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ULTRAFILTRATION OF CHEESE WHEY USING CHITOSAN MEMBRANE

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Abstract

Cheese whey protein recovery was improved by chitosan coagulation followed by ultrafiltration using chitosan membrane. The steady state permeate flux increased 49.1% for ultrafiltration using chitosan membrane with coagulation from 0.68 l/m^2h found during ultrafiltration without coagulation at 1.2 bar and 0.6 GPM. The corresponding decrease in protein rejection from 86.5% for ultrafiltration without coagulation to 68.8% at the first hour for ultrafiltration with coagulation. The experiment was compared for polysulfone membrane using the same condition. The percent increase in steady state permeate flux was 39.2% for polysulfone ultrafiltration with chitosan coagulation to that of without coagulation. The decrease in protein rejection was 76.9% for polysulfone ultrafiltration without coagulation to 50.0% at the first hour during ultrafiltration with coagulation.

Keywords : Cheese whey, coagulation, ultrafiltration, chitosan membrane, polysulfone membrane

Introduction

Cheese whey is a by product or effluent waste from the manufacture of dairy products where the coagulum is formed by acidification in a pH range of about 5.1 or below (Bough, *et al.* 1978). Cheese whey is a dilute liquid containing lactose, proteins, minerals and traces of fat and contains approximately 6% total solids of which 70% or more is lactose and about 0.7% whey proteins. Most whey comes from cheese making, but some of it

results from the production of casein. Separation of protein from cheese whey has been carried out in order to (a) leave behind a clear lactose fraction, (b) allow disposal (e.g., land application), and (c) recover protein by-products for foods or feeds (Zall, 1980).

Chitosan ($\beta 1 \rightarrow 4$ anhydro-D-glucosamine) (Robert, 1986) Figure 1 is an effective natural polymer coagulants on cheese whey protein recovery (Englewood and Fort Lee, 1989)

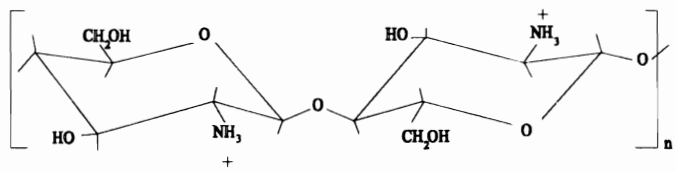


Figure 1 Chitosan structure

An earlier study on coagulation of cheese whey with chitosan demonstrated that the optimum percentage of chitosan to suspended solids was 2.0-2.5% at pH 6.0. A 90% reduction in suspended solid (SS) was achieved by this treatment. The applied dosages of the different chitosan products ranged from 50 to 150 mg/l (Bough, *et al.* 1978). Ultrafiltration (UF) can be used to separate and concentrate whey proteins (Cheryan, 1986). The permeate emerging from UF units contains lactose, minerals and low molecular weight nitrogen compounds. Generally, crossflow ultrafiltration, the feed solution under pressure flows over a supported membrane. Most water and solute molecules, smaller than the membrane pores, pass through and can be collected as permeate or ultrafiltrate. Unless there are leaks in the membrane, all larger molecules are retained in the feed solution which can be recovered as retentate (concentrate). Retentate can be recirculated back through the system so that more solvent and smaller molecules, not passed through the first time, have the opportunity to permeate on subsequent passes. Repeated recirculation further concentrates the retentate and removes more of the smaller molecules. Thus, control of the desired concentrate or separation of molecules can be achieved within physical limits of the osmotic system (Noble and Stern, 1995). Coagulation followed by ultrafiltration can achieve higher recovery or protein separation and can lead to a suitable cheese whey treatment and disposal process.

Materials and Methods

The cheese whey was collected from local dairy factory having 1008 mg/l total kjeldahl

nitrogen (TKN), 6300 mg/l protein nitrogen, 62177 mg/l total solid and pH of 4.7. Protein present in the supernatant of cheese whey were primarily in dissolved form. However, they may precipitate out after pH adjustment. Accordingly, effect of pH adjustment on protein precipitate and settlement from cheese whey supernatant was first investigated. 1 liter of supernatant cheese whey was taken into a number of 1 liter beakers and pH ion each beaker was adjusted to a specific value ranging from pH 6.0 to 10.0. Turbidity and protein content of the supernatant before and after pH adjustment were recorded for estimation of protein removal efficiency.

Investigation were conducted by jar test to estimate effect of operating parameters like pH and coagulation dosage on protein removal. Four different coagulants were used viz. chitosan, polyaluminium chloride (PAC), alum sulfate ($Al_2(SO_4)_3 \cdot 14H_2O$), and carrageenan. Separate stock solution of the four coagulants were first prepared with concentrations of 5g/l of chitosan in 1% acetic acid (Bough, *et al.* 1978), 50g/l of PAC, 50 g/l alum, and 5 g/l of carrageenan in water.

1 liter of cheese whey supernatant was taken into a number of 1 liter beakers and its pH was adjusted to 6.0. Different volumes of coagulant stock solutions were then added into these beakers to get final coagulation concentrations in the range of 20-90 mg/l for chitosan, 100-500 mg/l for PAC, 100-500 mg/l for alum, and 20-100 mg/l for carrageenan. The mixture was then stirred using flash mixing at 150 rpm for 2 minutes followed by slow mixing at 20 rpm for 15 minutes. Protein removal was then estimated by measuring turbidity and protein content before and after coagulation. First estimation of optimum coagulant dosage was obtained from maximum protein removal at pH 6.0. The operating conditions are summarized in Table 1.

Cheese whey separation was done by using chitosan as coagulant and then let to separate protein from supernatant cheese whey by chitosan and polysulfone membrane with the selected operation of ultrafiltration. Chitosan membrane is prepared from local commercial chitosan products form shrimp shells with a moisture content of 10.00%, ash content of 0.50% and degree of deacetylation of 74.85%. Filmtec flat sheet polysulfone membrane (GR61PP) is used for the experiment. The molecular weight cut-off (MWCO) for chitosan and polysulfone membranes were found in the range of 20000 Dalton that were suitable for application in ultrafiltration for protein separation from cheese whey (ASTM, 1990; and Persson, *et al.* 1993).

Table 1 Various operating conditions in coagulation

Operating conditions	Unit	Values
Coagulant: Chitosan	mg/l	20-90
PAC	mg/l	100-500
Alum	mg/l	100-500
Carrageenan	mg/l	20-100
pH	-	6.0-10.0
Rapid mixing: Speed	rpm.	150
Contact time	min.	2
Slow mixing: Speed	rpm.	20
Contact time	min.	15
Sedimentation in Imhoff cone	min.	60
Filtration	min.	10

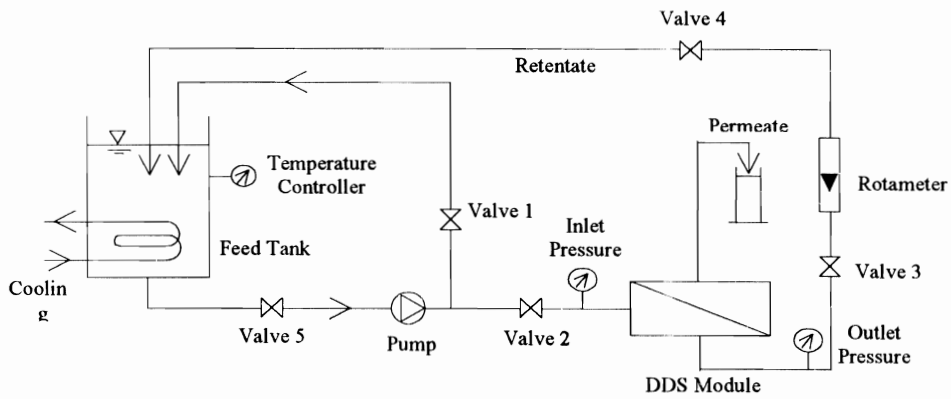


Figure 2 Experimental setup for cheese whey ultrafiltration

The ultrafiltration experiments Figure 2 were conducted in DDS mini lab 10 module by comparing between chitosan and polysulfone membrane of 20000 Dalton MWCO. The experiments were investigated by varying applied pressure at 0.9, 1.2 and 1.5 bar while keeping retentate flowrate constant at 0.6 GPM. The retentate flowrate was then varied at 0.2, 0.6 and 1.0 GPM keeping applied pressure constant at 1.2 bar. Temperature was maintained at 25°C for both

experiments. The pH of feed concentration was fixed at 7.0 for chitosan membrane. The permeate solution was collected every hour for 10 hours to analyse flux and protein content. Protein concentration of cheese whey in feed tank and permeate stream was measured spectophotometrically using calibration curve developed by Bradford Assay (Boyer, 1993). The permeate flux (Kesting, 1971) was measured by using the equation (1) :

$$J = \Delta V / (A \Delta t) \qquad (1)$$

where J is permeate flux ($l/m^2.h$), ΔV is permeate volume collected in a time interval (liter). A is effective area of membrane (m^2) and Δt is time interval (h).

The protein rejection (ASTM, 1990) was measured by using the equation (2) :

$$\% \text{ Rejection} = (Cb-Cp)/Cb \times 100 \qquad (2)$$

where Cp is the permeate concentration (mg/l) and Cb is the bulk concentration in retentate (mg/l).

The two types of membrane were compared using the relationship between permeate flux and protein rejection with time.

Results and Discussions

The coagulant dosage were a deciding factor based on pH. The optimum pH were adjusted to 7 for all the coagulants and results were described based optimum pH value Figure 3.

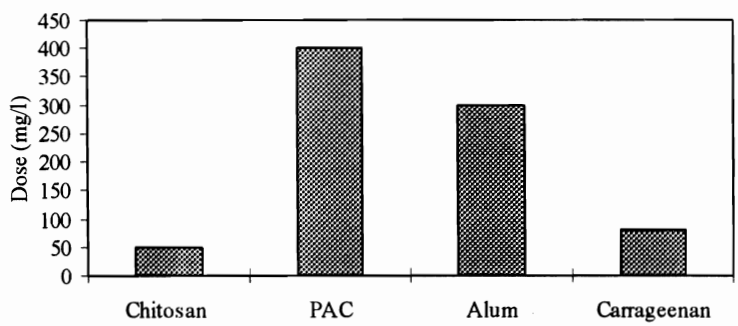


Figure 3 Dosage for coagulants at optimum pH

Turbidity removal was higher for chitosan at a dosage of 50 mg/l Figure 4. For PAC, alum and carrageenan the removal was lower even for the higher dosage.

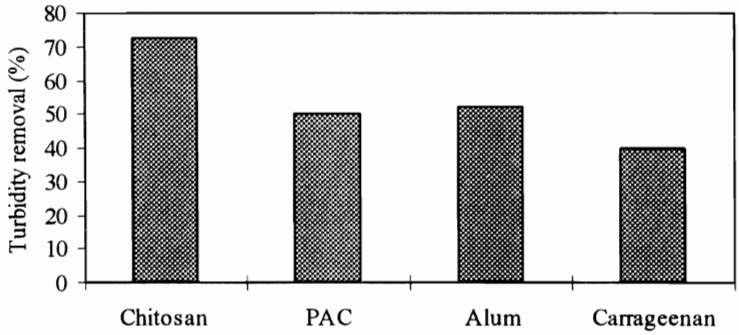


Figure 4 Turbidity removal for different coagulant dosage

The sludge volume was much higher in case of chitosan and PAC at the dosage where maximum removal was found Figure 5. Carrageenan gave the lowest sludge volume among all.

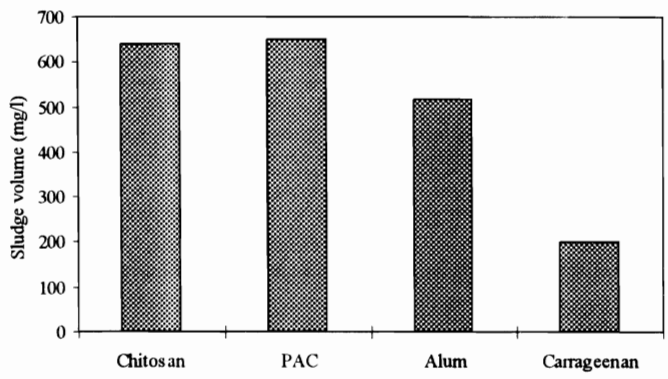


Figure 5 Sludge volume at different coagulant dosage

Protein removal was higher for chitosan at a dosage of 70 mg/l which was 82%. At optimum dosage chitosan maintained higher removal of protein comparing to other coagulants Figure 6.

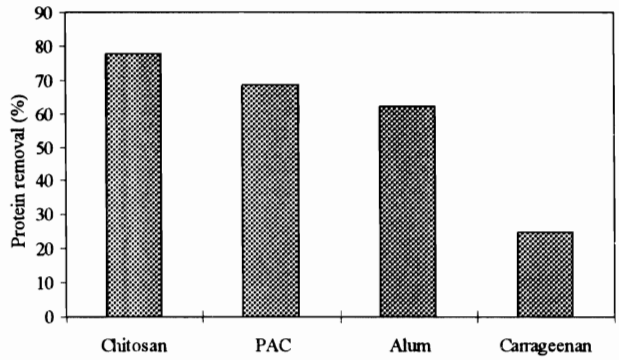


Figure 6 Protein removal for different coagulant dosage

Filtrate volume was higher for chitosan Figure 7 that indicates larger sludge particle size in chitosan coagulation.

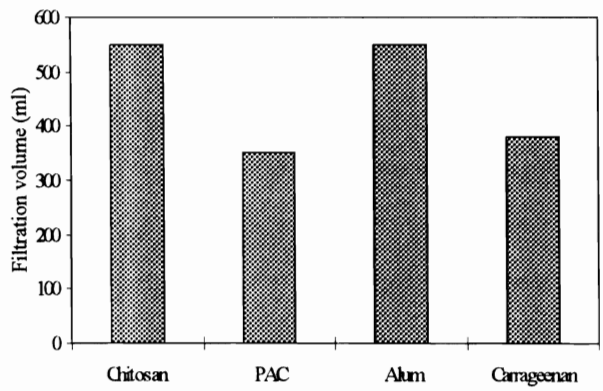


Figure 7 Filtration volume for different coagulant dosage

Figure 3 to 7 illustrates the performance of different coagulants at their selected operating conditions. As can be seen, performance by chitosan is superior than other coagulants in all respects. Chitosan yielded better protein as well as turbidity removal at lower dosage value. Coagulant dosage at selected operating conditions for chitosan was only 50 mg/l as compare to 400 mg/l for PAC, 300 mg/l for alum, and 80 mg/l for carrageenan while superior protein removal of 77.8% was obtained by chitosan in comparison with 68.5%, 62.0%, and 25.0% by PAC, alum and carrageenan, respectively. Turbidity removal of 72.5% was obtained by chitosan under selected conditions

while only 50.0%, 52.5% and 40.0% removal were recorded by PAC, alum and carrageenan, respectively. This data also suggests turbidity removal is not proportional to protein removal from cheese whey. Filtrate volume was higher at selected chitosan dosage compare to PAC and carrageenan. The highest value belonged to both chitosan and alum. 550 ml of filtrate volume from 1000 ml cheese whey supernatant was obtained at selected chitosan dosage, while only 350 ml and 380 ml of filtrate volume were recorded by PAC and carrageenan. Sludge volume was higher at selected chitosan dosage compare to alum and carrageenan. The highest value of filtrate volume belonged to PAC at 650ml. 640 ml of sludge volume from 1000 ml cheese whey supernatant was obtained at selected chitosan dosage, while only 520 ml and 200 ml of sludge volume were recorded by alum and carrageenan. Higher protein removal along with higher filtrate volume Figure 7 obtained by chitosan indicated compact nature of sludge.

Ultrafiltration experiments were conducted in 2 sets. In the first, effect of applied pressure on permeate flux and protein rejection was investigated while keeping retentate flowrate constant. While in the second set of experiments, effect of retentate flowrate on permeate flux and protein rejection was investigated while keeping applied pressure constant. Temperature in the feed tank was maintained at 25°C. Cheese whey with and without chitosan coagulation was tested for each set of experiment. Raw Cheese whey was necessary to maintain at a pH of 7.0 otherwise chitosan membrane was damaged and/or dissolved by lactic acid if pH lower than 5.8.

Ultrafiltration of chitosan membrane without coagulation

Effect of applied pressure on chitosan membrane without coagulation

The effect of applied pressure on permeate flux and protein rejection was investigated by performing experiments with three different applied pressures viz. 0.9, 1.2, and 1.5 bar. The flowrate from retentate stream was kept constant at 0.6 GPM in all the experiments.

Protein concentration in feed tank

Since the retentate stream was recycled back into the feed tank, it was expected that protein concentration in feed tank would increase with batch duration. Accordingly, Figure 8 shows the variation of protein concentration in feed tank with batch duration. It was found that protein concentration in feed tank did not increase during variation with batch time. For chitosan membrane, protein concentration increased typically about 7.2-8.0% and for polysulfone membrane it increases about 9.7-11.5% during 10 hour operation.

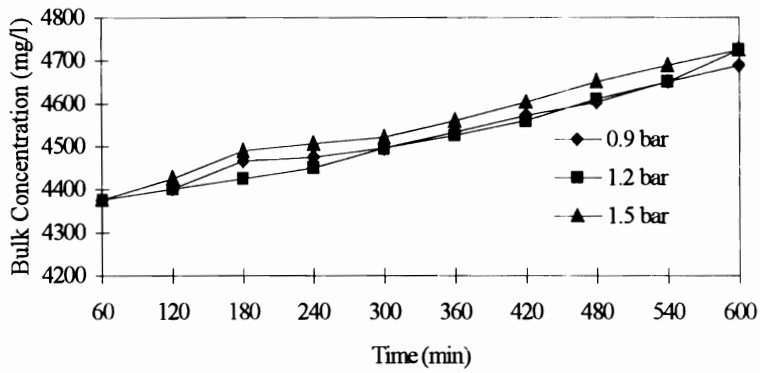
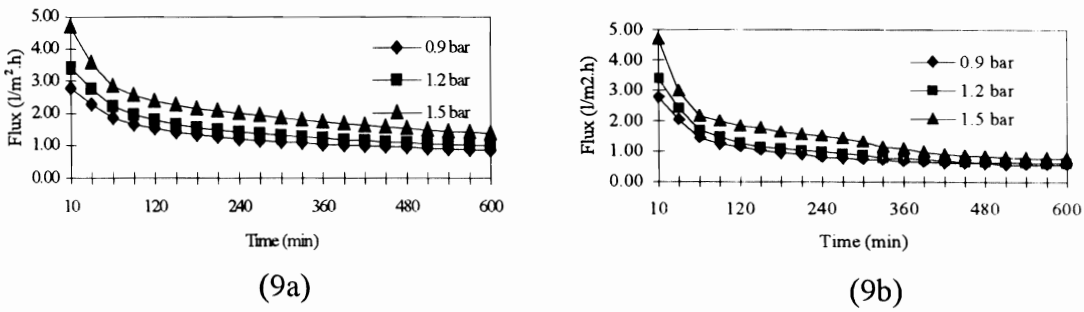


Figure 8 Illustration of protein concentration in feed tank for chitosan ultrafiltration

The Permeate flux

Permeate flux in all the batches showed marked reduction of 48.4%-54.3% and 43.7%-58.0% for chitosan and polysulfone membranes respectively during first hour of operation. Steady state flux was obtained after about 7 hours of operation in case of chitosan membrane and polysulfone membrane it was about 6 hours. Permeate flux was permeate flux for chitosan membrane (CTS) were 0.88, 0.68, and 0.61 l/m^2.h and for polysulfone membrane (PSF) were 1.21, 0.72 and 0.66 l/m^2.h for the applied pressure of 1.5, 1.2, and 0.9 bar, respectively Figure 9. The decrease in permeate flux during a typical batch operation can be attributed to the increase in thickness of protein gel layer which increases the resistance to permeate flux (Noble and Stern, 1995). Deposition and adsorption of colloidal proteins on external surface and internal pores of the membrane leads to reduction on membrane permeability, causing reduction of permeate flux (Noble, *et al.* 1995; Aiba, *et al.* 1986; and Coulter, 1992)



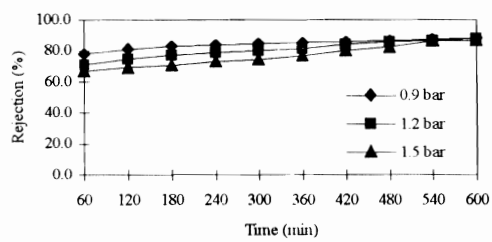
(9a) (9b)

Figure 9 Effect of applied pressure on permeate flux (9a: For chitosan membrane, 9b: For polysulfone membrane)

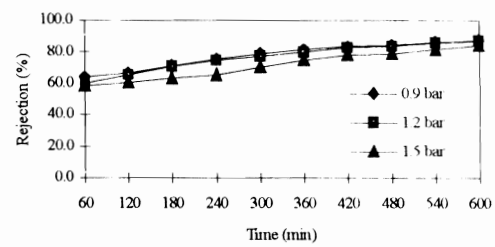
The Protein rejection

Average protein rejection during each hour of sampling interval with respect to batch time is plotted in Figure 10. As illustrated in the figure, protein rejection is lowest of about 66.8% with CTS and 58.0% with PSF for 1.5 bar applied pressure as compare to 70.5% and 78.0% with CTS and 60.0% and 63.5% with PSF for 1.2 and 0.9 bars during first hour of batch operation. This is primarily because at the beginning of batch operation, the external surface as well as the internal pores of the membrane are clean and hence more protein molecules can escape through the membrane at higher operating pressure. But as the times passes, the protein molecules are deposited on the external surface of membrane and they also partially block the internal pores of membrane (Noble, *et al.* 1995; Chandrkachang, 1996; and Frisch, 1978). Accordingly, the resistance for permeate flux increases (Cheryan, 1986). Although this reduces the permeate flux Figure 9 due to increased resistance, protein deposition on membrane and partial blocking of internal membrane pores also reduces any further leakage of protein molecules through the membrane which results in improved protein rejection, and this deposition occurs faster at the higher applied pressure.

The steady state protein rejection are 81.4%, 82.8% and 85.6% with CTS and 83.8%, 86.7% and 87.2% with PSF for 1.5, 1.2, and 0.9 bars, respectively Figure 10 implying higher steady state protein rejection for higher operating pressures. It is expected that this values will eventually reach time averaged protein rejection as batch operation time further increases.



(10a)



(10b)

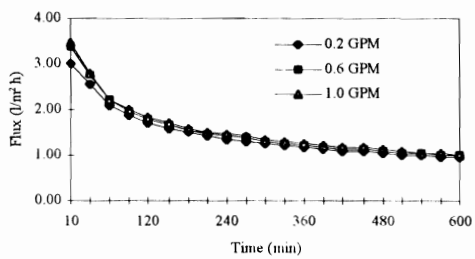
Figure 10 Effect of applied pressure on protein rejection (10a: For chitosan membrane, 10b: For polysulfone membrane).

Effect of flowrate on chitosan membrane without coagulation

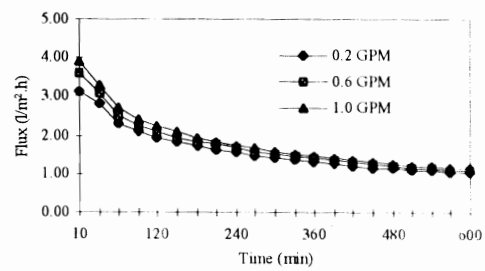
The effect of flowrate on permeate flux and protein rejection was investigated by performing experiments with different retentate flowrate viz. 0.2, 0.6 and 1.0 GPM. Applied pressure was kept constant at 1.2 bar in all the experiments.

The Permeate flux

Figure 11 shows that permeate flux was higher for higher retentate flowrate after first hour of batch operation. Steady state permeate flux of 0.71, 0.68, and 0.65 l/m^2.h with CTS and 0.74, 0.72 and 0.74 l/m^2.h with PSF were recorded for retentate flowrate of 1.0, 0.6, and 0.2 GPM, respectively. The increase in permeate flux with increased retentate flowrate can be attributed to the effect of high flowrate to remove gel layer on membrane surface (Noble and Stern, 1995). The decrease in permeate flux during a typical batch operation due to the deposition and adsorption of colloidal proteins on external surface and internal pores of the membrane which increases the resistance to permeate flux and thus causing reduction of permeate flux (Cheryan, 1986).



(11a)



(11b)

Figure 11 Effect of flowrate on permeate flux (11a: For chitosan membrane, 11b: For polysulfone membrane)

The Protein rejection

Since gel layer on membrane surface during batch operation could be partially removed by high flowrate (Cheryan, 1986), it was expected that protein rejection would decrease with increased flowrate. Accordingly, the variation of protein rejection with batch duration based on cumulative values was presented in Figure 12. As can be seen from these figures, protein rejection during batch operation does not show improvement while the variation with flowrate from 0.2 to 1.0 GPM.

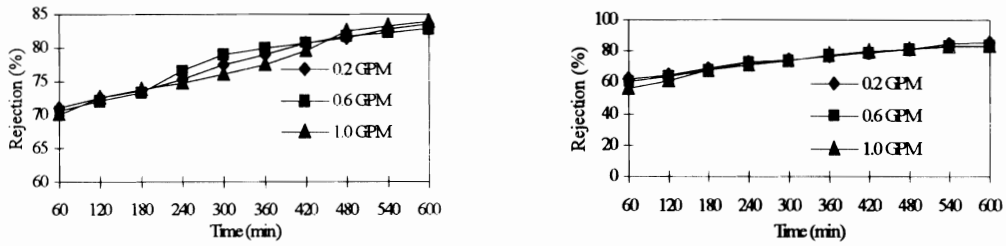


Figure 12 Effect of flowrate on protein rejection (12a: For chitosan membrane, 12b: For polysulfone membrane)

Chitosan and polysulfone membrane of MWCO 20000 Da ultrafiltration for separation of proteins from cheese whey showed the steady state permeate flux of 0.68 and 0.72 l/m^2.h and protein rejection of 86.5% and 79.6% respectively at optimum pressure of 1.2 bar and retentate flowrate of 0.6 GPM.

Ultrafiltration of chitosan membrane with coagulation

Ultrafiltration experiment was investigated while keeping the retentate flowrate constant at 0.6 GPM and applied pressure constant at 1.2 bar. Temperature was maintained at 25°C and pH of feed concentration fixed at 7.0. following the selected coagulation by using chitosan as coagulant. The result shows in Figure 13 and 14.

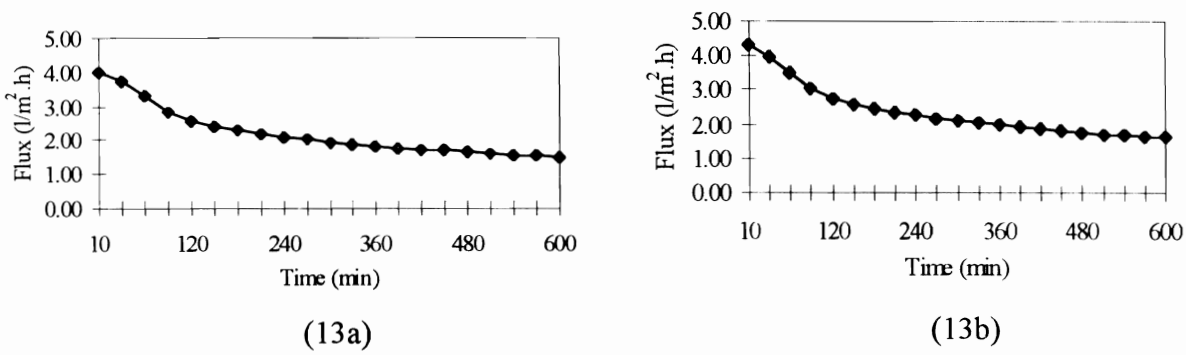


Figure 13 Flux variation with time (13a: for chitosan membrane 13b: for polysulfone membrane)

The Permeate flux

Figure 13 shows the cumulative permeate flux decreased around 17.6% and 18.9% during first hour of operation for chitosan (CTS) and polysulfone (PSF) membrane respectively. Steady state flux was obtained after about 8 hours of operation. The cumulative flux reduction for CTS membrane 62.6% at the end of operation compared to 62.7% for PSF membrane. Permeate flux for both membranes in case of cheese whey with chitosan coagulation was higher than that of without coagulation. . The increasing value was about 69.5% and 58.4% at the first hour of operation and 30.0% and 55.0% at the end of operation for CTS and PSF membrane respectively.

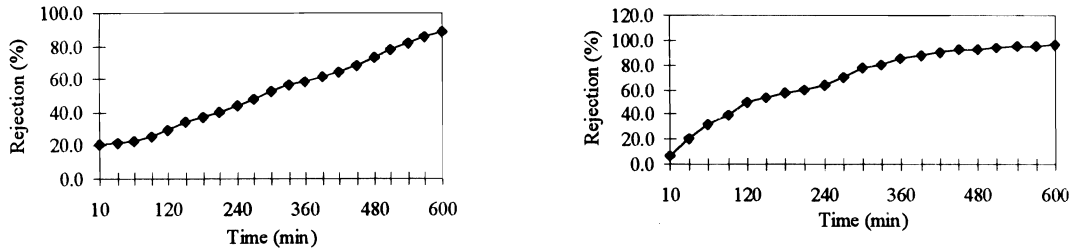


Figure 14 Protein rejection with time (14a: for chitosan membrane 14b: for polysulfone membrane)

The Protein rejection

Figure 14 shows protein rejection in case of cheese whey with chitosan coagulation were lower than in case of cheese whey without chitosan coagulation. The cumulative protein rejection started at 20% and increased to 88.8% for CTS membrane and 6.9% to about 94.6% for PSF membrane. Steady state protein rejection was found when it reached at 8 hours. The nature of the curve was similar although this reduces the permeate flux due to the increased resistance caused by protein deposition on membrane and partial blocking of inter membrane pores also reduces any further leakage of protein molecules through the membrane which results in improved protein rejection and this operation occurs lower when applied coagulation. It means coagulation can increase permeate flux and decrease protein rejection or the ultrafiltration and means a partial protein rejection from cheese whey. Permeate flux increased to about 69.5% and 58.4% and protein rejection decreased about 68.8% and 46.8% at first hour of operation when using chitosan as coagulant for CTS and PSF membrane respectively. However, pH of feed concentration was mainly limiting factor to chitosan membrane.

Table 2 Performance of membranes for ultrafiltration of cheese whey proteins

Condition	Chitosan	Polysulfone
Without Coagulation	Steady state permeate flux of 0.68 $l/m^2.h$, protein rejection of 86.5% at 1.2 bar and 0.6 GPM.	Steady state permeate flux of 0.72 $l/m^2.h$, protein rejection of 76.9% at 1.2 bar and 0.6 GPM
With Coagulation	Permeate flux increasing of 49.1% and protein rejection decreasing of 68.8% at first hour. Permeate flux reduction of 62.6% and protein rejection increase of 88.8% during 10h at 1.2 bar and 0.6 GPM	Permeate flux increasing of 39.2% and protein rejection decreasing of 50.0% at first hour. Permeate flux reduction of 62.7% and protein rejection increasing of 94.3% during 10h at 1.2 bar and 0.6 GPM

Conclusion

Coagulation of cheese whey using chitosan and other coagulant varied with pH of the cheese whey. For optimum pH at 7.0 chitosan showed better and more effective coagulant compared to alum, PAC, carrageenan. Chitosan flat sheet membranes showed their high performance as well as the commercialized polysulfone membranes for ultrafiltration of cheese whey proteins. The nature of the performance of chitosan membranes is similar to polysulfone membranes. Permeate flux and protein rejection depends on effect of applied pressure and effect of retentate flowrate. Permeate flux decreasing and protein rejection increasing caused by deposition and adsorption of colloidal proteins on external surface and internal pores of the membrane leads to reduction on membrane permeability (Aiba, *et al.* 1986). Cheese whey protein ultrafiltration followed by chitosan coagulation improved the flux as chiotsan coagulant removed portion of protein during coagulation. A decrease in protein rejection occurs during ultrafiltration with chitosan coagulation as solid to a large extend has already been removed during coagulation.

The performance of chitosan membrane in comparing with commercial polysulfone membrane for ultrafiltration of cheese whey protein was shown in Table 2.

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References

Aiba, S., Izume, M., Minoura, N. and Fujiwara, Y. 1989. Preparation and Properties of Dialysis Membranes. In : *Chitin in Nature and Technology*. R., Muzzarelli, C. Jeuniaux and G. W. Gooday. (eds.), New York, Plenum Press.

American Society for Testing and Materials. 1990. Standard Test Method for Molecular Weight Cutoff Evaluation of Flat Sheet Ultrafiltration Membranes. *ASTM Designation* : E 1343-90.

Bough, W. A., Salter, W.L., Bwu, A.C.M., Berkins, B.E. 1978. Influence of Manufacturing Variables on the Characteristics and Effectiveness of Chitosan Products. I. Chemical Composition, Viscosity, and Molecular-Weight Distribution of Chitosan Products. *Biotechnology and Bioengineering*. **10** : 1931-1966.

Boyer, R. F. 1993. *Modern Experimental Biochemistry*. 2nd ed. Redwood City, Calif., Benjamin/Cummings : 51-57.

Chandrkrachang, S. 1996. Chitin and Chitosan, Multipurpose Biodegradable Biomaterials. *Proceeding of MTEC annual meeting, 2-3 May 1996. Bangkok, Thailand.*

Cheryan, M. 1986. *Ultrafiltration Handbook. Lancaster, Pa.,* Technomic Pub.

Coulter, A. R. 1992. Water permeability in poly (orthoester)s. *J. Membrane Science:* **65** : 269-275.

Englewood and Fort Lee, NJ. 1989. *Chitin and Chitosan Specialty Biopolymers for Foods, Medicine and Industry*. Technical Insights, Inc.

Frisch, H. L. 1978. Diffusion in inhomogeneous films and membranes. *J. Membrane Science*. **3** : 149-161.

Kesting, R. E. 1971. *Synthetic Polymer Membranes*. New York, McGraw-Hill.

Noble, R. D. and Stern, S.A. 1995. *Membrane Separation Technology, Principles and Applications*. Amsterdam, Elsevier Science B.V.

Persson, K. M. and Tragardh, G. 1993. The Pore Size of Ultrafiltration Membranes : A Novel Approach in Development. In : *Food*

Engineering. Amsterdam, Elsevier Science B.V. : 647-649.

Roberts, G. A. F. 1986. *Chitin Chemistry.* London, Macmillan Press.

Zall, R. R. 1980. Cost-effective disposal of whey. *Dairy Ind. Int.* **45(4)** : 45.