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Studies on Membrane Processing of Sesame Protein Isolate and Sesame Protein Hydrolysate using Rotating Disk Module

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Abstract: Proteolytic enzyme modifications of sesame (*Sesamum indicum* L.) protein isolate produce a protein hydrolysate containing small peptides. These small peptides are mainly used for therapeutic applications. This work was carried out to fractionate protein hydrolysate into small peptides and large peptides fractions using ultrafiltration and to study the effect of different operating parameters to optimize the process. Membrane rotation reduces concentration polarization and enhances the permeate flux value and found to be more effective than stirrer rotation. Maximum rejection and minimum flux were observed at the isoelectric point of the protein. So, ultrafiltration is a potential route to produce sesame peptides of desired properties.

Keywords: Concentration polarization, flux, rejection, sesame protein isolates, sesame protein hydrolysate, small peptides, ultrafiltration

INTRODUCTION

Sesame (*Sesamum indicum* L.) seeds have long been cultivated as a source of oil and protein. Sesame is an East Indian flowering plant that comes from the family of Pedalliaceae and the genus Sesamum. It is a *Kharif*

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crop which is sown in June-July with the onset of the monsoon season and is harvested in January-February. In 2005, the production of sesame seed was 68,0000 metric tons in India, which is next to China. The defatted sesame seed meal contains about 40–50% protein, 10–11% fiber, 8–9% ash, and 4–6% moisture. The protein dispersibility index and the nitrogen solubility index of the dehulled sesame meal are 40.2 and 33 respectively (1). Sesame meal is very important as a protein source for human consumption due to a reasonably well-balanced amino acid composition. It also contains sulfur containing amino acids, mainly methionine, which is very rare in other plant proteins (2). Sesame seed is composed of globulin (67.3%), albumin (8.6%), prolamine (1.3%), and glutelin (6.9%) fractions (3). In the sesame globulin fraction, the ratio of the two branched chain amino acids (BCAAs), lysine and arginine, is 0.67, which is appropriate for cholesterol metabolism (4). Thus sesame protein has the potential to be used in therapeutic application. The characteristics of sesame protein isolate are mentioned elsewhere (1).

Proteolytic enzyme modifications of sesame protein isolate (PI) [containing about 90% protein] produce protein hydrolysate (PH). This protein hydrolysate shows improved functional properties like higher solubility, emulsification, and foaming and thus expands the field of application. Protein hydrolysates are widely used as nutritional supplements, functional ingredients, and flavor enhancer in food, coffee whitener, cosmetics, and confectionary and in the fortification of soft drinks and juices (5). Small peptides present in protein hydrolysate, are mainly used in therapeutic applications like a temporary decrease of systolic blood pressure (6) in the treatment of psoriasis, and age-related various chronic diseases (7). Synthetic small peptides containing three or six amino acid residues improve the key parameters of monoclonal antibody-producing mouse hybridoma cultures (8).

The use of membrane technology (MT) in isolation and fractionation of seed protein has generated considerable research interest as evidenced by several recent publications (9,10,11) and patent applications (12,13). Being simple, selective, and energy efficient, MT has significant advantages over other technologies. Considering the importance of sesame protein isolate (PI) and sesame protein hydrolysate (PH) it seems to be worthwhile in investigating the processing of the sesame protein isolate and the respective hydrolysate by membrane technologies. In the present study the rotating disk module was used for the purpose of ultrafiltration of sesame protein isolate and protein hydrolysate to enrich protein concentration. This type of module was also used for the treatment of kraft black liquor (14,15) and during ultrafiltration of casein whey (16). Some papers also report the effectiveness of enhanced shear effect on the improvement of permeate flux value and reduced fouling effect (17,18). Dynamic filtration technology

(rotating and vibrating filtration, RVF technology) has also been investigated for the clarification of rough beer (19). Vibrating membrane module to lower the problem of concentration polarization during the concentration of milk protein was also observed (20). A comparative study of the rotating disk and vibratory dynamic filtration system was also done in several papers in order to improve the permeate flux behavior (21).

The UF process has been an area of active research because of its potential application in the field of separation science. UF is primarily a size exclusion based pressure driven membrane separation process. In this UF process, one of the major problems is the decline of the permeate flux (J) with time, which mostly results due to the accumulation of solute particles on the membrane surface. This phenomenon is called concentration polarization. Different module design and flow pattern have so far been suggested to reduce the flux decline resulting from membrane fouling as well as the concentration polarization. In this work, our primary objective is the fractionation of protein hydrolysate into small peptides and large peptides fractions using a suitable ultrafiltration membrane, as well as their concentration for better application purposes. We are also studying the effect of different operating parameters like membrane rotation, stirrer rotation, and pH of the feed solution in order to optimize the ultrafiltration process parameters to assess the feasibility of the process with reduced effect of membrane fouling.

MATERIALS AND METHODS

Materials

Authentic sesame seed of brown variety was collected from district Hooghly, West Bengal, India. Dehulling was done in a low-speed laboratory grinder (Remi, mixture blender) followed by air classification. A deoiled meal was prepared in the laboratory using food grade hexane in Soxhlet apparatus for 24 h. The deoiled meal was desolventized in a vacuum oven at 60°C at 4 mm Hg for 5 h. The deoiled meal was stored in desiccators and used for further experiments. All other reagents were obtained from E. Merck (Mumbai, India) and Folin-Ciocaltau's Phenol reagent (AR Grade, 2 N, Batch no X/729505) was supplied by SISCO Research Laboratory Private Limited, Mumbai, India.

Preparation of Sesame Protein Isolate

Sesame protein isolate was prepared according to the standard method (1). Briefly, defatted sesame meal was milled and passed through an 80

mesh screen (about 80% through) and mixed with water (1:10 w/v). The pH was adjusted to 9.5 with 1(N) NaOH solution, and stirred for 1 hr at 50–55°C, and then centrifuged at 4000 g for 10 min to remove the residual meal. The supernatant liquid was separated and the pH adjusted to 4.9. After complete precipitation, the solution was centrifuged at 8000 g for 20 min. The precipitated protein (PI) was collected and then dried in a vacuum oven at 50°C for 10 hrs. The dried mass was stored at 4°C for further studies.

Preparation of Sesame Protein Hydrolysate

For preparation of the protein hydrolysate, protein isolate was dispersed in distilled water (1:20 w/v dry basis) at pH 10 and incubating at 50°C for 1 hr with shaking (1). The solution was then adjusted to pH 8 and hydrolysed using 0.5% (w/w) papain (activity 6000NF unit, batch no. 2807, supplied by Viral Rasayan, West Bengal, India). The hydrolysis was carried out for 30 min at 37°C, and the enzymes were rapidly inactivated by heating at 95°C for 5 min. The hydrolysate was stored at 4°C for further studies.

Analytical Methods

Measurement of Protein Concentration

Protein concentration of the feed, retentate and permeate were determined according to Folin-Lowry method of protein assay (22) at 750 nm against an appropriate blank. In this method, 5 mL of alkaline solution (50 parts of 2% Na_2CO_3 in 0.1 M NaOH + 1 part of 0.5% $CuSO_4$ in 1% sodium potassium tartarate solution) was added to 1 mL of the test solution, mixed thoroughly, and allowed to stand for 10 min at room temperature. 0.5 mL of the diluted Folin-Ciocalteau reagent was rapidly added to the above solution with immediate mixing, allowed to stand for 30 min in the dark, and the protein concentration was measured at 750 nm against BSA (bovine serum albumin) calibration curve (Fig. 2).

Methods of Capillary Gel Electrophoresis

Feed was characterized by determining the molecular weight distribution, which is an important parameter governing the ultrafiltration flux and rejection. For this purpose, a capillary gel electrophoresis (P/ACE) system of Beckman instrument Inc., Fullarton, CA was used with P/ACE UV absorbance detector. The method comprised of preparing protein test

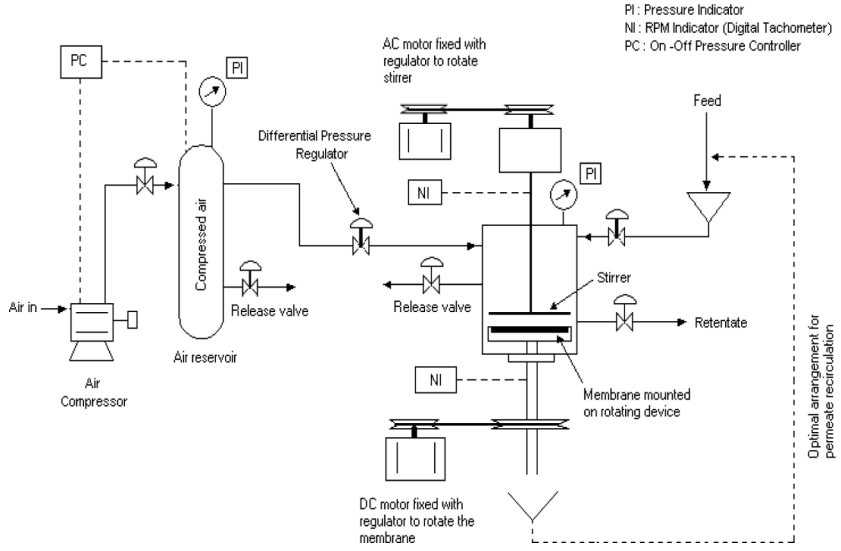

Figure 1. Rotating disk membrane module (in-house fabricated).

mix (SDS 14–200), containing seven standard proteins, ranging from 14.2 to 205 kDa of α-lactalbumin (14.2 kDa), carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase (97.4 kDa), β-galactosidase (116 kDa) and myosin (205 kDa), and dissolving in 750 μL of sample buffer (SDS sample buffer, 0.12 M, Tris HCl/1% SDS, pH-6.6). 750 μL deionized water was then added to it and mixed thoroughly. 200 μL of the test solution (~0.1–1 mg of protein) was mixed with 100 μL of sample buffer, 10 μL of Orange G reference

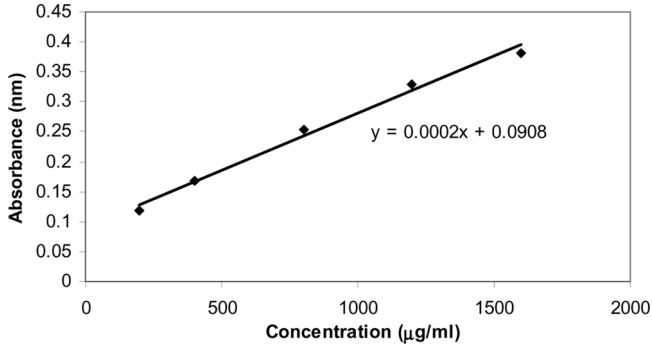

Figure 2. Calibration curve of BSA for protein estimation.

Table 1. Characteristics of sesame protein isolate

Properties	Compositions (%)
Yield	65.3 ± 0.87
Protein	78.4 ± 2.34
Fiber	2.41 ± 0.42
Ash	3.56 ± 0.65

marker (0.1% solution), 5 μL of 2-marcaptoethanol, 85 μL of deionized water in a 400 μL vial and vortexed for 2 min. Then the mixture was boiled in a water bath at 100°C for 10 min in a closed microfuge vial and then placed in ice bath to cool for 3 min before injection. A capillary gel electrophoresis system comprised of 0.27 m long and 100 μm i.d. capillary, and detection was made in the UV range at 214 nm. Molecular weight was calculated with reference to the standard protein test mix by using Beckman system Gold P/ACE control software. The detailed procedure for feed preparation in capillary electrophoresis was given elsewhere (23). Results of the investigation are reported in Tables 1 and 2.

EXPERIMENTAL SET-UP

Membrane and Module

Ultrafiltration of pretreated sesame protein isolate and sesame protein hydrolysate was carried out in a stirred rotating disk module (capable of being used as fixed disk module) using a cellulose tri-acetate (CTA) membrane. The module made of SS316 was manufactured by Gurpreet Engineering works, Kanpur, UP, India as per specified design. The

Table 2. Degree of hydrolysis of different Sesame Protein Hydrolysates

Product	Time of hydrolysis (Minute)	Degree of hydrolysis (%)
SPH_{10}	10	21.13 ± 0.18
SPH_{20}	20	21.70 ± 0.27
SPH_{30}	30	23.36 ± 0.20
SPH_{40}	40	25.80 ± 0.37
SPH_{50}	50	26.50 ± 0.26
SPH_{60}	60	28.96 ± 0.43

module (Fig. 1) was equipped with 2 motors with speed controllers to provide rotation of the stirrer (AC motor, HP = 1/20, maximum rpm-4000) and membrane housing (Direct current motor, HP = 0.25, maximum rpm-1500). The module has the facility to rotate the membrane and the stirrer in the opposite direction to provide maximum shear in the vicinity of the membrane. Adequate mechanical sealing (capable of withstanding maximum pressure of 9.8 bars) mechanism was provided to prevent leakage from the rotating membrane assembly. The magnetic drive stirrer mechanism prevents any leakage possibility from the top of the stirrer. Air compressor was used to provide compressed air for pressurization of the cell. An intermediate air reservoir coupled with an on-off controller based on the pressure sensor was provided which maintains the pressure within the reservoir between 9–11 bars. A differential pressure regulator was used to set the pressure at the desired level within the module. In this module the membrane rotates with a speed of 50 to 600 rpm. Membranes were placed properly within the membrane module using a gasket, such that there must not be any leakage, otherwise feed will bypass with permeate. The effective filtration area available for each membranes was 4.7414 cm^2. The gap between the stirrer and the membrane is 5.4 cm. The complete schematic diagram of the rotating disk module set up is given in Fig. 1. The module can withstand high temperature as it is made of SS316, but the maximum temperature during the run was fixed according to the membrane characteristics. The cellulose acetate membrane of 5 kD MWCO (cat no. 14529-76-D, dia 76 mm) was imported from Millipore Corporation, Bedford, MA (USA) through its Indian counterpart (Millipore India Limited).

Membrane Compaction and Water Run

Before the experiment started, the membrane was subjected to compaction for about 1 hr with ultra pure water at a pressure of 5.88 bars, higher than the highest operating pressure to prevent any possibility of change of the membrane hydraulic resistance (R_m) during ultrafiltration. Once the water flux becomes steady with no further decrease, it is concluded that the full compaction of the membrane has taken place. After compaction, R_m was determined at different trans membrane pressures (TMP) of 2, 3, 4, 5 bar. The ultrapure deionized water, was obtained from Arium 611DI ultrapure water system (Sartorius AG., Göttingen, Germany).

Feed Pretreatment

In order to reduce the extent of membrane fouling, the suspended matters from the feed solution were removed by UF, in a cross flow module

using a membrane with a nominal MWCO of 100 kDa and made of Poly Ether Sulfone(PES). The module was Vivaflow 200 (Serial no. 05VF20003) supplied by Vivascience Ltd, UK. The dimension of the membrane was, overall L/H/W of 126/138/38 mm; channel (W/H) of 10 mm/0.4 mm; the active membrane area was 200 cm^2; the hold up volume (module) 5.3 mL; the minimum recirculation volume of less than 20 mL, and the nonrecoverable holdup of less than 2 mL. The module could be operated up to the maximum pressure of 4 bars and maximum temperature of 60°C, with a pump flow rate in the range of 200–400 mL/min. The permeate from 100 kD UF was adjusted for pH by adding the calculated amount of hydrochloric acid and sodium hydroxide as required to produce the feed of subsequent UF in the rortating disk module. A digital pH meter (Sartorius, A.G., Göttinger, Germany) was used to measure solution pH resolution of 0.01. Feed pretreatment was done under transmembrane pressure of 2 bar upto a volume reduction factor (VRF) of 4. 500 mL feed (concentration 129.88 ppm) at pH 7 was processed within 9 min with the average flow rate of about 60 mL/min. The run was taken in an airconditioned room maintaining a temperature of 20°C. We have not varied the temperature during the run. Permeate concentration was the final permeate concentration which was 100.06 ppm and the retentate concentration was 225 ppm. The loss of mass may be due to the adsorption of protein molecules on the membrane surface.

Methodology

An experiment was carried out in a rotating disk membrane module at different operating conditions starting each time with an initial feed volume of 500 ml. All the experiments were performed with feed concentration of 100 ppm at room temperature for 1 h. In order to clearly identify the effect of membrane rotation, stirrer rotation, pH of feed we have used a fixed transmembrane pressure of 2 bars in most of the presentations if not mentioned otherwise. The objective of this study is to find out the effect of the operating parameters, membrane rotation (N_m), stirrer rotation (N_s), the pH of the feed solution on the permeate flux (J), and rejection. So, the pH of solution varied from 3 to 7 at 4.9 being the isoelectric point (IP) of the sesame protein. Membrane rotation varied from 50 rpm to 150 rpm, stirrer rotation varied from 100 rpm to 300 rpm. The stirrer rotation speed and the membrane rotation speed were checked with a digital tachometer working on stroboscopic principle. After experiment the membrane was thoroughly washed for 20 minute under running water and checked for membrane hydraulic resistance. To assess

the reproducibility of the results, all experiments were repeated for thrice and reported as the average of three readings.

RESULT AND DISCUSSION

Characterization of Protein Isolate and Hydrolysate

The sesame protein isolate was prepared from dehulled and defatted sesame seed meal. The chemical analysis of the protein isolate is presented in Table 1. The degree of hydrolysis of sesame protein isolate with time is presented in Table 2. Table 2 shows that the degree of hydrolysis increases with time. With increases the degree of hydrolysis solubility of protein increases, and this improves the functional properties (emulsifying and foaming properties) of the protein, but excessive hydrolysis may cause a negative impact on the foaming properties (1). We have used hydrolysate prepared in 30 min hydrolysis as feed in our experiments.

Effect of Hydrodynamic Conditions

After initial compaction as mentioned in section 2.5, a series of water runs were taken to evaluate the membrane hydraulic resistance (R_m), which was found $4.31810^{13}\,m^{-1}$ for 5 kDa cellulose triacetate membrane, used in this study. Experiments with sesame protein isolate and hydrolysate was calculated to understand the effect of membrane rotation (N_m) and other important parameters on permeate flux and rejection. The other independent variables being studied were solution pH, and stirrer speed. Figure 3 shows the variation of the permeate flux of PI and PH, as a function of time at different membrane rotation with fixed pH, N_s, TMP, C_b. At constant membrane rotation, the permeate flux falls gradually with time. This decline in flux is due to concentration polarization and fouling of the membrane surface, which behave as secondary membrane and resist the passage of solute molecules. For protein isolate and protein hydrolysate this fouling was irreversible in nature and lowers the initial water flux from $2.109 \times 10^{-5}\,m^3\,m^{-2}\,sec^{-1}$ to $1.58 \times 10^{-5}\,m^3\,m^{-2}\,sec^{-1}$. For protein hydrolysate the fouling is found much less. This is due to the presence of small protein molecules in the protein hydrolysate solution, which results in less concentration polarization on the membrane surface. The initial water flux was not totally recovered after the water wash in case of both PI and PH indicating an irreversible fouling of the membrane.

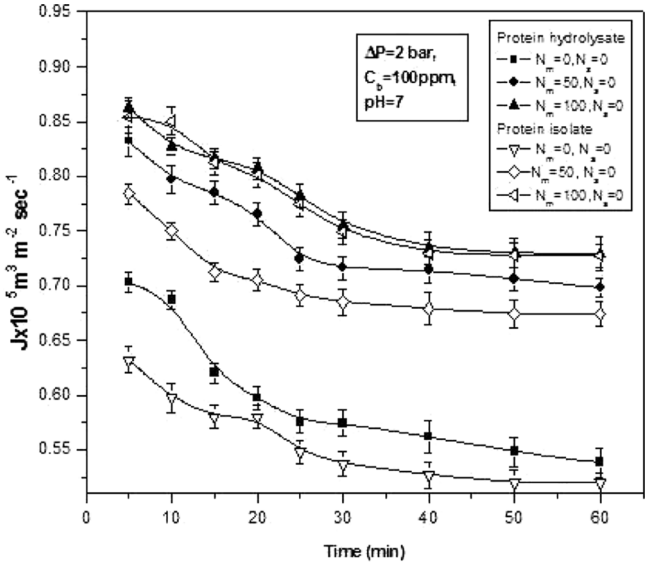

Figure 3. Variation of permeate flux as function of time at different membrane rotation for protein isolate and protein hydrolysate.

The membrane rotation causes an enhancement of permeates flux. For $N_m = 50$ rpm, at 5 min the flux enhancement is about 24.05% and 18.35% for the protein isolate and protein hydrolysate respectively, compared to the stationary membrane. This enhancement is higher for a longer duration of operation (at 30 min 27.56% and 24.90% respectively). For the stationary membrane the effect of the concentration polarization is very much prominent in reducing the permeate flux with time. The flux decline between 5 min and 60 min of operation for the stationary membrane was 17.72% whereas that for rotating membrane at 100 rpm was 14.87% for protein isolate (Fig. 4). For protein hydrolysate flux decline for stationary membrane was 23.47% and that for the rotating membrane at 100 rpm was 15.37%. These results clearly support the phenomenon that membrane rotation help in reducing the concentration polarization and enhancement of the flux value. The reason is that the membrane rotation produces shear at the vicinity of the membrane surface and prevents accumulation of solute on the membrane surface. Though the initial flux is higher for PH but the flux reduction value for protein hydrolysate is much higher compared to that for the protein isolate. Since the protein molecules in the protein isolate having larger sizes compared to that of protein hydrolysate (Table 3) so the decline in flux should be higher for protein isolate compared to the protein hydrolysate but in

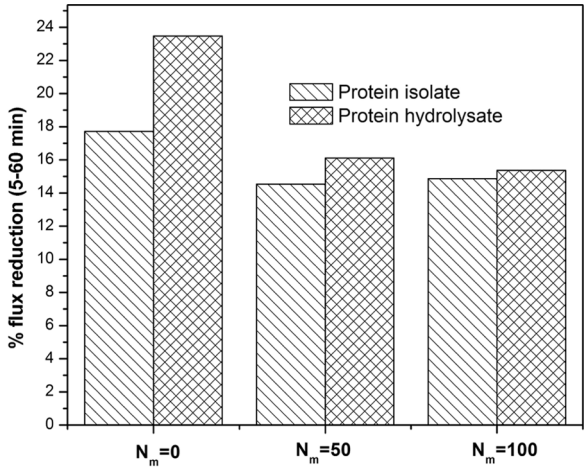

Figure 4. Percent flux reduction for protein isolate and protein hydrolysate at different membrane rotation.

our study the observation is totally different. According to the literature (24) high shear rate generated at the membrane surface tend to shear of the deposited materials and thus reduce the hydraulic resistance of the fouling layer. On the other hand shear induced diffusion is more important with the size of suspended particles. Shear induced lift velocity (V_L) is proportional to (γd_p^n) where $n = 1.3–4$. Thus, larger particles in the feed stream will experience a greater lateral lift away from the membrane surface. This causes a stratification of smaller particles on the membrane surface, which will foul membrane to a greater extent by pore plugging. So, in our experiment this different behavior is explained in terms of pore plugging by the smaller protein molecules, which is much severe compared to the concentration polarization on the membrane surface. Though the initial flux for protein hydrolysate is much higher (11.23%

Table 3. Molecular weight distribution of protein isolate and protein hydrolysate

MW (kDa) Protein isolate	%	MW (kDa) Protein hydrolysate	%
4	15.1	4.7	22.4
20.7	18.0	14.8	21.8
27.6	9.9	25.8	22.4
31.0	16.2	28.6	33.4
35.0	40.8		

of stationary membrane) but with a longer time of operation pore plugging cause the decline in flux value.

Figure 5 shows the percent average rejection (R_o) of protein isolate and hydrolysate at different membrane rotation value, for the protein isolate rejection is somewhat higher compared to the hydrolysate. This may be due to the presence of larger protein molecules in the sesame protein isolate compared to hydrolysate. Larger molecules face more resistance to pass through the membrane so average rejection is more for the protein isolate (about 6.16% higher than PH for stationary membrane). Increase in membrane rotation speed lowers the R_o, and this may be due to the shear produced on the membrane surface which help in lifting deposited molecules and lowers the tendency of concentration polarization, thereby enhancing the permeate concentration and lowering R_o. The effect of N_m on R_o is prominent both for the isolate and the hydrolysate. Figure 5 also shows that the difference between R_o values of the protein isolate and the hydrolyaste have an decreasing tendency with the increasing membrane rotation speed. For $N_m = 50$ rpm the difference is only 4.18% where as for $N_m = 100$ rpm the difference is 2.93% compared to 6.16% for the stationary membrane. This decreasing difference is due to the comparatively high shear effect produced by the higher speed of membrane rotation.

The effect of the stirrer speed on the permeate flux for PI and PH is shown in Fig. 6, which clearly shows the flux enhancement with increasing

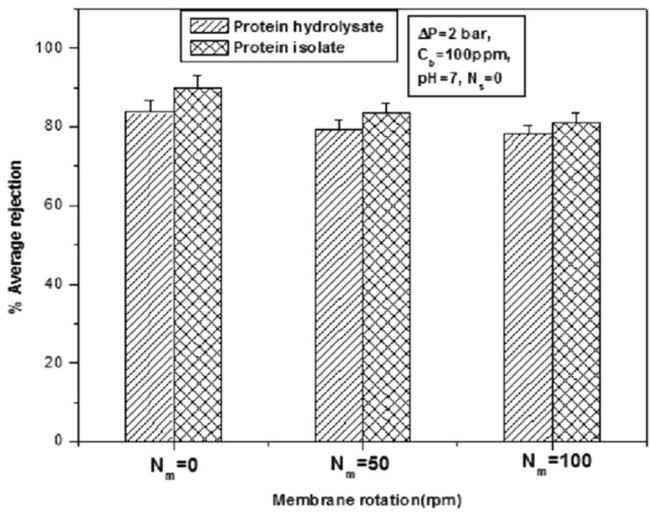

Figure 5. Variation of percent average rejection of protein isolate and protein hydrolysate as function of membrane rotation.

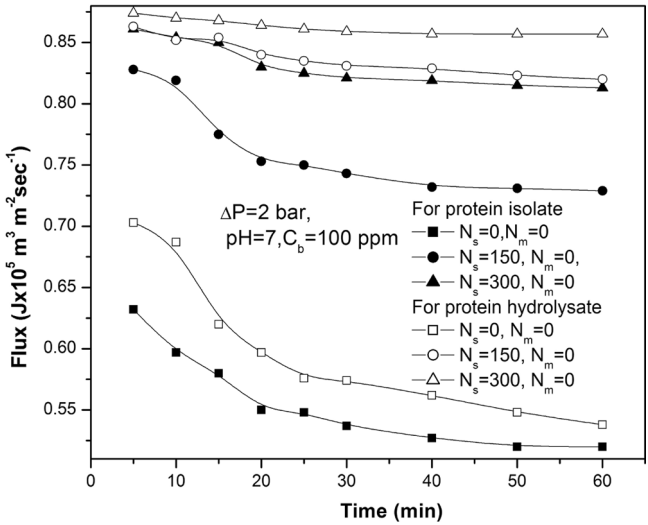

Figure 6. Permeate flux variation with time at different stirrer speed for protein isolate and protein hydrolysate with fixed TMP, N_m and pH.

stirrer speed, providing all other operating variables remaining constant. The increased stirrer speed creates more turbulence near the vicinity of the membrane, which subsequently reduces the concentration polarization effect and accordingly increases the permeate flux. It is evident from this figure that, irrespective of the fact that the stirrer rotation flux for PH is higher than PI, both increase with increasing stirrer speed. In the absence of stirrer rotation, the flux for PH was 11.23% higher than PI but in the presence of the stirrer rotation this difference decreases. At $N_s = 150$ rpm and 300 rpm J_5 for PH is 5.78% and 5.55% higher than PI. This can be explained in terms of the shear produced by the stirrer rotation, which lowers the tendency of concentration polarization and consequently increases the flux value. Flux enhancement of PI is somewhat higher (about 15% at $N_s = 150$ rpm) than PH as the shear lifting velocity is more effective on larger molecules in PI. The effect of stirrer rotation in combination with membrane rotation on flux decline is shown in Fig. 7. The percent flux reduction between 5 min−60 min of operation, expressed as $(J_5 - J_{60}) \times 100/J_5$ under constant pH and TMP at various values of N_m and N_s as shown in the figure. From this figure it is clear that membrane rotation is more effective in reducing the flux decline in comparison to the stirring action. This observation is true for both PI and PH. In case of PI the flux reduction is 9.92% for the rotating membrane at $N_m = 150$ rpm with a fixed stirrer, 17.72% for the stationary

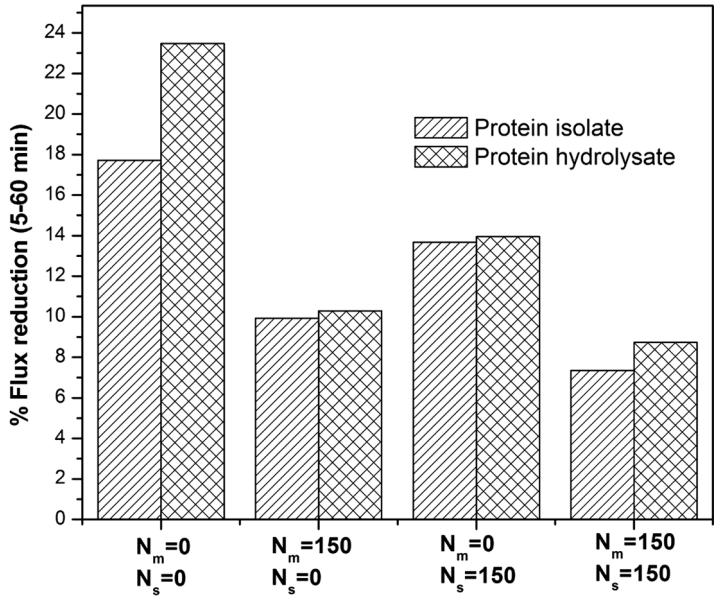

Figure 7. Percent flux reduction for protein isolate and protein hydrolysate under different combination of stirrer speed at fixed TMP and pH.

membrane, and 13.68% for the fixed membrane with stirrer rotation at 150 rpm. In the presence of both the stirrer and the membrane rotation (at 150 rpm) flux decline is much lower than all other cases, it is about 7.35%. In case of PH, flux decline is 23.47% for stationary membrane and that for the rotating membrane ($N_m = 150$ rpm) with a fixed stirrer is 10.28%. With a fixed membrane at $N_s = 150$ rpm flux reduction is 13.95% and in the presence of both the stirrer action and membrane rotation this value falls to 8.75%. From these observations it is clear that membrane rotation is much more effective in reducing concentration polarization as well as the resulting fouling of membrane. Membrane rotation is more effective as the high shear is produced in the vicinity of the membrane surface, which is not achieved from stirrer rotation.

Effect of Solution pH

In our observation another important variable is solution pH. Isoelectric point (IP) of the sesame protein being 4.9, observations are made at three different points, above IP at pH 7, below IP at pH 3, and at IP pH 4.9. Figure 8 shows the variation of the permeate flux for protein hydrolysate as a function of time at different pH value (inset is the same for protein

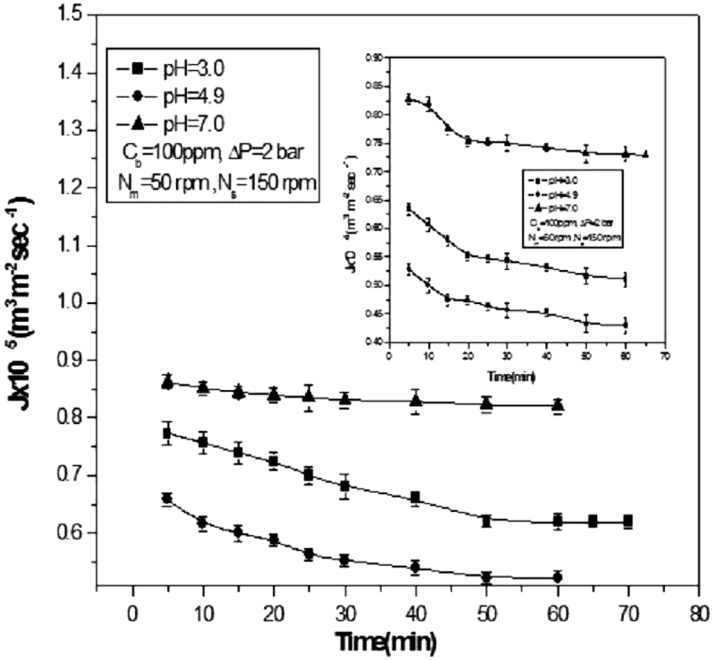

Figure 8. Variation of permeate flux as function of time at different pH value with fixed TMP, N_m and N_s for protein hydrolysate (Inset: The same for protein isolate).

isolate). Minimum flux is observed at pH 4.9 and maximum flux observed at pH 7. These observations can be explained in terms of the changing molecular sizes and shape of the protein molecules at different pH values of the feed solution and also concentration polarization on the membrane surface. In general protein adsorption and aggregation on the membrane surface is maximum at the isoelectric point (IP) because of the reduced isoelectric repulsion between themselves (25,26) and accumulates significantly on membrane surface, resulting in a significant fall in the permeate flux value. Another important point is that, permeability through the polarized layer near the membrane surface is lower at the isoelectric point as the protein molecules occupy small sizes and form a densely packed layer on membrane (27), thereby causing low permeate flux at isoelectric point. Some studies (28,29) also show that relatively high level of denaturation and aggregation takes place in acidic solution with pH value below IP. At such low pH values, the protein is more "bulky" with a part of the polymer chain "sticking out" (29,30). Due to these conformational changes, larger protein molecules accumulate on the membrane surface and offer a secondary membrane action and

lower the flux value. In comparison to the isoelectric point the flux value is higher, due to the more open structure of the polarized layer by the larger protein molecules. Since no such conformational changes take place at pH 7 the flux value is higher compared to both the flux values at pH 3.0 and pH 4.9.

Figure 9 shows the variation of rejection with time at different pH value. Rejection with time increases gradually with the progress of time. This is attributed to the increasing the polarized layer thickness, thus increasing the secondary membrane effect and providing more hindrance to the passage of solute molecules thereby increasing the rejection value. Figure 10 shows the variation of rejection at different solution pH, keeping other variables constant. This figure shows that the rejection value is minimum at pH 7 and maximum at pH 4.9. This can easily be explained on the basis of the permeate flux value. At pH 7 the flux value is maximum so a large fraction of the protein molecules pass through the

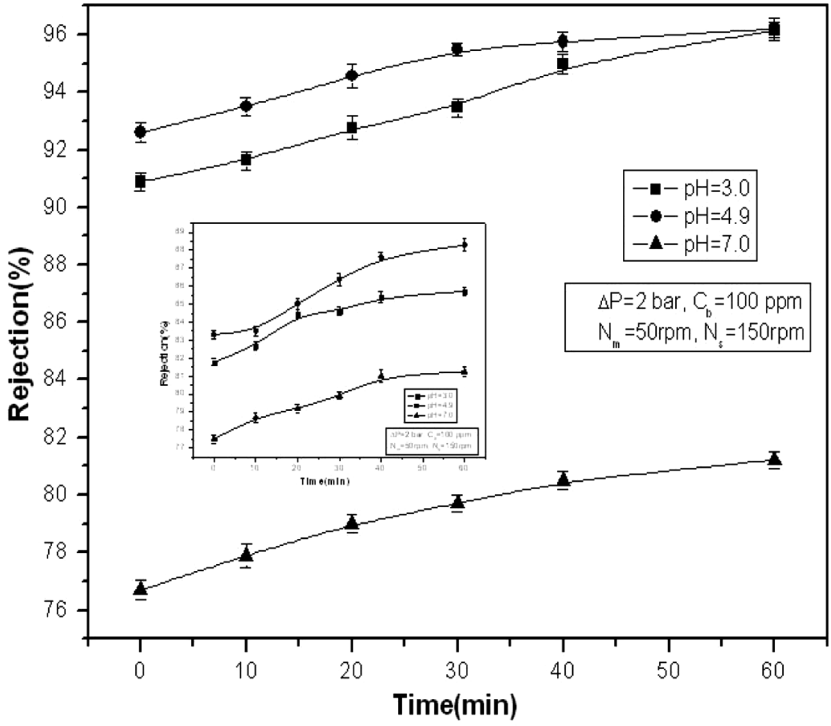

Figure 9. Variation of percent average rejection of protein hydrolysate as function of time at different pH, with fixed TMP, N_m and N_s. (inset: The same for protein isolate).

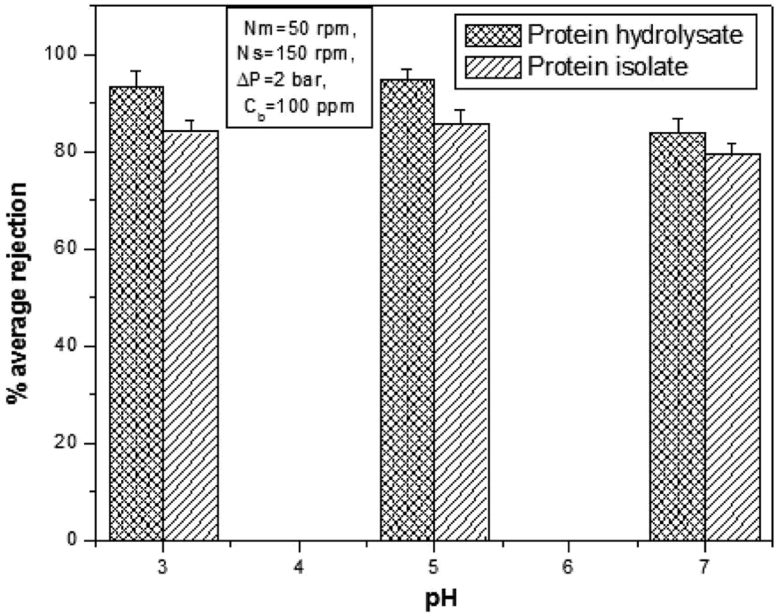

Figure 10. Variation of percent average rejection of protein isolate and protein hydrolysate as function pH, with fixed TMP, N_m and N_s.

membrane thereby lowering the rejection value. The flux value is minimum at the IP, as the polarized layer offers maximum resistance to the passage of the solute molecules, which ultimately increases the rejection value. In case of PI the percent average rejection values are 84.10%, 85.69%, 79.59% respectively at pH values of 3.0, 4.9, and 7.0. In case of PH the percent average rejection values are 93.29%, 94.68%, 83.72% respectively at the pH values as mentioned above. From this figure it is clear that the percent average rejection for protein hydrolysate is higher

Table 4. Sieving coefficient at different pH value under fixed operating conditions (TMP = 2 bar, $N_m = 50$ rpm, $N_s = 150$ rpm)

Protein isolate		Protein hydrolysate	
pH	S_o	pH	S_o
3.0	0.1533	3.0	0.0586
4.9	0.1382	4.9	0.0499
7.0	0.1979	7.0	0.1509

than the protein isolate. This is due to more pores plugging by the small protein molecules present in the protein hydrolysate compared to the large protein molecules present in the protein isolate.

The sieving coefficient (S_o), is a measure of permeation of solute through the membrane. It is defined by the ratio of concentration of solute in UF permeates to that in feed (C_p/C_b). The sieving coefficient of the sesame protein and protein hydrolysate at different pH values are shown in Table 4. At pH 4.9 sieving coefficient both for PH and PI minimum, supporting the phenomenon of minimum solute permeation at the isoelectric point.

CONCLUSION

In this work, a detailed investigation on the UF of sesame protein by the rotating disk membrane module have been presented with an objective of improving the functional properties of the protein isolate and fractionation of the small peptide fractions from the protein hydrolysate at the expense of minimum concentration polarization or fouling of membrane in a dead end membrane module. Since increasing turbulence in the feed lowers the problem of solute deposition, we have modified the dead end module by introducing stirrer rotation and membrane rotation. Membrane rotation was found to be more effective in reducing the concentration polarization and enhancing the permeate flux compared to the stirring action. A combined stirrer rotation and membrane rotation were found to be very effective in reducing the fouling phenomenon. The solution pH was also found to be an important factor to be considered during UF processing of the sesame protein. Maximum permeate flux and minimum rejection was achieved at pH 7. Minimum permeate flux and maximum rejection was achieved at the isoelectric point of the protein. An irreversible fouling of the membrane was observed during the ultrafiltration of the sesame protein. Further work regarding the determination of the molecular weight distribution of the permeate and retentate is in progress.

LIST OF ABBREVIATIONS

UF	Ultrafiltration
TMP	Transmembrane prssure
C_b	Bulk concentration of the feed
C_p	Permeate concentration
R_m	Hydraulic resistance
J	Flux

N_m Membrane rotation speed
N_s Stirrer rotation speed
PI Protein isolate
PH Protein hydrolysate
IP Isoelectric point
S_o Sieving coefficient

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