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A Review on Application of ABSM in Protein Isolation

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ABSTRACT

Proteins can be obtained from a wide variety of samples like cells or tissues and microorganisms. In any case, endogenous proteins that we do not want are present in a much greater quantity than the proteins we do want. Regardless of the source, it is not easy to isolate a specific proteins and special methods are induced as there is no way to amplify proteins. Hence using overexpression and enrichment is the validated methods. Current applied techniques are ultrafiltration, gel filtration, ion exchange or precipitation and coagulation. An alternative but still less investigated method for the enrichment of whey proteins is called foam fractionation and solvent sublation, belonging to Adsorptive Bubble Separation Method (ABSM). It is a suitable method for the enrichment of surface-active substances like enzymes and other proteins under mild conditions. The efficiency of separation is high for smaller undesirable molecules present in highly diluted solutions. In recent times more advanced processes as made to use. There are several criteria like recovery time, purification factor, enrichment factor, operation expenditure, investment costs, running costs and environmental pollution which are to be considered before selecting a recovery method. Operation expenditure, investment costs, running costs and environmental pollution are higher in precipitation and chromatographic techniques. These are significantly lower in foam fractionation technique. But it is restricted to lower concentrated feed.

KEYWORDS: Protein isolation, Adsorptive Bubble Separation Method (ABSM), Whey Proteins, Foam Fractionation, Solvent Sublation.

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1. INTRODUCTION

Whey becomes a strong pollutant when discharged into streams, its high organic matter enhances biochemical oxygen demand (BOD5) ranging from 30 to 40 g of oxygen per liter. Since large quantity of whey is produced worldwide each year so the risks of pollution are therefore extremely high. There are various methods of utilizing or disposing of whey. It can be dumped at the production site, provided that the land area is large enough and the soil permits the absorption of the mineral elements and the organic matter. The principal constituents of whey can also be separated by various methods of separation available. The resulting proteins can be utilized for various purposes and the lactose obtained by the crystallization of the raw or concentrated product could be utilized for human food or by the pharmaceutical industry. Whey is also an excellent substrate for the cultivation of yeasts. There are various procedures which make it possible to obtain large quantities of protein, lactic acid, ethyl alcohol and vitamins. Purified whey protein is , natural, high-quality product that contains little or no fat, lactose or cholesterol and is a rich source of essential amino acids. In its purest form known as whey protein isolate provides innumerable benefits to athletes and dieters, boosts the immune system, helps bone strength and improves overall wellness. Currently large number of people recognized its potentiality. Now whey protein is regarded to be beneficial in cardiovascular health, athletic sector and the proper growth of infants and toddlers.

Thus, a cost-effective method for the enrichment or isolation of such protein fractions is of high economic and ecological interest. Current applied techniques are ultrafiltration, gel filtration, ion exchange or precipitation and coagulation. An alternative but still less investigated method for the enrichment of whey proteins is called foam fractionation and solvent sublation, belonging to adsorptive bubble separation method. It is a suitable method for the enrichment of surface-active substances like enzymes and other proteins under mild conditions. The efficiency of separation is high for smaller undesirable molecules present in highly diluted solutions. Foam fractionation is a well-documented protein separation technique, which has potential use in the preliminary stages of the downstream processing of recombinant and other proteins. The advantages of the process include, ease of operation, mechanical simplicity and therefore low cost compared to existing purification methods. Much research has been conducted using single protein in solution but limited work exists for protein mixtures in order to successfully purify one protein component from any mixture of proteins.

Foam fractionation or adsorptive bubble separation processes had been widely applied in mineral flotation. The techniques were based on the difference of surface tension between materials to be separated. This technique is also applied in waste water treatment. The recovery or removal of

dilute organic compounds contained in industrial waste water is possible, because organic compounds often show low surface tension and could be enriched at air-water interface. Although this technique has been known since the beginning of the twentieth century, there is still a need for research to elucidate the suitable condition and feasibility of separation of proteins and of single whey fractions using foam fractionation with an aim to optimize most important process parameters. Solvent Sublation is a non-foaming adsorptive bubble separation process, which is capable of removing trace levels of non-volatile and volatile organic compounds from waste waters. The advantage of solvent sublation over bubble fractionation is that higher removal efficiencies are possible. The Sublation process does mixer and phase separators, which is needed in the solvent extraction. Furthermore, the effluent water from a sublation remove residual solvent. Handling is simple and expenses are cheap, so the solvent sublation has the potential in the environmental pollution treatment. On the other hand, the recovery of valuable trace metals is a very important aspect in the future. Although many works have been done about the solvent sublation, but many aimed at the laboratory studies, the investigations on the large-scale studies are sparse. Though the solvent sublation technique has many advantages over many traditional techniques, the road is very long to utilize the technique in the wastewater treatment and the recovery of usable materials in near future.

2. REVIEW ON WHEY PROTEINS

Whey protein is the collection of globular proteins that can be isolated from whey, which is a by-product of cheese and sweet manufactured from cow‘s milk. Whey has the highest biological value (BV) of any known protein. The protein fraction in whey (approximately 10% of the total dry solids within whey) comprises four major protein fractions and six minor protein fractions. The major protein fractions in whey are beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin and immunoglobulins. Each of these components has important disease- fighting effects. In addition, whey protein is easily digestible. Whey protein can be denatured by heat. High heat (high temperatures above 72^{o}C associated with the pasteurization process) denatures whey proteins, destroying some bioactive compounds, such as the amino acid cysteine. While native whey protein does not aggregate upon renneting or acidification of milk, denaturing the whey protein triggers hydrophobic interactions with other proteins, and the formation of disulfide bonds between whey proteins and casein micelles, leading to aggregation with other milk proteins at low pH.

Whey protein products are typically available in three major forms: concentrate, isolate, and hydrolysate in the market:

2.1. ***Concentrates*** contain a low level of fat and cholesterol but,in general, havehigher levels of bioactive compounds, and carbohydrates in the form of lactose

They are 35%-95% protein by weight.

2.2. ***Isolates*** are processed to remove the fat, and lactose, but are usually lower inbioactive compounds as well — they are 90%+ protein by weight. Both of these types are mild to slightly milky in taste.

2.3. ***Hydrolysates*** are predigested, partially hydrolyzed whey proteins that, as aconsequence, are more easily absorbed, but their cost is generally higher. Whey protein hydrolysate also tends to taste quite different than other forms of whey protein, usually in a way that many find undesirable but can be masked when used in beverages.

Table 1. Whey Proteins present in Commercial Protein

Protein	Approx % of Whey Protein
Beta-lactoglobulin	50-55
Alpha-lactalbumin	20-25
Immunoglobulin	10-15
Bovine Serum Albumin	5-10
Glycomacropeptide (GMP)	2-5
Lactoferrin	1-2

3. ADSORPTIVE BUBBLE SEPARATION TECHNIQUE [6-11]

Adsorptive bubble separation technique, the generic namewas first proposed by elc16)Woitoue ta Asbon differences in surface activity. Material, which may be molecular, colloidal, or macro particulate in size is selectively adsorbed or attached at the surface of bubbles rising through the liquid and is thereby concentrated or separated. A substance, which is not surface active itself, can often be made effectively surface active through union with or adherence to a surface-active collector. The substance so removed is termed as colligend. The material is concentrated or separated in a small volume of collapsed foam, which is called foamate.

In principle, gases (e.g. air, nitrogen, or carbon dioxide) are passed through a porous glass frit, which is placed in the aqueous solution at the bottom of a foam fractionation column. Thereupon, foam develops by first becoming spherical and then polyhedral bubbles in the upper

column part. The adsorption of surface active molecules takes place at the gaseous bubble/liquid interface either during drainage of the laminar liquid or because of collapsing lamellas, leading also to a reflux.

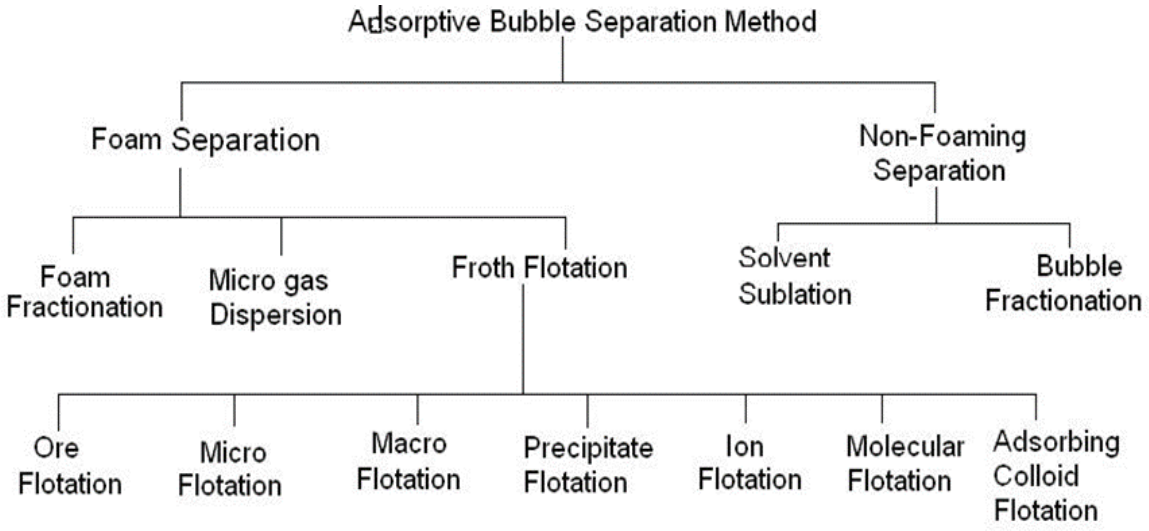

Fig1. Classification of ABSM

The Adsorptive Bubble Separation Method can be operated by following methods:

a) Simple Batch

b) Simple continuous flow

c) Continuous flow enriching by reflux

d) Continuous flow stripping

e) Combined enriching and stripping

f) Staged operation.

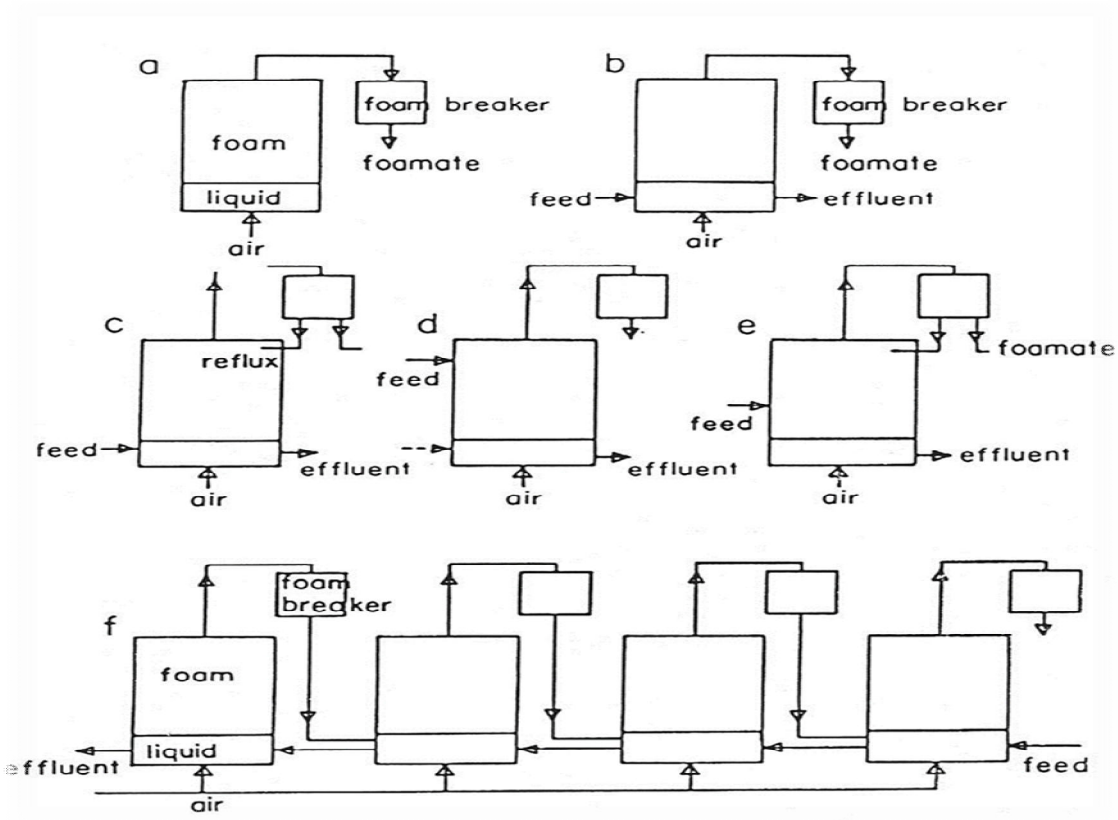

Fig 2. Mode of operation in Adsorptive Bubble Separation Method

4. FOAM FRACTIONATION [12-22]

4.1. ***Principle:*** Adsorptive bubble separation method, depends upon the differences in physico-chemical properties of particles. The particles of interest must selectively attach to the gas-liquid interface of foam bubble rising through the liquid pools. If the particles are not surface-active, can be made active by using collectors. Under equilibrium conditions of dilute solutions (assuming activity to unity), adsorption of surface active species from bulk solution at a gas- liquid interface.

4.2. Factors Affecting the Efficiency of Foam Fractionation

The performance and efficiency of a foam separation method depends on manyfactors. The relative importance of each factor depends on the specific condition.These include basic variables such as.

i. Concentration of collector
ii. Colligend concentration
iii. Collector-colligend ratio
iv. Pulsed addition of collector
v. pH

vi. Ionic strength

vii. Temperature

viii. Gas flow rate

ix. Presence of other auxiliary reagents

x. Surface area of bubbles

xi. Foam height

xii. Foam density

xiii. Foam drainage

xiv. Equipment design

5. SOLVENT SUBLATION[23-27]

The solvent sublation technique, a non-foaming adsorptive bubble separation in which enriched material on bubble surfaces is collected in immiscible liquids, rather than infoams. Surface active material will be present in bulk aqueous phase on top of which is placed an immiscible liquid. Gas bubbles are generated in aqueous media and buoyed upward into organic phases. The bubbles selectively adsorb surface active material while in the water (as in any adsorptive bubble process) and transport this material to the non aqueous phase. The material is either deposited in the top phase after the bubbles burst at the air- liquid interface or is dissolved during the passage of the bubble through the immiscible phase. The name solvent sublation arises from the fact that an ionic species, called the colligend is removed by addition of a surfaceactive collector of opposite charge to that on the colligend. The complex formed bycoulombic attraction is called as ―sublate‖ and the process of lifting sublate by gasbubbles is called ―sublation This technique was originated by Sebba as an option for ion flotation, if excessively copious foam formation occurred. Although theadsorptive bubble separation techniques have been studied extensively, the literature on the sub area of solvent sublation is rather sparse. Lemlich‘s book on adsorptive bubble separation includes an excellent review on solvent sublation. The solvent sublation technique has shown promise for the removal of certain types of organic compounds from aqueous systems.

The other important advantages of this technique are:

a) The possibility of easy handling of large volumes of aqueous samples, thus making the techniques of great potential interest for the analysis of natural, residual and marine waters for trace elements.

b) The active material is carried by the gas bubbles and goes into the upper immiscible liquid layer without bringing about mixing of the aqueous and organic phases, so that the separation process can offer high selectivity, potentially greater than that of other flotation techniques. On the other hand, as

a minimum fraction of the aqueous phase comes into contact with the organic phase, the solvent extraction thermodynamic parameters controlling the extent of extraction can be defined from the establishment of an equilibrium state. In solvent sublation this equilibrium state cannot be established in the bulk of the system but only at the aqueous–organic interface, which can remain virtually immobilized when the gas flow–rate is kept sufficiently low. As a result, the solvent sublation process is not limited by the equilibrium constant; the recovery of trace elements can eventually reach 100%.

c) In addition, the phase stirring process associated with liquid- liquid extraction frequently leads to the formation of undesirable emulsions, especially when surface–active species have to be extracted, where as in solvent sublation processes emulsion formation is negligible owing to the absence of phase mixing processes.

d) On the basis of similar arguments, the extent of recovery in extraction processes is dependent on the organic to aqueous phase volume ratio whereas solvent sublation is independent of this ratio.

5.1. Theory and Mechanism

Sebba was the first person who explained the mechanism of the solvent sublation. The sublate moved across the water-organic solvent interface in one direction only, namely into the organic phase with the rising bubbles. But the mechanism is very simple, the factor is not considered that sublate moved downwards across the liquid-liquid interface should be rapidly carried into the bulk of aqueous because of the marked turbulence. The sublate moved in both directions across the liquid-liquid interface. Greater upward movements of sublate occurred because the flow of bubbles is in this direction. The bubbles arriving the organic-aqueous interface are small and have too little kinetic energy to overcome the interfacial tension; coalescence must occur before the bubbles transfer across the interface. Since the surface of the bubbles possess a zeta-potential. The volume below the interface, therefore, contains many stationary bubbles, and the liquid trapped between them is effectively protected from the turbulence of the solution below. Some of the liquid entrained in this interfacial region will be dragged into the organic phase by the larger bubbles moving upward. Collector and colligend contained in this water and adsorbed on the bubbles readily dissolve in the organic phase. When the bubbles finally burst into the atmosphere the water surrounding each of them form globules, which then returns across the liquid-liquid interface. Because of small size of these droplets, a larger area of organic aqueous interface is exposed, and it is likely that a liquid liquid equilibrium is established between the two phases. Since the total volume of water in the organic phase is very small, the amount of sublate carried back into the aqueous solution is minimal.

Herein lies an essential difference between solvent extraction and solvent sublation. In the former process the organic phase contents are in equilibrium with the bulk of the aqueous solution, while in solvent sublation, equilibrium exists with only the small amount of water that is entrained. Because of the protection afforded by the stationary bubbles at the interface, the small amount of water that moves down from the organic layer is not carried into the bulk of the aqueous solution. Rather it is transported back to the organic phase in the form of layers around other bubbles and carries with it a small amount of sublate. A steady state is ultimately attained in which the amount of sublate traveling into the organic layer was equal to that carried back across the interface by the returning droplets.

There are mainly three mass transport mechanism in solvent sublation. They are as following.

a) Transport by air bubbles.

b) Entrainment of water droplets along with the rising air bubbles into the solventlayer at top.

c) Molecular mass transfer across the solvent-water interface.

5.2. Selection of Solvent

A wide variety of immiscible organic layers are potentially possible in solvent sublation, however certain consideration must be kept in mind. First the solvent must dissolve or at least wet the sublate. Polar solvents are therefore to be desired from this point of view. On the other hand, if the solvent is too polar, it may be too soluble in water. Consequently, a compromise must be drawn in terms of polarity. A second consideration is solvent volatility. As gas is being passed continuously through the organic phase, it is important that the solvent have low volatility, lest significant evaporation occur. Finally, the viscosity of the organic solvent should be low.

5.3. Factors Affecting Solvent Sublation

Several factors influence the process: some affect the solution and others are operational factors. Some of the first group are of the operational parameters include gas flow-rate, temperature.

i) Factors affecting the solution:

a) Surfactant Concentration

b) pH

c) Ionic strength.

d) Ethanol.

e) Organic solvent.

ii) Operational parameters include:

a) Gas flow rate

b) Temperature

6. APPLICATION OF ABSM

a. separation of heavy metals like uranium from aqueous solution
b. recovery and separation of ore
c. Waste water purification by removing toxic inorganic like mercury, lead etc.
d. removal of oxyions from solution
e. foam fractionation for separation of enzymes, proteins of biological materials
f. recovery of protein and microorganism from cultivation medium
g. surfactant removal
h. removal of active radio isotopes
i. separation of fatty acids
j. separation of organic dyes
k. separation of protein from beet root juice and potato juice
l. enrichment of plant proteins
m. foam fractionation in protein skimming
n. foam fractionation of bile acids.
o. separation of drug components or purification of drugs from a mixture of components
p. separation of enantiomeric drugs mixture
q. separation of chemical constituents from plant source, e.g. salts of alkaloidmixture, separation from soap, and enrichment of active components by foamfractionation method
r. enrichment of plant proteins with adsorptive foam separation method
s. foam fractionation of fruit juice enzymes for example, bromelin from pineapple
t. removal of drug components from waste water.

7. ACKNOWLEDGEMENT

Dairy council of California (2004)[1] published a monograph on whey, where they discussed mainly about the major available proteins in whey and their distribution andalso indicated about the different varieties of available whey proteins mainly concentrates, isolates and hydrolysates. They also studied about the lipid content, mineral content, lactose content and different properties of whey proteins such assolubility and many other parameters.

Casper J.L., Wendorff W.L., Thomas D.L. Functional Properties ofWhey Protein from Caprine and Ovine Specialty Cheese Whey Concentrates. Journal of Dairy Science 1999; 82(2): 265-271[2]prepared whey protein concentrates from twocaprine and one ovine specialty cheese whey by ultra filtration, diafiltration and freeze-drying processes. They compared the composition and

functional properties(foam overrun, foam stability, gelation, and emulsifying capability). Ovine whey protein concentrate showed significantly better foam overrun, foam stability, and gel strength than did bovine and caprine whey protein concentrates, and both caprine whey protein concentrates showed better gel strength than did bovine whey protein concentrate. Caprine whey protein concentrate, produced from rennet whey, showed significantly better emulsifying capability at low pH than did both bovine and ovine whey protein concentrates. Caprine whey protein concentrate, produced from direct acidified whey, had less emulsification capability than did bovine whey protein concentrate produced from rennet whey.

Krastanka G., Marinova, Elka S.,BashevaA, BorianaNenova, Temelska M, Campbell B., Ivan B. Ivanov, Physico-chemical factors controlling the foam ability and foam stability of milk proteins: Sodium caseinate and whey protein concentrates. Food Hydrocolloids 2009, 1864–1876[3]investigated and explored the foaming behavior of the two main types of milk proteins: flexible caseins andglobular whey proteins. Direct foam comparison was complemented with measurements in model experiments such as thin foam films, dynamic surface tension, and protein adsorption. Foaming was studied as a function of pH (from below to above isoelectric point, pI) and range of ionic strengths. Maximum foam ability was observed near pI 4.2 for WPC in contrast to sodium caseinate which had minimum foaming near pI 4.6. Good foam ability behavior correlated well with an increased adsorption, faster dynamic surface tension decrease and increased film lifetime. Differences in the stability of the foam sand foam were explained with the different molecular structure and different aggregation behavior of the two protein types. Far from its isoelectric pI, casein adsorption layers are denser and thicker thus ensuring better stabilization. Added electrolyte increased further the adsorption and the repulsion between the surfaces (probably by steric and/or osmotic mechanism). In contrast the globular molecules of WPC probably could not compact well to ensure the necessary films and foams stabilization far from pI, even after electrolyte addition.

Geoffrey W. Krissansen, PhD Emerging Health Properties of Whey Proteins and Their Clinical implications, Journal of the American College of Nutrition, 2007; 26(6):713-723[4]Bovine milk-derived products, in particular wheyproteins, exhibit beneficial properties for human health, including the acquired immune response. However, their effects on innate immunity have received little attention. Neutrophils are key cells of innate defenses through their primary functionsof chemotaxis, phagocytosis, oxidative burst, and degranulation. A whey protein extract (WPE) purified from bovine lactoserum was evaluated for its direct and indirect effects on these primary functions of normal human blood neutrophils invitro.

Yoshiyuki Okamoto and Eng J. Chou, Foam Separation Processes in Philip A.(ed.), 'Schweitzer, Handbook of separation techniques for Chemical Engineers',McGraw Hill York,1979[7]discussed and described the several factors influencing foamseparation.

Samita Bhattacharjee, R. Kumar, K. S. Gandhi Journal of Chemical Engineering and Processing, 2001; 56(19): 5499-5510[8] worked on modeling of protein mixture separation in a batch foam column. In that work separation of proteins from their binary mixture has been investigated using static foams.

Yoshihiro Suzuki, Toshiroh Maruyama, Journal of Water Research, 200236: 2195-2204[9] worked on the process by using several kinds of surface-active proteins as a chemical agent that combined collector with further, removal of suspended substances by coagulation and foam separation with dispersed air was examined .Milk casein showed the great capability of suspension removal, and coagulating flocs formed by clay particles and iron hydroxide were almost perfectly recovered in foam generated from the liquid, even in the case of fresh water and seawater suspension at neutral pH. In contrast the removal efficiency was extremely low using sodium dodecyl sulphate (SDS). Foam separation experiment with out coagulation was carried out. Two cases of fresh water and sea water suspension were examined. A group of proteins: Eel mucus, casein, albumin, hemoglobin, gelatin, and soy protein and a group of surfactants: SDS, oleate, LAS were used. In sea water, the foaming capacity of proteins, SDS, LAS was increased and a sufficient amount offoam was generated at the lower doses.

ThompsonL. , Enrichment of Biologically Active Compounds form selected Plants Using Adsorptive Bubble Separation, Separation Science And Technology**,** 2004; 29(3): 1015-1028[10]worked on enrichment of biologically active compounds from selected plants using adsorptive bubble separation**.** Foam fractionation is a separation method suitable for the enrichment of active principles contained in plant materials. The goodness of the method was proven by the application of the theoretical foundations of foam fractionation, to enrich active principles contained in those plants.

Marlene. B., Peritian. E.,Gunher. L., Mehmet. C., Harun. P. Enrichment of glycoalkaloids alpha-solanine and alpha chaconine from potato juice byabsorptive bubble separation method using a pH gradient Journal of Separation science, 2004**;** 27(12):1042-1044 work on Enrichment of the glycol alkaloids alpha solanine and alpha-chaconine from potato juice by adsorptive bubble separation using a pH gradient. For the first time, Adsorptive Bubble Separation (ABS) could quantitatively enrich the solanidine alkaloids alpha-solanine and alpha-chaconine from potato juice with a pH gradient. The enrichment into the foam was influenced by the pH value, bubble size, and gas flow rate. The efficiency was highest on using diluted samples with a concentration between 2

and 6 mg/L of the alkaloids at pH. The experiments with a standard solution of each alkaloid confirmed that these substances could be quantitatively enriched into the 'spumat' without surface-active potato proteins. The transfer under these conditions was similar to that from theaqueous potato extract.

Schugrel K, Recovery of proteins and microorganisms from cultivation media by foam floatation method Advanced in Biochemical Engineering/ Biotechnology, 2000; 68: 191-233[12]had studied the recovery of proteins and microorganisms fromcultivation media by foam floatation method. He also presented a clear description. Offoaminess of proteins and microbial cell cultivation system in his paper. He investigated the recovery of Bovine serum Albumin (BSA), a globular protein from aqueous solution by foam flotation. The foaming device used consists of athermostated column with 23mm internal diameter, 49cm bubbling liquid height and30 cm foam layer.

Ziad. S. S, Hussain. Md. M., The separation of proteins from multicomponent mixtures by a semi-batch foaming process Chemical Engineering and processing, 2001; 40: 371-378[13] studied the separation of proteins from multi-component mixtures by a semi-batch foaming process. Their experience was conducted to obtained the values of the average bubble size, gas holdup, interfacial area, the bulk phase concentration and the heat desorption (which) determines theconcentration of adsorbed components) in the liquid solution using a 75mm diameter,530mm long glass column fitted with stainless steel sparger for bubble generation. Here the mass transfer coefficient was determined from the analysis of the concentration of foam and feed at times using the above mention parameters.

Suzuki. A., Yasuhara. K., Selective Foam Separation of Binary Protein Solution by SDS complexion Method., Journal of Colloid And Interface Science, 2002; 253: 402-408[14] worked on selective foam separation of binary protein solution by SDS complexation method. They conducted a fundamental investigation about the selective foam separation of protein mixture. They used a solution containing two proteins, ovalbumin (OA) and lysozyme (LZ), and an anionic surfactant, sodium doedecylsulphate (SDS) protein molecules are positively chargedwhen the solutions pH is below isoelectric point. Sodium dodecyl sulphate (SDS) ananionic surfactant is always negatively charged in usual PH range.They observed thata proper addition of SDS greatly improved the selective recovery of LZ to OA.

Liping, Prokop. A., and Robert, D.T., Effect of pH On the Startup of A continuous Foam Fractionation Process Containing Ovalumin, Separation Science And Technology, 2003;38(5): 1093-1109[15] investigated the effect of pH on the bubble size distribution, void fraction and enrichment ratio of a continuous foam fractionation column containing ovalbumin. The bubble size & void fraction were measured using a photoelectric capillary probe for different solution pH (3.5, 4.5, 6.5 and 9.7). The bubble diameters for pH 3.5 and 4.5 were the largest of the four pH studied. At these

twopH, the foam was less stable & formed aggregates, leading to lower enrichment &mass recovery. For nearly neutral & basic pH (6.5, 9.7), the bubble size was smaller& the foam was more stable resulting high enrichment & mass recovery. In the lower foam phase, the calculated specific area increased as the pH increased from 3.5 to 9.7which may partially contribute to the higher enrichments of pH 6.5 & 9.7. During experiment, the fresh feed solution was fed to the column at two different flow rates(24 and 45 cm3/min). The liquid pool height was kept constant of 28 cm with the bulkliquid output near the bottom of the column.

Darton. R. C., Supino. S., Sweeting. K. J., the development of a multistage foam fractionation column, Journal of Chemical Engineering and processing, 2003;43: 477-483[16] worked on the development of a multistage foam fractionation column. Here, surface-active materials stabilize foam by adsorption at the gas/liquid interface. In foam fractionation, the foam is condensed to give a 'foamate', liquid rich in surfactant. They had developed equipment and a process able to supply a number of stages of separation, working with an inert stripping gas. They had tested that with aqueous surfactant, which extracted and concentrated on organic solute. The measured liquid compositions were in good agreement with a model, which described the equilibrium using adsorption isotherm, which makes a mass balance for each stage in the column.

Ekici. P., Backleh-sohrt. M., Parlar. H., High effiency enrichment of total and single whey proteins by pH controlled foam fractionation, International Journal of Food Science and Nutrition, 2005; 56(3): 223-229[17]worked on high effiency enrichment of total and single whey proteins by pH controlled foam fractionation**.** The feasibility of foam fractionation, as an alternative to other more commonly used methods, to effectively separate whey protein concentrate and single fraction has been studied. The investigation focused on the effects of different process parameters such as the pH value, the initial protein concentration as a surfactant. Whey proteins could be almost completely enriched into the foam fraction at pH values between 2and 3; but some claimed that % recovery and enrichment were maximum atisoelectric point only traces of them remained in the residual whey solution. Albuminbovine, b- lactoglobulin and a- lactalbumin from whey solutions in the presence of sodium dodecyl sulfate could be transferred into the foam fraction with enrichment ratios of up to 30 and with % recovery between 64.5% and 99.8%. The results demonstrate that enriching whey proteins using foam fractionation can be quantitative and effective according to process parameters.

Burapatuna. V., Tannev. R., comparison of the activity reduction occurring in two detergent-assisted protein (Cellulase and Lysozyme) foam fractionation process, Separation Science and Technology, 2005; 40: 2445-2461[18] worked on comparison of the activity reduction occurring in two detergent-assisted protein (Cellulase and Lysozyme) foam fractionation process. Foam fractionation has the potential to be an inexpensive alternative to current separation methods; however, it has a

few drawbacks. One is the fact that not all proteins form a foam layer when aerated at low concentrations. The other is the possible protein denaturation caused during the foaming process. Addinga detergent to the non-foaming protein solution causes it to foam when aerated. Here, cellulase and lysozyme were studied as model proteins in this process. By them selves, both cellulase and lysozyme solutions hardly form a foam layer when aerated atconcentrations below 1000 mg/L (1000 ppm). The addition of 100 mg/L of cetyltrimethyl ammonium bromide (CTAB) to a 200 mg/L cellulase solution increasesthe foam volume and makes it possible to almost quadruple (relative to the initial bulk concentration) the concentration of the resulting cellulase foam solution. The foaming, however, reduces the cellulase activity. Diluting the foam with ß-cyclodextrinregains some of the lost activity because ß-cyclodextrin strips CTABaway from the cellulase, which allows the cellulase to refold to its native state. CTABdetergent does not work well with lysozyme, but the addition of SDS detergent leadsto a tripling of the concentration of lysozyme solution without any reduction inenzymatic activity.

Zaid.S. Stanley. R., and Nigam. M., Extraction of Polyphenolics from Apple Juice by Foam Fractionation**,** International Journal of FoodEngineering, 2006; 2(2): 2[19]investigated the extraction of polyphenolics from Apple Juice by Foam Fractionation for use as functional food ingredients. The separation performance like enrichment ratio, selectivity & percentage recovery, was determinedas a function of operating variables, namely N2-flow rate, initial feed concentration, bubble size, solution pH and the presence of alcohol to modify the surface tension. Measurements were made of the average bubble size and gas hold up volume tocalculate interfacial area. The bulk phase concentrations of the polyphenolics in the feed & foam fractions were analysed for total phenolic content by Folin assay and phenolic composition of the Polyphenolics in the feed & foam fractions were analysed for total phenolic content by Folin assay and phenolic composition by reverse phase HPLC. Enrichment factors of upto 6 were found under optimum conditions of low sugar concentration, low flow rate (0.2-0.6 cm3 /min) and acidic pH (3-4).Recoveries were low at around 30% of total phenolics and selectivity was poor.

Ko. S., Cherry. J., and Prokop. A., Effect of Natural Contaminant on Foam Fractionation of Bromelin, Applied Biochemistry and Biotechnology, 2007;91-93:405-411[20]studies the effect of a natural contaminant on foam fractionation of bromelin. They found a dilute bromelin solution with a pH (2.0 – 7.0)foams very well when bubbles were introduced into a foam fractionation column. It was observed that the dilute enzyme solution only foamed between pH – 2.0-3.0 when inner wall of the fractionation column was coated with a natural contaminant. They studied the separation ratio and the protein mass recovery to explore the effect of anaturalbifoaming agent on the foam

fractionation of a dilute bromelin solution. The control variables used in this process were initial bulk solution pH, which rangedfrom 2.0 – 7.0 and superficial air velocity (1.7 – 6.2) cm/sec.).

Stevenson. P., and Jameson. G.J., Modelling Continuous Foam Fractionation with Reflux, Chemical Engineering and Processing, 2007; 46(12): 1286-1291[21]developed an equilibrium stage approach to modeling the performance of a continuous foam fractionation column with reflux. Such anapproach had been facilitated by recent developments in the understanding of pneumatic columns of foam. It was shown that the recovery of surfactant into the product stream increases monotonically with increasing reflux ratio but this is at the expense of reduced product rate.

Qu. Y.H., Zeng. G.M., Huang J.H., Xu. Ke, Fang. Y.Y., Li Xue,LiuL.H., Recovery of Surfactant SDS and Cd2+ From permeate In MEUFusing continuous Foam Fractionator, Journal of Hazardous materials, 2009; 155(1-2): 32-38[22]investigated on solvent sublation of l- lysineand found out that it can be used to separate the low concentration l- lysine from theaqueous solution by using dodecylbenzene sulfuric acid (DBSA) as the surfactant, di-(2-ethylhexyl) phosphoric acid (D2EHPA) as the extractant, *n*-heptane as theextraction solvent. Especially the effects of initial pH, D2EHPA concentration in theorganic phase, DBSA and NaCl concentration in the aqueous phase and air flow rate on the separation efficiency were investigated. The results showed that the optimum conditions of initial pH, D2EHPA concentration in the organic phase, DBSA and NaCl. Concentration in the aqueous phase and air flow rate were 0.2 gmol/l and 250ml/min, respectively, under room temperature and the enrichment ratio and recovery ratio were 13.26 and 79.57%, respectively. The separation efficiency of solvent sublation was higher than that of the traditional foam floatation and solvent extraction. Furthermore, the separation mechanism of solvent sublation was also discussed.

Zhaoliang Wu, Bo Liang, Bin Hu, Huijie Zheng, Separation of l- lysine bysolvent sublation**,** Separation and Purification Technology, 2009; 66: 237–241[22] investigated and found out that as a soft separation technology with bubble mass transfer, solvent sublation can replace solvent extraction because of many advantages. In this study, solvent sublation was used to separate and purify Penicillin G from fermentation broth. The effects of pH of the solution, NaCl concentration, concentration of butyl acetate(BA) in organic phase, nitrogen flow rate and sublation time on the separation efficiency of Penicillin G were investigated in details, and the optimal conditions for solvent sublation were obtained. The flotation product, which was separated and purified from the fermentation brothunder optimal conditions, was quantitatively analyzed by HPLC. Compared with traditional solvent extraction, solvent sublation can not only increase the separation efficiency, but also efficiently reduce the BA emulsification.

P.Y. Bi, H.R. Dong, Q.Z. Guo, Separation and Purification of Penicillin G from fermentation broth by solvent sublation, Separation and Purification Technology, 2009; 65; 228–231[24]combining solvent sublation with complexation extraction, flotation complexation extraction (FCE) is proposed for the first time. Thisnew technique was used to separate and purify l-phenylalanine from fermentation liquid with good results. The effects of extraction solvent, pH of the solution, NaCl concentration, concentration of di-(2-ethylhexyl) phosphoric acid (D2EHPA) inorganic phase, nitrogen flow rate and flotation time on the separation efficiency of phenyl alanine were investigated in detail, and the optimal conditions for FCE were obtained. The flotation product from the fermentation liquid under optimal conditions, after back-extraction and re-crystallization, was characterized by FTIR and HPLC,and its purity was more than 98%. Furthermore, the mechanism of FCE was also primarily discussed.

8. CONCLUSION

'Foam fractionation' under adsorptive bubble separation method is a well-known operation. Nutritional values of whey proteins are now recognized worldwide. At present, the whey proteins are available at high cost because their total cost of recovery is extremely high. So, whey produced as one of the by-products in confectionery units (small units) is unutilized because of the high cost of existing method of treatment and the whey is lost as an effluent resulting higher BOD in aquatic system. Foam fractionation method is adopted in the present study to establish its feasibility in the recovery of proteins at low cost. There is no report that Foam fractionation method is used in downstream processing like other methods for the recovery of biomolecules. There are several criteria like recovery time, purification factor, enrichment factor, operation expenditure, investment costs, running costs and environmental pollution which are to be considered before selecting a recovery method. Operation expenditure, investment costs, running costs and environmental pollution are higher in precipitation and chromatographic techniques. These are significantly lower in foam fractionation technique. But it is restricted to lower concentrated feed.

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