

Separation of Polyphenolics and Sugar by Ultrafiltration: Effects of Operating Conditions on Fouling and Diafiltration

Diqiao S. Wei, M. Hossain, and Zaid S. Saleh

Abstract—Polyphenolics and sugar are the components of many fruit juices. In this work, the performance of ultra-filtration (UF) for separating phenolic compounds from apple juice was studied by performing batch experiments in a membrane module with an area of 0.1 m² and fitted with a regenerated cellulose membrane of 1 kDa MWCO. The effects of various operating conditions: transmembrane pressure (3, 4, 5 bar), temperature (30, 35, 40 °C), pH (2, 3, 4, 5), feed concentration (3, 5, 7, 10, 15 °Brix for apple juice) and feed flow rate (1, 1.5, 1.8 L/min) on the performance were determined.

The optimum operating conditions were: transmembrane pressure 4 bar, temperature 30 °C, feed flow rate 1 – 1.8 L/min, pH 3 and 10 Brix (apple juice). After performing ultrafiltration under these conditions, the concentration of polyphenolics in retentate was increased by a factor of up to 2.7 with up to 70% recovered in the permeate and with approx. 20% of the sugar in that stream.. Application of diafiltration (addition of water to the concentrate) can regain the flux by a factor of 1.5, which has been decreased due to fouling. The material balance performed on the process has shown the amount of deposits on the membrane and the extent of fouling in the system. In conclusion, ultrafiltration has been demonstrated as a potential technology to separate the polyphenolics and sugars from their mixtures and can be applied to remove sugars from fruit juice.

Keywords—Fouling, membrane, polyphenols, ultrafiltration.

I. INTRODUCTION

POLYPHENOLICS are the most plentiful secondary metabolites in plants [1]. In apple juice, polyphenols are responsible for flavour, colour, bitterness, polymerized complex and astringency [2]. Polyphenols with their natural antioxidant ability can prevent oxidation of high-density lipids (HDL), remove low-density lipids (LDL) and they can fight against ulcer and cancer [1]. Because of the health-promoting effects the consumption of food and beverage has been increasing in the last decade and this trend is of vital importance to fruit and vegetable markets. The various waste streams from apple juice processing are good sources of

polyphenolic compounds, which can potentially be added as functional ingredients into foods and beverages with enhanced health benefits [3]. The current methods for polyphenol extraction involve organic solvents, such as methanol, ethanol and hexane. This method is safe and efficient, however, it involves high capital cost and the high temperature required to increase the extraction rate may denature the polyphenols [1].

Although they are effective, the extracts may contain residual solvents and considered unsafe for human consumptions [1]. Another method currently being used for large scale is superficial fluid extraction (SFE).

Recently efforts have been devoted to evaluate processes based on membrane filtration because of their potential advantages and possibility of avoiding toxic solvents in the separation [1]. The advantages of ultrafiltration-based process, are easy automation [4] and scale up [1], shorter process time [4], lower labour and energy costs, less waste disposal [5] and mild operation conditions [1]. However, membrane filtration has the disadvantage of fouling resulting in a decline of performance. Therefore, research continues to evaluate the process for separating polyphenols from sugars and to determine the operating conditions where the fouling effects are less. In this research the applicability of ultrafiltration for the separation and recovery of polyphenolics in apple juice is evaluated. The effect of the operating parameters: pH, temperature, concentration and feed flow rate on the membrane performance are investigated.

II. MATERIALS AND METHODS

A. Materials

Clear apple juice concentrate (EPAJC), with enhanced level of polyphenols and 75 °Brix sugar was supplied by ENZAFOODS New Zealand Ltd. Catechin and Folin Ciocalteu's phenol reagent (2N), BAK (Benzylalkonium Chloride) powder, anhydrous sodium carbonate (GR grade) was from Merck (Germany), sodium hydroxide pellets were from BDH (UK), phosphoric acid and ethanol (AR grade) were from Orica-Chemnet, New Zealand.

B. Membrane Apparatus

Ultrafiltration experiments in cross-flow mode were conducted to separate polyphenolics and sugar from apple juice (Fig. 1). The temperature of feed solution was measured using a thermometer and maintained by a 20-L hot water bath. Feed flow rate was controlled by the pump speed controller. Transmembrane pressure was controlled by the valve on the

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retentate side. The retentate from the membrane was recycled back to the feed and the permeate was collected in a separate bucket. Pellicon-2 regenerated cellulose membrane with area of 0.1 m² was supplied from Millipore, USA. It is a hydrophilic membrane with nominal MWCO of 1000 Da. The maximum operating temperature is about 45°C and the maximum operating pressure is about 5 bar. The membrane Mini Cassettes and Mini Cassette Holder are shown in Fig. 2.

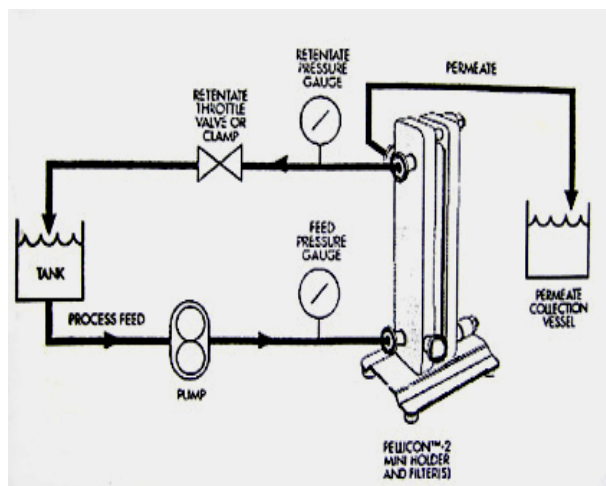


Fig. 1 A schematic of the experimental set up

C. Experimental Procedure

This investigation involved ultrafiltration of polyphenolic compounds from diluted apple juice. The steps involved in an experiment were: initial water flushing to remove the storage solution, measurements of water flux first, then filtration of feed solution and finally flushing with water followed by a cleaning solution. Feed solution was prepared to specific concentration, temperature and pH. Phosphoric acid was used to decrease the pH while sodium hydroxide is used to increase the pH. De-ionized water was used for diluting apple juice concentration.

The feed was pumped using a Hydro-cell G-03 series (shaft-driven) pump at feed flow rate of 1-2 L/min. Transmembrane pressure was controlled by the retentate valve to give a pressure of 2-5 bar. The permeate flow was measured by a measuring cylinder every minute until the permeate flow is constant and 2-5 minutes afterward. Samples were collected from initial feed solution, permeate and final feed solution (retentate) during filtration process.

Final water flushing was done to remove any sample solutions left on the membrane. The membrane was then cleaned by recycling 0.1M NaOH solution at 35°C for 30-60 minutes at a cross flow rate of 1.0-1.5 L/min at a TMP of 1 bar. After cleaning, membrane was stored in 0.1% BAK solution in the fridge, which is recommended by membrane supplier.

Sugar concentration was measured by an ATAGO digital hand-held Refractometer in terms of °Brix. The total content of mono- and polyphenolic was determined by using Folin-Ciocalteu reagent and the results were presented as catechin or gallic acid equivalents. The amount of major polyphenolic compounds were measured by RP-HPLC separation, using a

Phenomenex packed with Synergi 4μ Hydro RP 80 Å column (250 X 4.6mm). The analysis was at 35°C with a 40μl injection volume and at 1.5 ml/min using the binary mobile phases: (A) acetonitrile (water/acetonitrile/formic acid 92:5:3 v/v) and (B) acetonitrile (containing 0.1% v/v formic acid).

D. Calculation of Membrane Performance

The membrane performance is measured by concentration factor ($C_{Fi(R,P)}$) and recovery ($C_{i(R,P)}$) of a certain species. Concentration factor is the concentration of species *i* in either permeate or retentate solution divided by its concentration in the feed solution:

$$C_{Fi(R,P)} = \frac{C_{i(R,P)}}{C_{i(f)}} \quad (1)$$

Recovery (%) of a species *i* is obtained by total its mass in either permeate or retentate divide by its total mass in feed solution:

$$R_{i(R,P)}(\%) = \frac{C_{i(R,P)} \times V_{(R,P)}}{C_{i(f)} \times V_{(f)}} \times 100\% \quad (2)$$

III. RESULTS AND DISCUSSION

The effect of transmembrane pressure, feed solution temperature, pH, concentration and flow rate on separation and concentration of polyphenolics from apple juice were studied by using 1 kDa membrane. The operating variables examined included: transmembrane pressure of 3-5 bar, temperature of 30-40 °C, pH of 2-5, feed concentration of 3-15 °Brix and feed flow rate of 1-1.8 L/min.

The standard operating conditions are: transmembrane pressure 4 bar, temperature 35 °C, pH 4, feed concentration 5 °Brix and feed flow rate of 1 L/min. While comparing one of the operating conditions, the others are kept constant at the standard value.

Ultrafiltration was stopped when the volume concentration factor (VCR defined as the initial volume divided by the retentate volume) reached 4. Therefore, the process time varied according to different flux. All experiments were replicated, the mean values were reported and reproducibility was ca. ± 5%. The polyphenolics concentrations were measured by the Folin assay and the sugar content was measured in Brix by a refractometer. A mass balance of polyphenolics in the final retentate and permeate compared with the initial feed solution indicated that up to 4.57 gm of polyphenolics were bound per m² of membrane. Results are presented in Table I below.

TABLE I
RESULTS FROM ALL MEMBRANE SEPARATION EXPERIMENTS

Run	Process condition				Polyphenolics content (g/L)			Sugar content (°Brix)			Fouling deposits (gm ⁻²)
	pH	TMP (bar)	Feed flow rate (L/min)	Temp (°C)	F	Ret	Per	F	Ret	Per	
1	4	4	1	35	0.125	0.207	0.094	3.2	3.2	3	0.24
2	4	4	1	35	0.196	0.353	0.139	5.1	5.8	4.8	0.37
3	4	4	1	35	0.379	0.74	0.215	7.1	6.7	7.8	2.2
4	4	4	1	35	0.509	0.98	0.265	10	10.9	9.6	3.92
5	4	4	1	35	0.844	1.717	0.375	15.2	14.5	17.2	4.57
6	4	3	1	35	0.187	0.345	0.143	5	5.5	4.7	0.18
7	4	5	1	35	0.205	0.383	0.149	5.1	5.9	4.8	0.63
8	4	4	1	30	0.198	0.362	0.136	5	5.6	4.7	0.46
9	4	4	1	40	0.195	0.337	0.148	5.1	5.8	4.8	0.13
10	2	4	1	35	0.196	0.45	0.073	5.1	6.1	4.7	0.73
11	3	4	1	35	0.191	0.455	0.085	5	6.1	4.6	0.81
12	5	4	1	35	0.208	0.347	0.159	5.1	6.2	4.7	0.13
13	6	4	1	35	0.217	0.33	0.175	5.1	6.2	4.7	0.26
14	4	4	1.5	35	0.256	0.598	0.142	5.2	6	4.6	0.17
15	4	4	1.8	35	0.234	0.626	0.119	5.2	6.8	4.4	0.14

TMP = trans-membrane pressure, Temp. = temperature, F = feed, Ret = retentate, Per = permeate.

A. Effect of Trans-membrane Pressure

According to Darcy's law, pressure is the driving force for mass transfer through the membrane. The average permeate flux is therefore expected to be higher with increased transmembrane pressure, as shown in Fig. 2. However, flux could become independent of pressure if the pressure is beyond a critical point due to concentration polarization and membrane fouling [5].

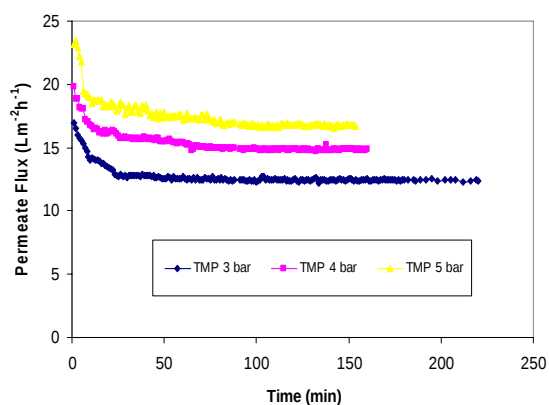


Fig. 2 Comparison of permeate flux at different transmembrane pressure for apple juice ultrafiltration

It is noticeable in Fig. 2 that when increasing transmembrane pressure from 3 bar to 5 bar, the permeate flux increases from 13 to 18 Lm⁻²h⁻¹. This is because the rate of deposition and fouling would be higher at a higher transmembrane pressure. This would compress the rejected solute into a denser fouling layer with increased fouling resistance [6].

Fig. 3 compares the effect of transmembrane pressure on the concentration factor and recovery of sugar and polyphenolics in permeate and retentate respectively. As transmembrane pressure was increased from 3 bar to 5 bar, the sugar concentration factor in the permeate decreased from 0.96 to 0.92 and recovery in the permeate decreased from 73% to 69%. In contrast, the polyphenolics concentration factor in the retentate increased from 1.68 to 1.85 and recovery increased from 41% to 47%. The reason for this result is that more fouling formed at a higher transmembrane pressure which can prevent solute flowing through the membrane. This is in accordance with flux decline as illustrated in Fig. 2. Increasing the transmembrane pressure from 3 to 5 bar increased the amount of polyphenolics deposited on the membrane by a factor of 3.5, as presented in Table I. Therefore, the optimum separation achieved when the transmembrane pressure is between 3 - 4 bar.

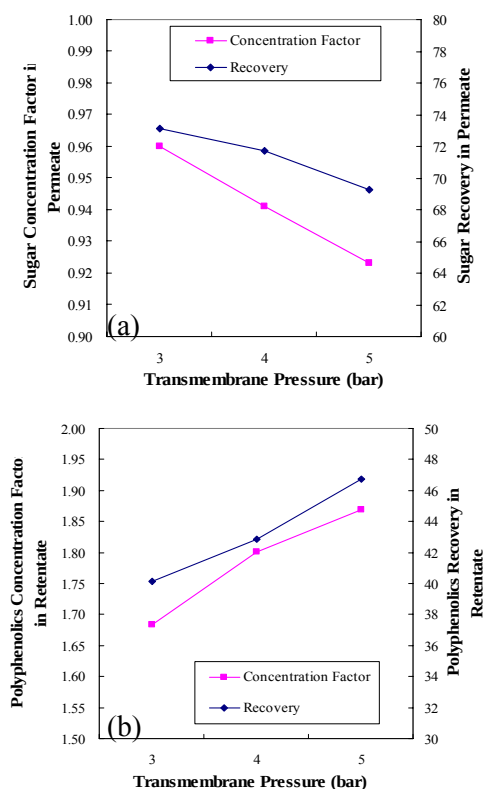


Fig. 3 Effect of TMPs on concentration factor and recovery (a) of sugar in the permeate and (b) polyphenolics in the retentate

B. Effect of Temperature

As mentioned before, temperature can influence membrane filtration by altering the fluid characteristics which produce deposits. The viscosity of fruit juice is inversely related to temperature; therefore it is important to study the temperature effect on apple juice viscosity and filtration efficiency [7].

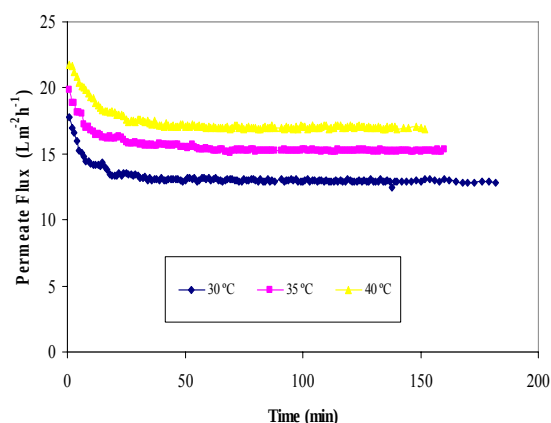


Fig. 4 Comparison of permeate flux at different temperature for apple juice ultrafiltration

The changes of permeate flux during the filtration process are illustrated in Fig. 4. The flux declined immediately after

starting the process and it accounts for 60-70% of the total decline during the first 10 minutes.

The lower the temperature, the faster the flux declined. It has been claimed that the flow regime may have changed at a higher temperature due to the increasing turbulence and cross flow velocity which can provide higher shear force to remove fouling solutes [8].

The effects of temperature on the separation of sugar and polyphenolics are shown in Fig. 5. As temperature is increased from 30 °C to 40 °C, the concentration factor and recovery of sugar in the permeate was almost the same, whereas both the concentration factor and recovery of polyphenolics in the retentate decreased by a factor of 1.1. Although regenerated cellulose membrane is stable to temperature [9], the lower temperature could favour the formation of insoluble aggregates and a secondary membrane which would restrict the flow through the membrane [7]. According to Table I, the amount of polyphenolics fouled on the membrane decreased from 0.46 to 0.13 gm^{-2} . The optimum temperature is therefore considered as 30 °C

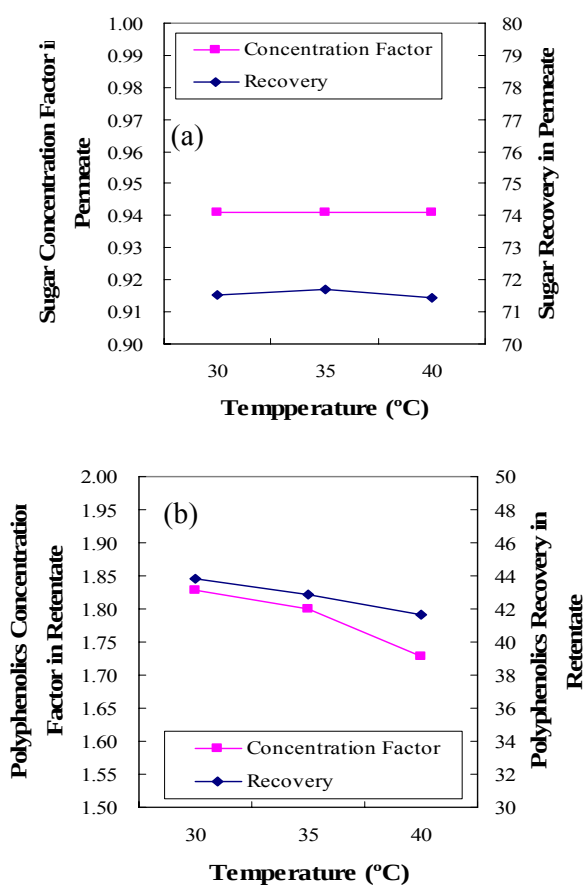


Fig. 5 Effect of temperature on concentration factor and recovery (a) of sugar in the permeate and (b) polyphenolics in the retentate

HPLC analyses (Fig. 6) revealed that, although there was a significant increase in the concentration of phenolic compounds on the retentate side, the composition of the retentate and permeate fractions were similar.

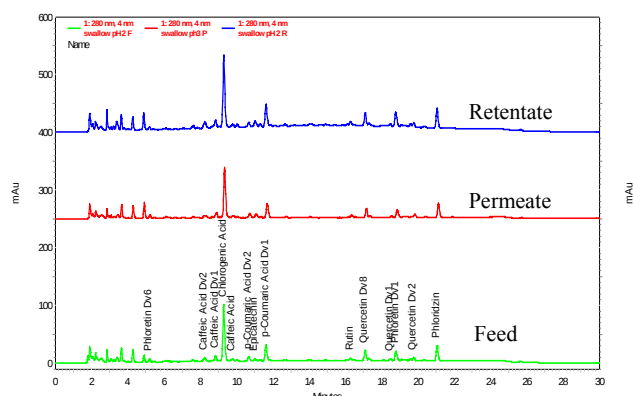


Fig. 6 HPLC chromatograms (A280nm) for feed, retentate and permeate from apple juice ultrafiltration

C. Effect of pH

The best separation of polyphenolics and sugar occurred at pH 3, in which the polyphenolics concentration factor and recovery achieved 2.4 and 60% respectively while 69% of sugar had been removed from the retentate. The amount of polyphenolics deposited on the membrane at pH 3 is 0.81, which is 5.3 times more than at pH 5. As a result, the optimum pH is suggested to be 3. However, this is in contrast with a previous study which found that the juice filterability is poor at acidic pH but improves dramatically at pH 7.5 [10].

D. Effect of Feed Concentration

The effect of feed concentration is very important in the filtration process because the ultrafiltration process is very sensitive to a critical concentration of moderately high molecular weight molecules [11].

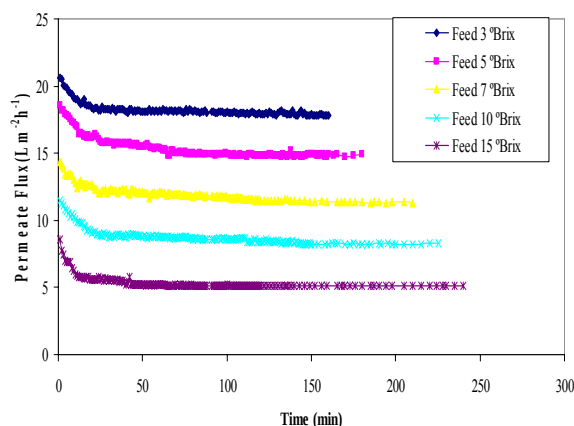
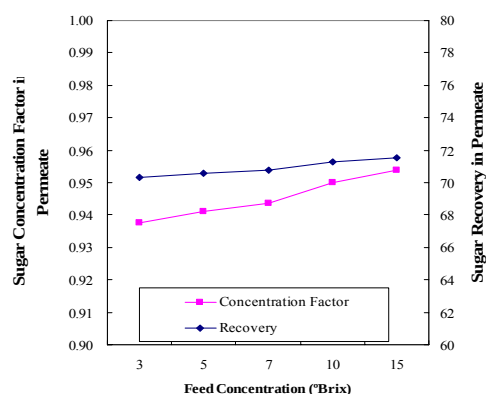


Fig. 7 Comparison of permeate flux at different feed concentration for apple juice ultrafiltration

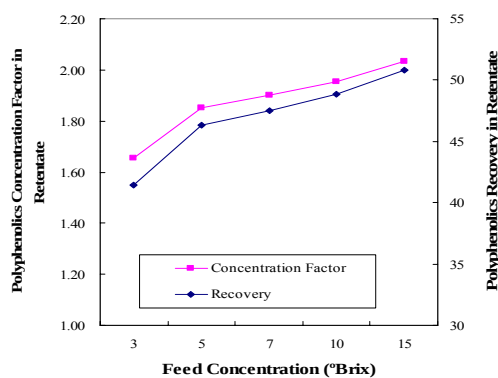
Fig. 7 illustrates the permeate flux for different feed concentrations, starting from 3 °Brix to 15 °Brix. The flux declined more rapidly at the start of the experiment at higher feed concentration. By increasing the feed concentration from 3 °Brix to 15 °Brix, the flux decline increased by a factor of

1.8 and the amount of polyphenolics deposited on the membrane surface increased by a factor of 19 (Table I).

The effect of feed concentration in terms of °Brix on separation of sugar and polyphenolics is presented in Fig. 8. By increasing the feed concentration from 3 °Brix to 15 °Brix, the polyphenolics concentration factor in the retentate increased from 1.65 to 2.05, and its recovery in retentate increased from 41.4 % to 50.9%. However, the effect of feed concentration on sugar recovery in the permeate is not as profound as on the polyphenolics recovery in the retentate, because the concentration factor and recovery for sugar in the permeate increased with increasing feed concentration by a factor of only 1.02. Although the separation of polyphenolics and sugar is best at the highest feed concentration of 15 °Brix, the trade off is the highest flux decline and low steady state flux which can shorten the continuation of the process time and reduce process efficiency. For this reason, an optimum feed concentration of 10 °Brix is suggested for ultrafiltration of apple juice.



(a)



(b)

Fig. 8 Effect of Feed Concentration on concentration factor and recovery (a) of sugar in the permeate and (b) polyphenolics in the retentate

E. Effect of Feed Flow Rate

The effect of feed flow rate is important in the membrane filtration process because a higher flow cross flow rate can reduce membrane fouling by providing a shear force to sweep

away deposited materials [5]. This can slightly increase the retention of most components. However, at a higher flow rate, the product passes through the pump more in a given period. This can lead to degradation of product quality. In addition, a higher flow rate requires larger pumps and piping, which increase the system hold-up volume and product loss. Therefore, it is important to choose a flow rate which can balance the increase in flux with the increase in pump passes and hold-up volume.

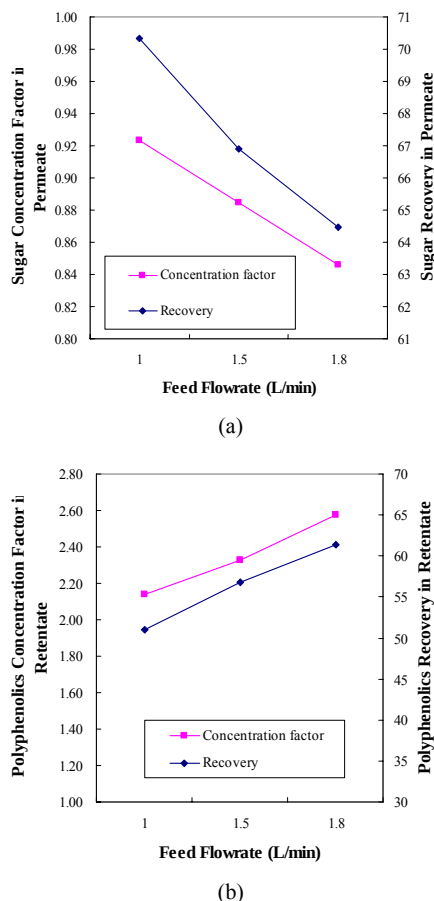


Fig. 9 Effect of Feed Flow rate on concentration factor and recovery (a) of sugar in the permeate and (b) polyphenolics in the retentate

The separation of polyphenolics and sugar from apple juice at different feed flow rate is depicted in Fig. 9. The effect of feed flow rate on permeate flux was found to be little. In this range of flow rate (1-1.8 L/min) the internal fouling is small and independent of flow rate yielding very similar values of flux [12]. As the feed flow rate is increased from 1 L/min to 1.8 L/min, both the polyphenolics concentration factor and recovery in the retentate were increased by a factor of 1.2. However, the trade off is less sugar being separated into the permeate stream. Therefore the optimum feed flow rate is suggested to be 1.5L/min.

F. Effect of Feed Volume

The normal experiments were carried out with 6 litre feed solution, finishing with 1.5 litre retentate. All the other

operating parameters were kept constant during this run. In order to determine whether it is practicable to scale up the process capacity, an 18-litre feed solution was compared with a 6-litre feed solution as shown in Fig. 10. It can be seen that the permeate flux decline was the same for both experiments during the first 20 minutes. It reached a quasi-steady state after about 50 minutes for the 6-litre trial, whereas it kept on declining for the 18-litre trial.

The experiments were expected to stop when the VCR achieved 4; however, in the 18-litre trial, the experiment stopped when fouling was too serious, that is, the feed pressure exceeded 9 bar when the retentate valve was fully opened. Compared with the 6-liter trial, the total flux decline for the 18-litre trial was $11.4 \text{ Lm}^{-2}\text{h}^{-1}$ and increased by a factor of 4. More fouling formed during the 18-litre trial due to the larger feed volume which has more fouling materials and leads to exceeding the membrane working capacity. However, the surface area of this membrane is only 0.1 m^2 . By using a larger membrane, ultrafiltration with a greater feed volume can be achieved.

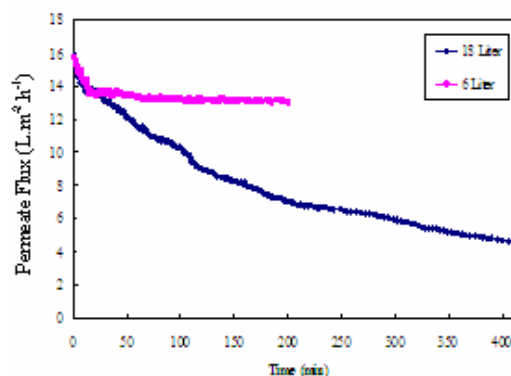


Fig. 10 Comparison of permeate flux for 6-liter and 8-liter feed solution

G. Fouling Rate

The concentrations of polyphenolics in both permeate and retentate were measured during the apple juice filtration process under standard operating conditions, i.e. a transmembrane pressure of 4 bar, temperature 35°C , feed concentration 5°Brix , feed flow rate 1L/min and pH 4, as illustrated in Fig. 11. Since the permeate volume was also recorded at each measurement time, the fouling mass and fouling rate at each measurement time were obtained from a mass balance of polyphenolics in the final retentate and permeate compared with the initial feed.

The accumulation of fouling mass and fouling rate during the filtration process is shown in Fig. 12. The fouling mass curve suggests that during the initial 20 minutes, fouling increased dramatically. After that it increased at a much faster rate, and after 75 minutes it increased linearly. The increase pattern of fouling mass can be explained by the fouling rate, which decreased with time exponentially. The initial high fouling rate was due to internal fouling which occurred immediately after filtration process. When the concentration polarization of the gel layer formation became the dominant

factor in fouling, the fouling rate gradually reached a steady level [5]. As a results the concentration of polyphenolics in the retentate increased exponentially whereas it in the permeate is increased much more slowly as shown in Fig. 11.

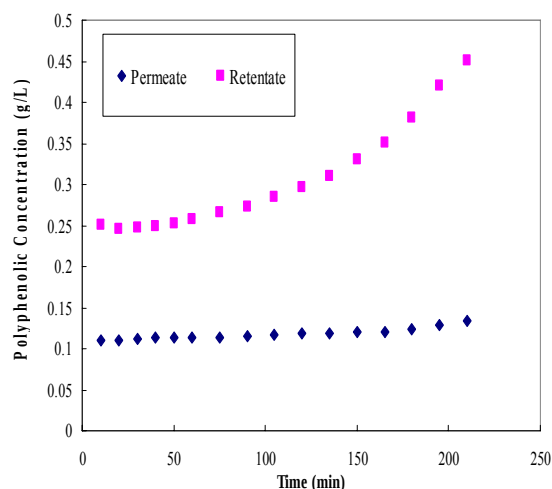


Fig. 11 Polyphenolics concentrations in retentate and permeate during apple juice filtration process

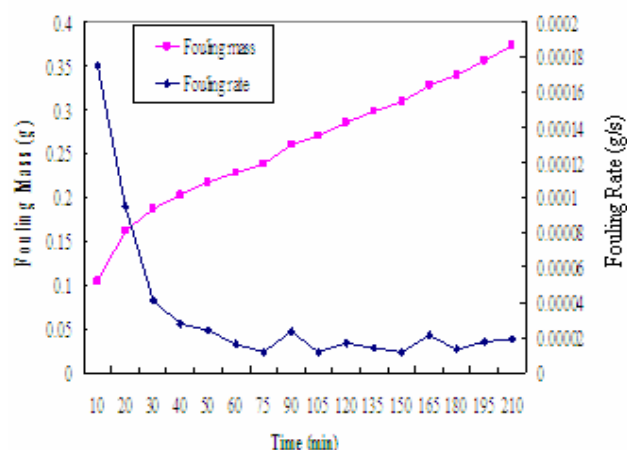


Fig. 12 Fouling and fouling rate during apple juice filtration process

H. Diafiltration of Apple Juice

Apple juice diafiltration was conducted at the optimum operating conditions of TMP 4 bar, temperature 35 °C, pH 3, feed concentration 10 °Brix and feed flow rate 1.5 L/min. The permeate flux declined immediately after starting the process. By diluting the retentate, i.e. by adding pure water to the retentate at 1 to 1 proportion, flux can be recovered. Flux decline also decreased from step 1 to diafiltration step2, indicating less and less fouling formed at each step (Fig. 13). In diafiltration, the percentage removal was 1.5 times higher. The overall process produced a retentate stream of approx. 54% polyphenolics and 21% sugar, and a permeate stream of 45.5% polyphenolics and 79% sugar.

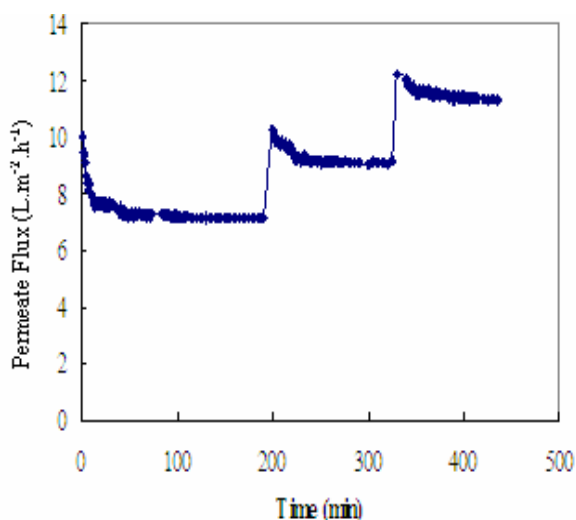


Fig. 13 Permeate flux of apple juice during diafiltration

I. Membrane Surface Analysis (SEM)

The 1st membrane was cut to obtain its the surface and cross section. Samples were dried in the desiccator then coated by a Polaron SC7640 Sputter Coater from VG Microtech. All samples were coated for three minutes using 1.5 kV and plasma current of 5 mA. Before coating, all samples were rinsed with purified water to remove sodium hydroxide which may crystallize under vacuum. Membrane surface morphology was studied using Philips XL30 S FEG Scanning Electron Microscope (SEM), along with Energy Dispersive X-ray Spectroscopy (EDS).

Fig. 14 shows the surface of regenerated cellulose membrane under 500 magnification. Since polyphenolic compounds consist mainly of carbon and hydrogen, fouling deposits on the membrane surface are confirmed to be polyphenolics, using EDS spectrum.



Fig. 14 SEM image of the surface of regenerated cellulose 1kDa membrane under 500 x magnifications

IV. CONCLUSION

The investigation of separation of polyphenolics and sugar from apple juice led to following conclusions:

- ✧ Flux increased with increasing transmembrane pressure up to a certain limit; thereafter the increase was minimal and the optimum value of TMP was found,
- ✧ Flux increased with increasing feed temperature, the optimum was around 30°C. The optimum pH was found to be 3 as the retention of polyphenolics was higher at this pH,
- ✧ Flux declined with increasing feed concentration. By increasing the feed concentration from 3 °Brix to 15 °Brix, the polyphenolics concentration factor in retentate increased from 1.65 to 2.05. The optimum feed concentration was suggested to be 10 °Brix,
- ✧ At high feed flow rate, more polyphenolics was retained by the membrane with less sugar permeating through it. The optimum feed flow rate was 1.5L/min,
- ✧ Fouling was reduced by increasing feed temperature and by reducing feed flow rate, pH, feed concentration and transmembrane pressure,
- ✧ Application of diafiltration recovered the flux, approx. 54% of polyphenolics and 21% of sugar was retained in the retentate after diafiltration,
- ✧ Ultrafiltration has been demonstrated as a potential technology to separate the polyphenolics and sugars from their mixtures and can be applied to remove sugars from fruit juice.

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