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Extraction of Cheese Whey Protein from Dairy Effluent by Using Polyethylene Glycol and Sodium Sulphate

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ABSTRACT: Whey is a byproduct which contains nutritional value added proteins like, α-Lactalbumin, α-Lacto globulin. Although several possibilities for cheese-whey exploitation have been assayed, approximately half of world cheese-whey production is not treated, but is discarded as effluent. It is necessary to develop an effective and environmentally benign aqueous two phase system (ATPS) for the recovery of value added cheese whey proteins. Recently ATPS have drawn more interest among the other separation processes, particularly in the field of biotechnology due to the mild conditions of the process, short processing time, and ease of scale up. In order to design a two stage ATPS process the effect of system parameters such as pH, types and the concentrations of the phase forming components , temperature, etc., on partitioning of proteins were addressed a view to maximizing recovery. In the present study, partitioning of whey proteins from dairy effluent by using polyethylene glycol and sodium sulphate was done. The results showed that PEG-4000 at 50% concentration and salt at 40% concentration under conditions of pH 5.4 and temperature 35ºC will give maximum yield and partition coefficient.

KEYWORDS: Cheese whey, aqueous biphasic system, Extraction, dairy effluent, Protein recovery.

I. INTRODUCTION

Whey protein is a mixture of globular proteins isolated from whey, the liquid material created as a by-product of cheese production. Some studies have suggested that whey protein may possess anti-inflammatory or anti-cancer propert. The effects of whey protein on human health are of great interest and are currently being investigated as a way of reducing disease risk, as well as a possible supplementary treatment for several diseases such as heart disease, cancer and diabetes. Whey protein is typically a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%), which are soluble in their native forms, independent of pH. Whey is an abundant source of branched-chain amino acids (BCAAs), which are used to fuel working muscles and stimulate protein synthesis. In particular, leucine plays a key role in initiating the transcription of protein synthesis. When leucine is ingested in high amounts, such as with whey protein supplementation, there is greater stimulation of protein synthesis, which may speed recovery and adaptation to stress (exercise). Another studies suggested that large amounts of whey protein can increase cellular glutathione levels.

Whey proteins were usually obtained by a combination of membrane concentration and chromatographic methods (i.e. ionic exchange, affinity and radial flow chromatography). Although these techniques are very effetive in partitiong the proteins they have some disadvantages i.e, they may induce the denaturation of proteins. Partitioning in aqueous two phase systems (ATPS) is a good alternative method to be employed as a first step purification. Many studies have been carried out using this technique for the separation of various products. Aqueous two phase system (ATPS) is an alternative method for separation of biomolecules which reduces number of steps and thus reduces the overall cost. It is a liquid – liquid extraction method which makes use of two aqueous phases. The two aqueous phases consists of two

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wa-ter-soluble polymers or a polymer and a salt. It is a potential technique that has applications in the field of biotechnology for the separation and purification of biological materials such as proteins, enzymes, nucleic acids, virus, antibodies and cell organelles. The simple process and low cost of phase forming materials allow this method for large-scale purification also. ATPS is used for the extraction of biomolecules due to their high water content which will help to prevent the denaturation of these biomeolecules. [1]

Polyethylene glycol (PEG) is used as one of the phase forming polymers in ATPS because it is available at low cost and forms a two-phase system with other neutral polymers as well as salts. Also PEG has the ability to enhance the refolding of proteins. Because of the high cost and high viscosity of the polymer/polymer system, the aqueous two phase polymer/salt systems are preferred over the polymer/polymer systems. Most commonly used salts were phosphates, sulphates, carbonate and citrate. Different studies shown that various factors will affect the partitioning of proteins into two phases such as such as the concentration of phase forming components, molecular weight of PEG, type of salt used for phase formation, pH and temperature of the system.

In the present study we have evaluated the effect of various salts, concentration of PEG and salts, temperature and pH conditions for the effective partioning and yield of proteins.

II. MATERIALS AND METHODS

A. Materials

Analytical grade (Merck) polyethylene glycol with a molar mass average of 4000 and 6000, sodium sulphate, sodium carbonate and sodium citrate were used. Bovin serum albumin (BSA),alkaline copper reagent, folin's ciocalteous reagent and cheese whey. Cheese whey is prepared by heating milk to 80 °C and appropriate amount of lime water is added (500 ml of milk require 20 ml of lime water). The prepared cheese whey is kept in refrigerator for storage.

B. Aqueous two phase system preparation

Prepare appropriate weight percentage of polyethylene solution(50%,40%), potassium phosphate solution, sodium sulphate solution and sodium chloride solution(30%,35%,40%). Aqueous two phase systems were prepared by mixing suitable amounts of PEG and given salts. Then centrifuge all the tubes at 15000 rpm for 10 minutes for separation in to two phases. The protein concentration in the individual phases was determined by the method of folin's lowry method. Calculate the partition coefficient and yield by using following equations. [2]

Partition coefficient, K= C(top)/C(bottom)

C (top) - concentration of protein in the top phase.

C (bottom) - concentration of protein in the bottom phase.

Yield,

$$Y_T = V_T C_{AT} / V_0 C_0$$

V_T- Top phase volume.

C_{AT}- Concentration of protein in the top phase.

V_0 -Total volume.

C_0 -Total concentration.

C. Estimation of protein by folin's lowry method

Graded volume of standard bovine serum albumin was pipette out in to a series of test tubes and then 0.4ml of top and bottom phase was pipette out into test tubes and making up to 1ml using distilled water. A blank was simultaneously prepared by taking 1ml of distilled water. 5ml of alkaline copper reagent was added to all tubes mixed well and incubate at room temperature for 30 minutes. Then add 0.5ml of folin's ciocalteous reagent and incubate for 15 minutes. Blue colour developed was read at 620nm.

A graph was plotted with concentration of BSA along X-axis and optical density on Y-axis. Then concentration of protein in the top and bottom phase was calculated from the graph.

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III. RESULT AND DISCUSSION

In the present study of, partition of cheese whey using different salts, sodium sulphate, sodium carbonate, and sodium citrate and 50% PEG based aqueous two phase system. The system was studied at different conditions. The effect of different salts, salt concentration, polymer concentration, molecular weight of PEG, pH, and temperature on partition coefficient and yield was studied.

A. Effect of different salts on yield and partition coefficient

In this study 3 salts such as sodium sulphate, sodium carbonate and sodium citrate(40%) and 50% polyethylene glycol were used for phase separation studies. The yield and partition coefficient was found out using the above discussed equations. It has been found that sodium sulphate give maximum yield and partition coefficient as compared to other salts. Fig 1 and 2 shows that sodium sulphate gives maximum yield and partition coefficient respectively.

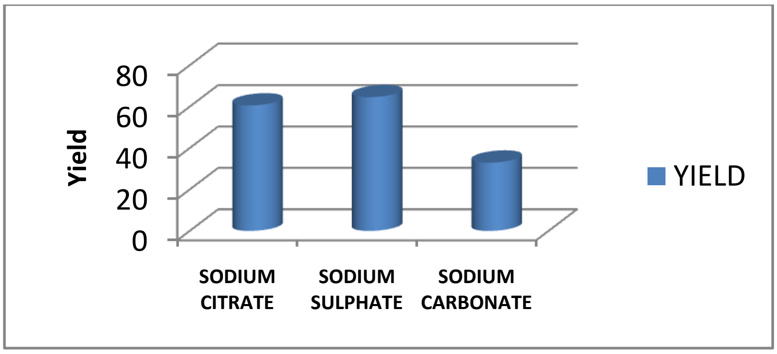

Fig 1: Effect of different salts on yield.

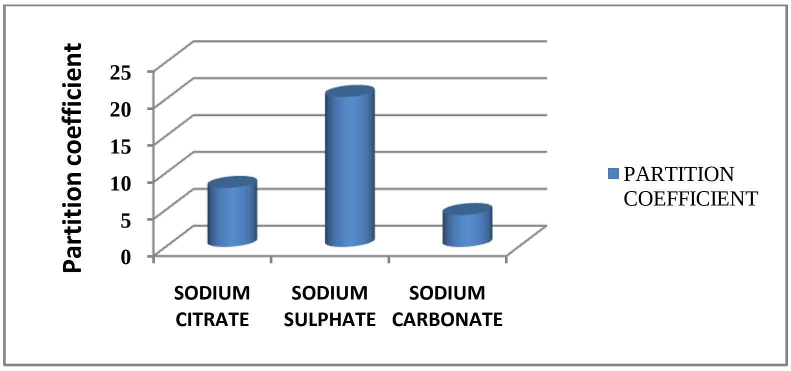

Fig 2: Effect of different salts on partition coefficient.

B. Effect of salts on yield and partition coefficient

In this work the effect of different salt concentration on yield and partition coefficient was studied. For this mainly two salt concentrations were selected say 30% and 40% and 50% PEG. The system was prepared for 3 salts, sodium sulphate, sodium carbonate and sodium citrate. From this study it has been shown that sodium sulphate at 40% concentration give maximum yield and partition coefficient. If salt concentration is high, the ionic strength increases in the bottom phase which improves biomolecule partition to the top phase due to this reason the yield and partition coefficient found to be increased at high salt concentrations. Fig 3 and 4 shows that sodium sulpahte at 40% concentration gives maximum yield and partition coefficient as compared to other salts.

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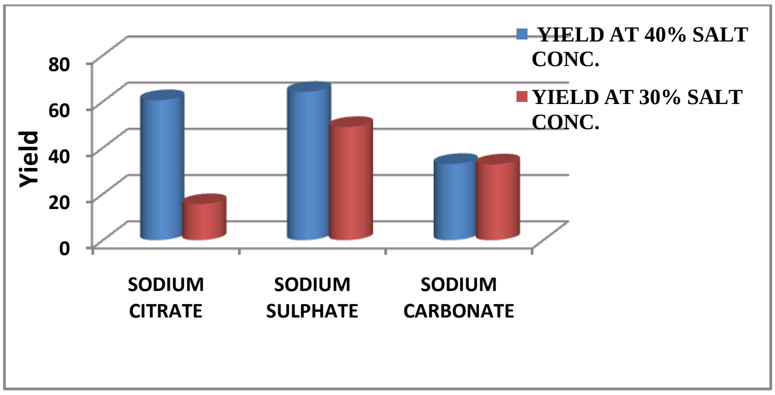

Fig 3: Effect of salt concentration on yield

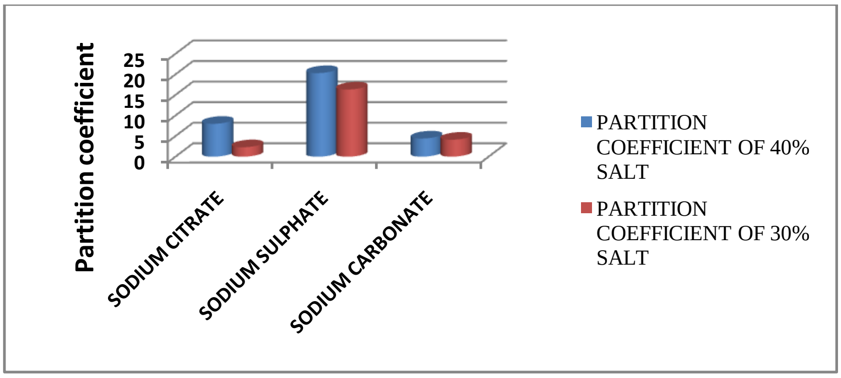

Fig 4: Effect of salt concentration on partition coefficient

C. Effect of polymer concentration on yield and partition coefficient

In the present work effect of different concentrations of polyethylene glycol on yield and partition coefficient has studied. The system was prepared for varying concentrations of polyethylene glycol say 30%, 40% and 50%. From this it has been found that PEG at 50% gives maximum yield and partition coefficient which is shown in the fig 5 and 6. An increase in polymer concentrations relates to high density, refractive index and viscosity of the phase. Thus high concentration of polymer provides large difference in properties between the phases. In case of polymer-salt system, lower concentration of salt is required for ATPSs preparation when using the higher the concentration of polymer. The role of molecular weight also concerns with concentration used in phase forming. The higher the molecular weight of the polymer, the lower the concentration required for phase separation. The viscosity of the phase is affected by the molecular weight of polymer. Since the viscosity of a polymer solution mainly depends on the concentration. High viscosity might impact further process. The viscosity of one phase might be decreased by employing a higher molecular weight of the polymer. The interfacial tension between the two phases of polymers system is very small in comparison to the interfacial tension between an aqueous phase and an organic solvent phase. The interfacial tension is dependent on the polymer and salt composition. [2]

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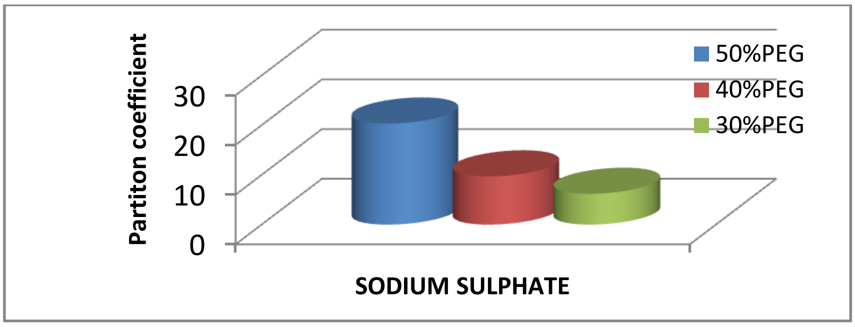

Fig 5: Effect of polymer concentration on partition coefficient

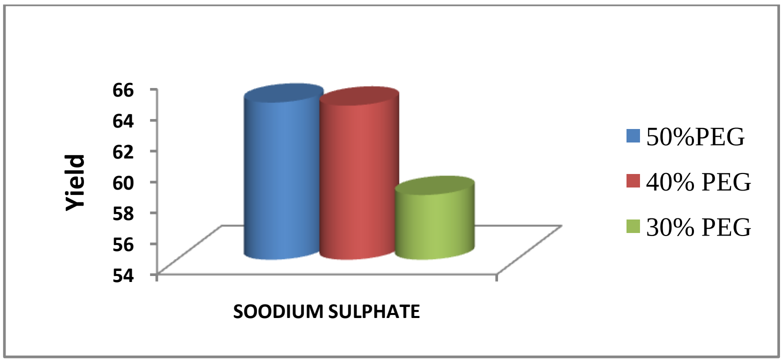

Fig 6: Effect of polymer concentration on yield

D. Effect of polymer molecular mass on yield and partition coefficient

For studying the effect of different molecular mass of polymer in yield and partition coefficient polymer of molecular mass 4000 and 6000 were selected. The systems were prepared using 50% PEG-4000 and 40% sodium sulphate, and 50% PEG -6000 and 40% sodium sulphate. From this study it has been found that PEG-4000 gave more yield and partition coefficient as compared to PEG-6000. Graphical representation of the results are shown the fig 7 and 8. Increasing the molecular weight of the phase polymers resulting in the distribution of biomolecule towards more strongly into the other phase as the repulsive interactions between the polymer and biomolecule become stronger. When the same molecules are added into phase system with different molecular weight of polymer, theirs partition coefficient decrease as molecular weight increase. The reason of this phenomenon is that an increase in molecular weight of polymer results in an increase in the chain length of the polymer and the exclusion effect, which lead to the reduction in the free volume. Thus, polymer acquire a more compact conformation with intramolecular hydrophobic bonds and hindered the partition of biomolecule into the top phase. [2]

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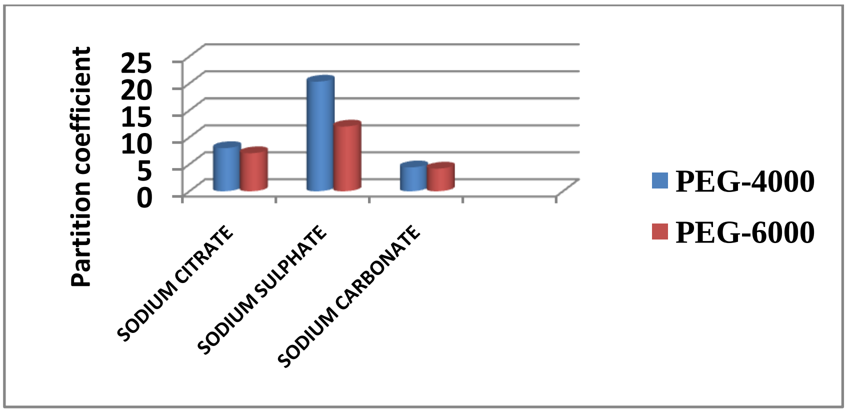

Fig 7: Effect of polymer molecular mass on partition coefficient

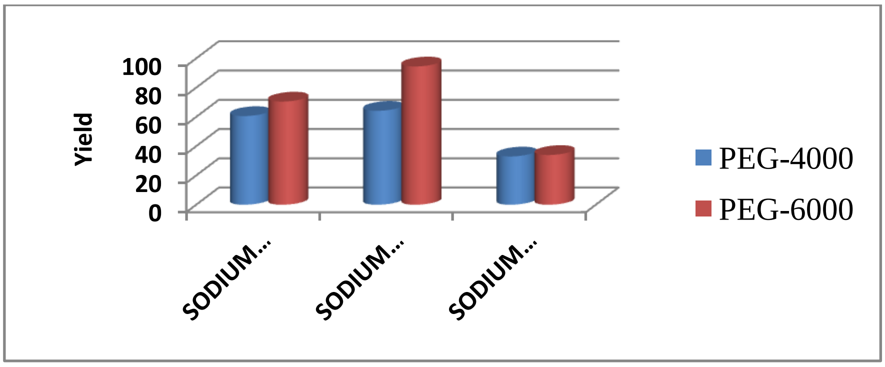

Fig 8: **Effect of polymer molecular mass on yield**

E. Effect of pH on yield and partition coefficient

Effect of different pH on the yield and partition coefficient was studied in this work. The system was set up at different pH say 2.56, 4.3, 4.4, 5.4, 6.2 and 7.2. From the study it has been found that the yield and partition coefficient is high at pH 5.4 and after this pH it was found to be decreased which is clear from the fig 9 and 10. Since the isoelectric point of whey proteins are in the range of 4.6-4.9 above this pH proteins will get a negative charge and all the proteins will migrate towards the positively charged top phase. At high pH the partition coefficient and yield were found to be decreased due to the salting out effect. [3]

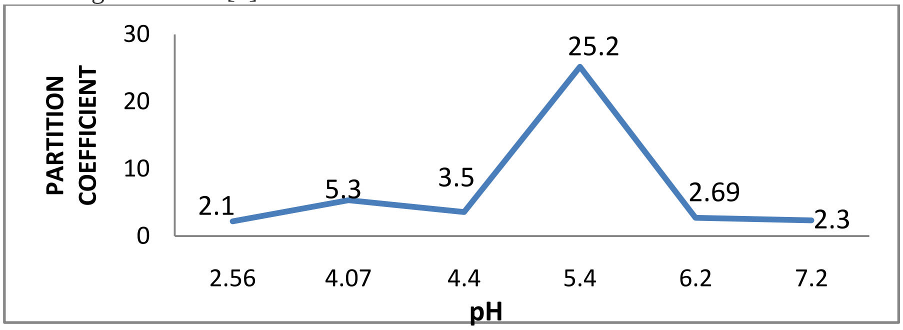

Fig 9: Effect of pH on partition coefficient

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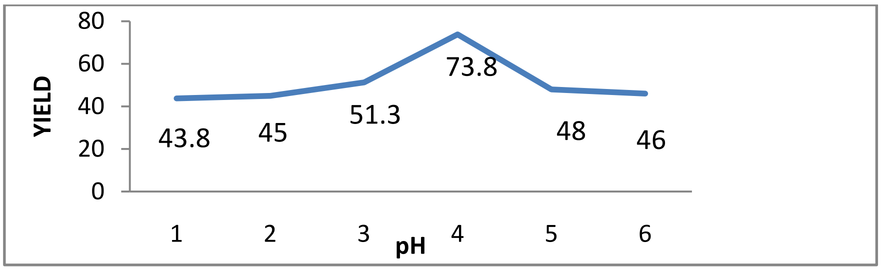

Fig 10: Effect of pH on yield

F. Effect of temperature on yield and partition coefficient

In this study the effect of temperature on yield and partition coefficient was done at different temperatures. The system was done at different temperatures. From the fig 11and 12 it has been found that at 35°C the yield and partition coefficient was maximum. And at high temperatures it found to be decreased. An increase in temperature results in increased differences in the phase composition. It enhances the concentration of PEG and salt in the top and bottom phase respectively. Consequently, the number of water molecules available for solute salvation in the bottom phase decrease due to an increase in salt concentration. This also reduces the solubility of biomolecules in the phase. The partition coefficient of the biomolecules probably influences by this variation in the phase compositions.

Furthermore, increasing temperature can destroy the bonds of biomolecule. As these bonds are weakened and broken, the biomolecule becomes more flexible structure. Water in two phase systems can interact and form new hydrogen bonds with the functional group of the biomolecules. The presence of water further weakens nearby hydrogen bonds by causing an increase in the effective dielectric constant near them. As the structure is broken, hydrophobic groups are exposed to the solution. As a consequent, losses in solubility of molecule are observed. [2]

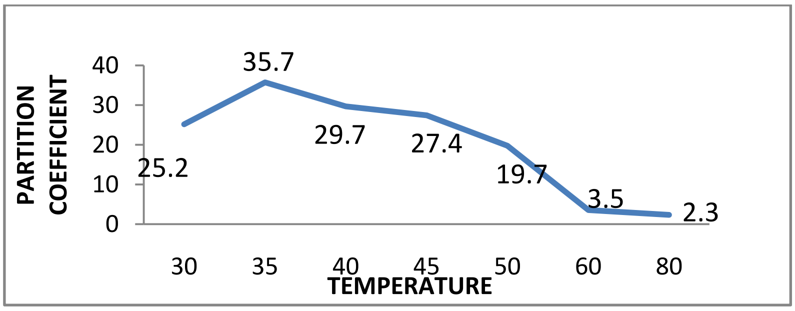

Fig 11: Effect of temperature on partition coefficient

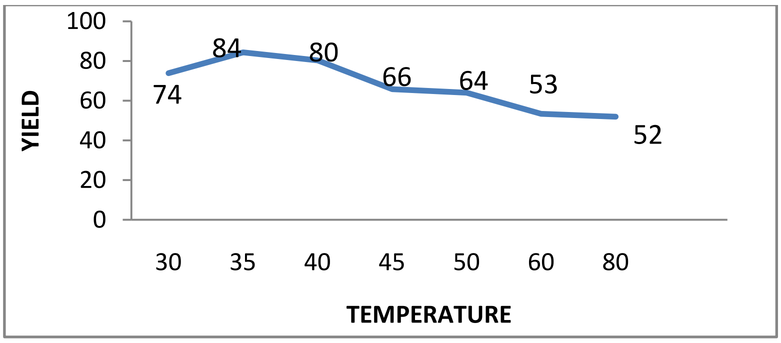

Fig 12: Effect of temperature on partition coefficient

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IV. CONCLUSION

In the present study, partitioning of cheese whey was carried out in PEG-sodium sulphate and water based aqueous two-phase systems. The influence of salt concentration, polymer concentration, polymer molecular weight, pH, and temperature was studied. The yield and partition coefficient were evaluated for each case. From this work we concluded that PEG-4000 at 50% concentration and salt at 40% concentration under conditions of pH 5.4 and temperature 35ºC will give maximum yield and partition coefficient. In future studies the results can be validated by using a software mini-tab.

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