and 8.5) on E_R and %

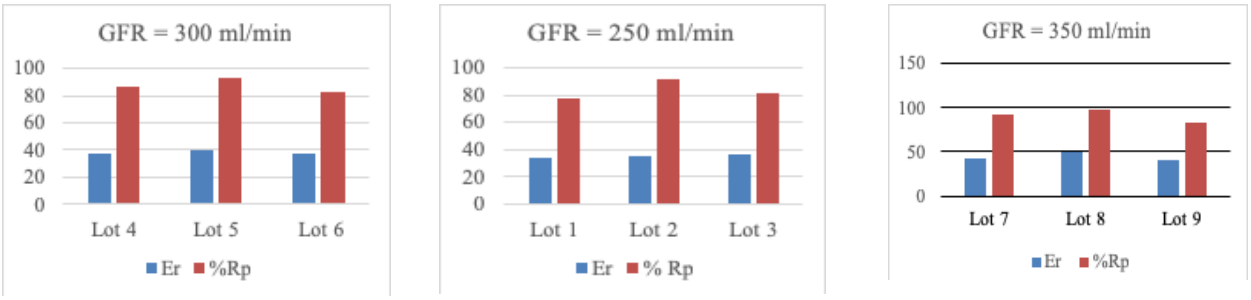

Figure 9: Effect of (a) [GFR 250ml/min], (b) [GFR300ml/min], (c) [GFR300ml/min] on E_R &%Rp

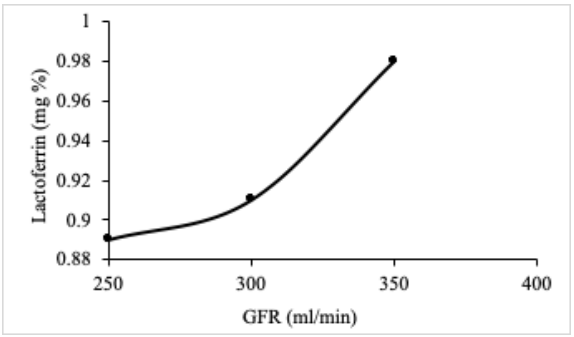

Figure 10: Effect of GFR on mass %(w/w) of Lactoferrin at pH 5.5 and feed concentration 500µg/mL

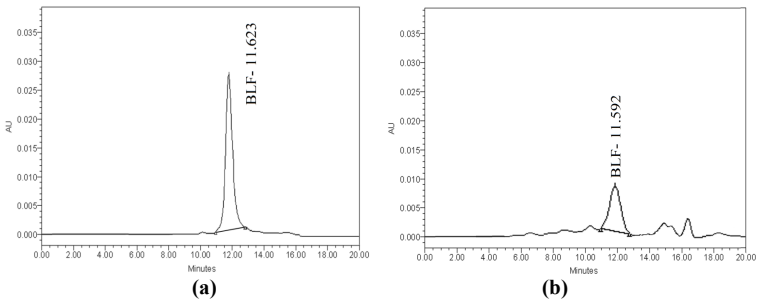

Figure 11: RP-HPLC chromatogram of BLF in (a) Standard; (b) Enriched foamate

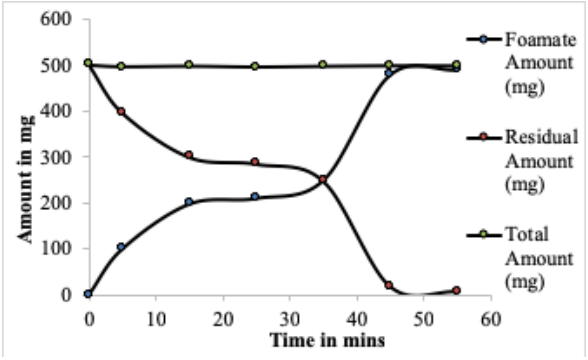

Figure 12: Material balance diagram of experiment no 2 of lot 8

DISCUSSION

From Table 1, it was observed[dγ/dc] value of waste solution without SDS (-0.0329 dynes /cm^2 / µg) more than that of SDS and protein waste solution mixture, so addition of SDS have no impact on CMC. "Ekichi et al. (2005) have also indicated that optimum foaming process is achieved below 750 µg /mL with appropriate gas flow rate which will have positive effect on protein enrichment" (Figure1).[21]

In foam fractionation experiment, pH of feed solution plays vital role in controlling adsorption of protein at the gas-liquid interface (bubble's surface). Iso-electric points (IEP) of all the major and minor dairy waste proteins are nearly at pH (5.5) and (9.0) respectively as mentioned in Table1.[16, 17]From Table 4 and Figure 8, it is noted enrichment factor and percent recovery are in the order of pH5.5>pH2.5>8.5. This is because at pH5.5, all the major proteins prefer hydrophobic adsorption at their isoelectric pH. Minor proteins such as lactoferrin and lactoperoxidase having IEP approximately 9.0>5.5 will behave cationic and form sturdy hydrophobic complex with anionic surfactant SDS to adsorb maximum on the bubbles surface [22, 23].At pH2.5<IEP, major proteins(more than 95% of total protein) become cationic and attached by columbic attraction with anionic SDS to form surfactant bridges between lamella (intra space of foam) by enhancing the foam's tackiness and rigidity which will resist the rising flow of liquid causing reduction of enrichment and recovery. At pH 8.5(>IEP), major proteins become anionic and columbic repulsion between proteins and SDS-protein complex molecules will repel each other reducing thickness and viscosity of film than that at pH 2.5. So, foam at pH 8.5 is quite wet and less dense than at pH 8.5. Minor proteins being heavy find difficulty for adsorption at pH8.5(adjacent to PI=9.0) due to weak hydrophobicity of raw protein than protein-SDS complex at pH 5.5 and 2.5.[22]

From figure 3and 4 and (Table 2and 6), increase of E_R and %Rp were found to increase due to gradual enhancement of SGV and GFR. This can be explained by the fact that gradual increase of both the values generate more of bubbles followed by increase of interfacial area of adsorption. From figure5 and table3,it was observed the effect of inorganic ions (NaCL) enhances the gas hold up volume, E_R and %Rp at the maximum of 0.1(M) of ionic concentration which is below critical concentration of NaCL [0.145 (M)] due to inhibition of coalescence between the bubbles and increase of interfacial area of adsorption by formation of micro bubbles.[20] The SDS – protein complex increases the foam properties like width, flexibility, and solidity of the interfacial membrane and foaming ability of protein for enhancement of adsorption.

In figure 12, linearity indicates the principle of material balance of proteins of total mass (M_T) equal to the sum of masses in foamate and residue ($M_T=M_S+M_R$) indicating least loss of material. Rate of removal and time for 50% removal ($t_{50\%}$) were obtained from the slope and point of intersection of curves. Based on the experimental condition of exp no 2 of lot no 9, λ and K were determined at the values of 3140cal/mol and 12.686 * 10^{-9} gm mol cal^{-1} cm^{-2} s^{-1} respectively. The highest separation outcomes [E_R = 49.09, % Rp = 98.18] were observed in lot no 8 of exp no 2 at pH 5.5, GFR (350 mL/min), WSR (1.5:1) and I_C (0.1gram mol of NaCL /L of feed.).

CONCLUSION

It is observed that the foam fractionation is the constructive unit operation to boost concentration of medicinal proteins from dilute dairy waste as well as to reduce pollutant proteins to certain extant from dairy waste water for controlling environmental pollution. The method was found to have best effectiveness at initial concentration of 500µg/ml, gas flow rate 350mL/min, waste surfactant mass ratio 1.5:1 and ionic concentration 0.1gm-mole of NaCL per litre of feed at pH5.5. Superficial gas velocity and ionic concentration enhance interfacial area for adsorption by escalating the number of micro bubbles. The observed mass transfer coefficient was to some extent high than that of earlier studies. The difference in value may be due to the impact of SDS and inorganic electrolyte (NaCL) resulting adsorption of high molecular weight proteins such as lactoferrin and lactoperoxidase. Evaluation of performance of experiment number 2 of lot 8 was found acceptable. The study focused foam fractionation as profitable unit operation to enrich thermo labile therapeutic proteins as well as remove pollutant proteins from dairy waste water.

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