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Fouling evaluation on nanofiltration for concentrating phenolic and flavonoid compounds in propolis extract

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Abstract. Nanofiltration is useful to concentrate propolis extract. During the selection of membrane, both compound rejection and permeate flux are important indicators of process economy. Brazilian green propolis extract was studied to evaluate the separation performance of Startmen 122 and NF270 membranes. Compared to Starmen 122, NF270 membrane showed better rejection of bioactive compounds. The flux decline patterns were further studied using Hermia's model. Cake formation is the major fouling mechanism on the hydrophobic surface of Starmen 122. While the fouling mechanism for NF270 is pore blocking. The fouled membranes were further characterized using SEM and FT-IR to confirm on the predicted fouling mechanisms.

Keywords: nanofiltration; fouling; propolis; polyphenols; flavonoids

1. Introduction

Propolis is of great interest to neutraceutical industry because it displays a wide spectrum of bio-active composition (Farooqui and Farooqui 2012). It is a popular functional ingredient which has been extensively used in beverages, health foods, nutritional supplements and cosmetics products. Unlike honey, propolis cannot be directly consumed. The bioactive compounds in the crude propolis have to be separated from the wax, ashes and other undesired components via solvent extraction. The advancements in propolis extraction such as the application of tensoactive compounds (Konishi *et al*. 2004) and the induction of hydrolysis reaction (Mello and Hubinger 2012) have been developed. Regardless of which method to be used, the solvent in propolis extracts must be reduced or eliminated to produce the concentrated extract. Traditionally, vacuum distillation, evaporation or lyophilization is used to concentrate propolis extract (Krell 1996). The mentioned techniques not only consume great energy, they also involve high temperature which may cause the degradation of bioactive compounds especially the phenolic and flavonoids compounds in the propolis extract.

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The use of membrane in food concentration attracted a lot of attention recently because it involves low temperature, single phase and reasonable energy requirement (Matta *et al*. 2004). Nanofiltration had been successfully used to purify and concentrate wine (Banvolgyi *et al*. 2006), xanthophyll (Tsui and Cheryan 2007) and juices (Díaz-Reinoso *et al*. 2009). Nanofiltration had also been applied in propolis researches that focused on the concentration (Tylkowski *et al*. 2010, Mello *et al*. 2013) and fractionation (Tsibranska *et al*. 2011) of propolis extract. Propolis contains bioactive compounds with molecular weight ranging from about 180 to 410 g/mol (Tsibranska *et al*. 2011). Nanofiltration fits well in such separation requirements because nanofiltration membranes possess the molecular weight cut off (MWCO) of 1000 Da and below. Using hydrophilic NF90 membrane, Mello *et al*. (2010) concluded that water extracted propolis could be concentrated faster than ethanol extracted propolis (80% ethanol solution). Both concentrated extract samples showed the similar polyphenol content although the water extracted propolis possessed much lower polyphenol content initially. Mello and co-workers (Mello *et al*. 2013) continued on the similar work using GE EZ2 Reverse Osmosis Kit and a spiral wound membrane module made from polyamide and polysulfone. The membranes possess an effective permeation area of 1.2 m^2 and a MWCO ranging from 150 to 300 g/mol. The concentration of aqueous extracted propolis was studied under the effects of temperature, pressure and pH in a central composite design of experiments. The flux decline patterns were further studied using Hermia models and they suggested that pore blocking instead of cake formation is predominant in aqueous propolis nanofiltration without much investigation on the membrane characteristics. Tylkowski and colleagues (Tylkowski *et al*. 2010) used the solvent resisted membranes, Starmen 122 and DURAMEM 200 to concentrate ethanol extracted propolis (70% ethanol solution). Operating at 30 and 50 bar, DURAMEN 200 showed 95% rejection of phenolic compound and nearly 2 times higher flux than Starmen 122. Both membranes exhibited surface fouling, which could be resulted from the accumulation of waxes, fatty acids, essential oil, pollens and minerals. In their further study (Tsibranska *et al*. 2011), DURAMEN membranes with higher MWCO were tested. Although surface fouling was not observed, only fraction of active compounds were rejected during nanofiltration.

From literatures mentioned earlier, membrane fouling is found to be the upmost important research topic before scaling up the nanofiltration process to concentrate propolis extract (Van der Bruggen *et al*. 2008). Propolis extract is a heterogeneous mixture of various groups of compounds (Cheng *et al*. 2013). At the present, most membrane fouling studies (Mah *et al*. 2012, Cathie Lee *et al*. 2014) focus on the well-characterized single foulant such as humic acid, oleic acid and phosphorus, with rather homogenous or well-defined properties. These studies do not yield the complete understanding of actual fouling mechanism, especially the concentration process of propolis extract. As mentioned earlier, the findings of Mello and coworker (Mello *et al*. 2013) are not universally true because it only studied the fouling mechanism for water extracted propolis. The fouling mechanisms are best explained if the membrane characteristic before and after nanofiltration could be also studied. The objective of this study is to investigate the fouling phenomena when nanofiltration is applied for the concentration of ethanol extracted propolis. Such understanding was achieved by investigating the propensity of permeate flux and rejection of Brazilian green propolis which will then be fitted with the Hermia's model to postulate the fouling phenomenon that occurred. Furthermore, the membranes are characterized to confirm the postulation.

Table 1 Specification of nanofiltration membranes

Membrane	Starmem™ 122	NF270
Manufacturer	Membrane extraction technology	Dow-FilmTec
Materials	Polyimide	Polyamide, Polysulfone (support)
MWCO (Da)	220	150-200
Average pore diameter (nm)	-	0.84 (Nghiem *et al*. 2004)
Pure water permeability ($L/m^2.hr.bar$)	-	13.5
Max. temperature (℃)	50	45
Max. pressure (MPa)	6	4.1
Recommended pH range	-	3-10
Water contact angle (°)	71	30

2. Experimental

2.1 Materials

Brazilian green propolis extract was acquired from Eu Yan San International Limited. Methanol, ethanol and sodium carbonate were purchased from Merck (Darmstadt, Germany), Folin-ciocalteu reagent and aluminum chloride from Sigma-Aldrich (St. Louis, MO). Laboratory standard of gallic acid and quercitin were purchased from Sigma-Aldrich (St. Louis, MO).

Two membranes were used to carry out the dead-end filtration, namely Starmem™ 122 and NF270. The surface hydrophilicity of these membranes was determined using a goniometer (Ramé-Hart Instruments Co.). The surface and cross-section of these membrane samples were studied using a scanning electron microscope (SEM, Quanta FEG 450 Oxford Instrument, Netherlands) after fractured using liquid nitrogen. The membranes were also characterized using a Fourier Transform Infrared (FT-IR) spectrometer (Nicolet iS10, Thermo Scientific, USA) to detect the foulant accumulated on the membranes. The specifications of the membranes are stated in Table 1.

2.2 Experimental setup and procedures

The dead-end filtration was conducted using a dead-end stirred cell (HP4750, Sterlitech Corporation, USA) pressurized by nitrogen and a stainless steel reservoir. The diameter of the membrane is 4.9 cm with an effective area of 14.9 cm^2. Before filtration, the new membrane was soaked overnight in deionized water. Then, it was placed in the stirred cell and 300 ml deionized water was allowed to flow through the membrane at high pressure for compaction purpose. The stirring rate was kept constant at 350 rpm.

For the filtration of the propolis extract (5 v/v% in ethanol solution with 30wt% of deionized water), 200 ml of feed solution was pressurized using nitrogen gas operating pressure for 3 hours and the stirring rate was kept constant at 350 rpm. The permeate (V_p) was continuously collected and measured by time (t). Then, the permeate flux (J) was determined by using Eq. (1).

$$J = \frac{V_p}{t.A} \tag{1}$$

2.3 Determination of total polyphenols content

The total polyphenols content of the propolis extracts was determined using the Folin-Ciocalteu method. Two hundred and fifty microliter of the propolis extract was mixed with 250 μL of Folin-Ciocalteu (diluted in water, 1:1) and 500 μL of 20% Na_2CO_3. The absorbance was measured at 760 nm after 30 min of incubation at room temperature and centrifugation. A blank was prepared using 70% ethanol in water. The total phenolic content in samples was determined in term of gallic acid equivalent (GAE) concentration using a calibration curve of 50-250 mg GAE/L (y = 0.0046x + 0.029; r^2 = 0.977). The analyses were done in triplicate.

2.4 Determination of total flavonoids content

The modified aluminum chloride colorimetric method was applied for the estimation of the flavonoids content. 500 μL of propolis extract were mixed with 250 μL of $AlCl_3$ (5wt.% in methanol) and 4.25 mL of methanol were added. The mixture was incubated under room temperature for 30 min before the absorbance was measured at the wavelength of 425 nm. Seventy percent of ethanol was used to substitute quercetin for the blank solution. The total content was determined using a calibration curve of 10-100 mg Quercitin/L (y = 0.0069x + 0.0158, r^2=0.9984). The analyses were done in triplicate.

2.5 Rejection of total phenolic content and flavonoids

After determining the total phenolic content and flavonoids contents of the feed (C_f) and permeate collected after 1 hr (C_p), the rejections (R) for both phenolic compounds and flavonoids were determined using Eq. (2).

$$R = \frac{C_f - C_p}{C_f} \times 100\% \tag{2}$$

2.6 Hermia's model data fitting

Hermia's models can accurately predict the permeate flux decline at different experimental condition (Vela *et al*. 2008, Salahi *et al*. 2010). Hermia's model is applicable for dead-end filtration at constant pressure and the fitting equations of this model were summarized in Table 2. The equations are expressed in terms of permeate flux (J_p), pure solvent flux (J_0), correlation coefficient (K) and time (t).

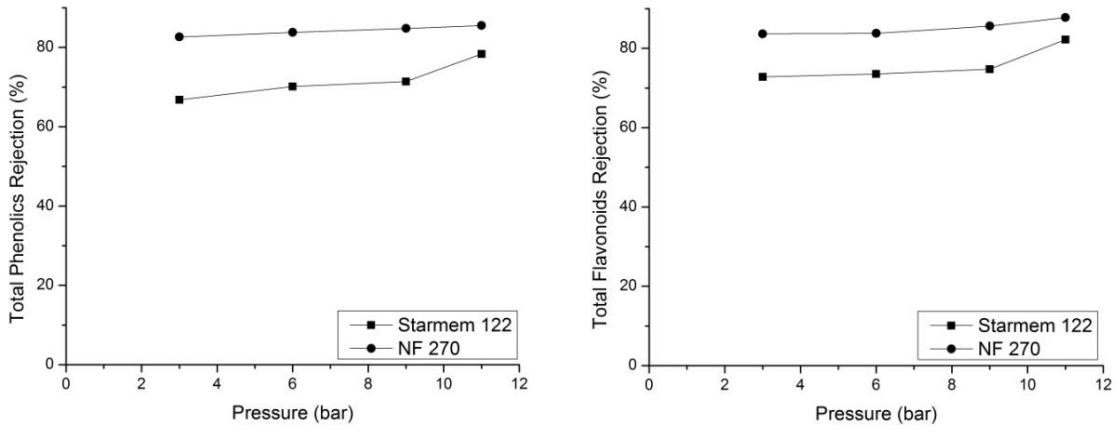
Table 2 Linearized equations of Hermia's model

Fouling mechanisms	Linearized equations	Equation
Complete blocking	$lnJ_p = lnJ_o - K_c t$	(3)
Intermediate blocking	$\frac{1}{J_p} = \frac{1}{J_o} + K_i\, t$	(4)
Standard blocking	$\frac{1}{\sqrt{J_p}} = \frac{1}{\sqrt{J_o}} + K_s\, t$	(5)
Cake layer formation	$\frac{1}{{J_p}^2} = \frac{1}{{J_o}^2} + K_{cl} t$	(6)

3. Results and discussion

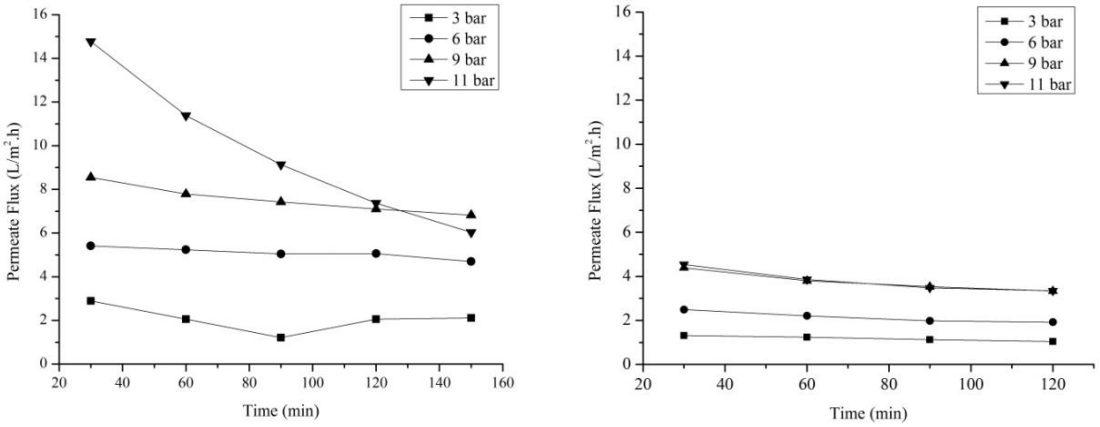
3.1 Rejection study on bioactive compounds in propolis extract

During the rejection study on the phenolic and flavonoid compounds in propolis extract, the feed temperature was kept constant throughout the nanofiltration experiments. Fig. 1 showed that the rejection of phenolic and flavonoids compounds increased for Starmem™ 122 and NF270 membranes when the operating pressure was increased. The similar trend was also observed by Conidi *et al*. (2011), whom had carried out similar research on bergamot juice. The rejection of bioactive compounds by the nanofiltration membranes usually increases with pressure due to the formation of fouled surface with smaller pore size for enhanced rejection. Within the operating pressure range of 3-11 bar, NF270 membrane exhibited the rejection of phenolic and flavonoid compounds more than 80%. Starmem™ 122 membrane showed a lower rejection of phenolic and flavonoid compounds compared to NF270 member, only in the range of 66-79% and 72-83% respectively. Tylkowski and colleagues (2010) reported better results during the study on nanofiltration of propolis extract using Starmem™ 122 membrane. They achieved satisfactory rejection up to 88% for both phenolic and flavonoids compounds, but a high operating pressure of 30 bar was applied. The increment of pressure as high as 50 bar could yield a higher rejection of 89%, but such improvement is uneconomical. In this study, the satisfactory rejection of both phenolic and flavonoid compound as much as 88% was achieved at a low pressure of 11 bar using NF270 membrane which is more economically favorable. The retention of solutes by nanofiltration membranes is commonly governed by the following mechanisms, e.g., steric hindrance, electrostatic and hydrophobic interactions. Steric hindrance, or size exclusion, appears to be the most prevalent mechanism affecting the solute retention and the occurrence of membrane fouling (Nghiem and Hawkes 2009). Membrane with lower MWCO tends to have greater sieving effect as more solutes are hindered by the membrane from passing through, resulting higher solute rejection than membrane with higher MWCO. Since NF270 membrane possesses a lower MWCO compared to Starmem™ 122, it resulted in the higher rejection of phenolic and flavonoid compounds.



(a) (b)

Fig. 1 The rejection of total phenolic and flavonoid compounds using Starmen 122 and NF 270 membranes



(a) NF270 (b) Starmen™ 122

Fig. 2 The flux decline during propolis nanofiltration using NF 270 and Starmen™ 122 membranes

3.2 Flux decline study

Fouling occurs during the concentration process using nanofiltration membranes and the occurrence of fouling could result in the flux decline as shown in Fig. 2. The permeate flux of propolis filtration was monitored throughout the nanofiltration conducted at varied operating pressure using the initial feed concentration of 0.5%. Generally, the permeate flux decreased more significantly at higher pressure for both membranes. For NF270 membrane operated at 11 bar, the steep decrease in permeate flux was initially observed and it was due to concentration polarization (Prudêncio *et al*. 2012). Meanwhile, the gradual decrease in flux at the later stage of nanofiltration was believed to be caused by the fouling occurred on the membrane surface during filtration process (Stroller 2011). As shown in Fig. 2, the permeate flux of NF270 membrane only increased with the increasing operating pressure until 9 bar. The limitation may be due to fouling on NF270 after 120 min. The higher operating pressure could also induce more severe flux decline for Starmem™ 122 membrane. Even though comparatively the flux decline of Starmem™ 122 membrane was less than NF270 membrane, NF270 membrane showed a higher permeate flux than Starmem™ 122 membrane. The great permeability of NF270 membrane could be related to its hydrophilic surface measured in term of water contact angle (Table 1). However, the occurrence of fouling which caused by the deposition of solutes will alter the membrane surface properties, consequently affecting the solute rejection by the membrane in the long term operation (Dolar and Košutić 2013). The fouling mechanism of the studied membranes will be addressed in details through fitting of Hermia's model in the following section.

3.3 Fouling mechanisms during nanofiltration of propolis extract

Four different fouling mechanisms were proposed in Hermia's model, namely complete blocking, intermediate blocking, standard blocking and cake layer formation. The fitting of the model with the permeate flux pattern of Starmem™ 122 and NF270 membranes was shown in Figs. 3 and 4. The fitting of the model with the experimental data were measured through the correlation coefficient, R^2. Based on Table 3, the cake layer formation model is the best model to

describe the fouling mechanism for Starmem™ 122 membrane at varied pressure, while the complete blocking model fits poorly with the experimental result. The poor fitting of all fouling equations at 3 bar may be due to the insignificant fouling on Starmem™ 122 membrane at low operating pressure. Cake layer formation on Starmem™ 122 membrane could result in the increment of membrane resistance for mass transfer. Hence, Starmem™ 122 membrane showed low permeate flux during propolis filtration as discussed earlier. For NF270 membrane, the cake layer formation model was not the best model to describe the fouling mechanism at varied pressure as shown in Table 4. It is apparent that pore blocking caused the fouling of the membrane when the phenolic and flavonoid compounds were well rejected by NF270 membrane.

The factors of fouling mechanism are membrane properties, solution properties and operating condition. In this study, the interaction between the selected membranes and compounds in propolis extract was the major factor of fouling. There are more than 300 compounds that have been identified in propolis including flavonoids, phenolic acids and phenolic acid esters which are reported as the major components in propolis (Bankova 2005). However, its composition varied in the propolis samples collected from different botanical origin. Brazilian green propolis contains major components of artepillin C, isoprenylated ρ-coumaric acid derivatives and flavonoids

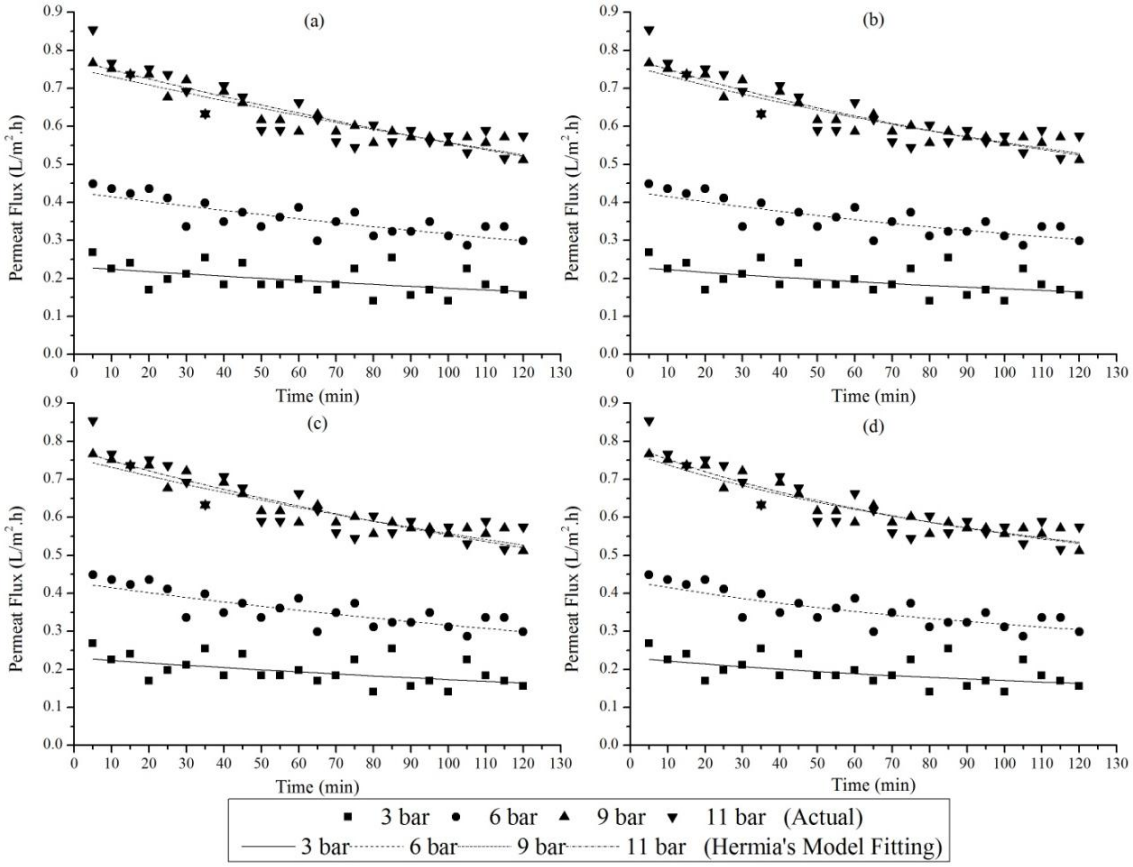


Fig. 3 Hermia's model fitting for Starmem™ 122 membrane: (a) complete blocking; (b) intermediate blocking; (c) standard blocking; and (d) cake formation

Table 3 The coefficient of determination for Starmem™122 membrane

Pressure (bar)	R^2			
	Complete blocking	Intermediate blocking	Standard blocking	Cake layer formation
3	0.5227	0.5272	0.5250	0.5315
6	0.8242	0.8339	0.8293	0.8421
9	0.9410	0.9482	0.9448	0.9533
11	0.8927	0.9057	0.8997	0.9162

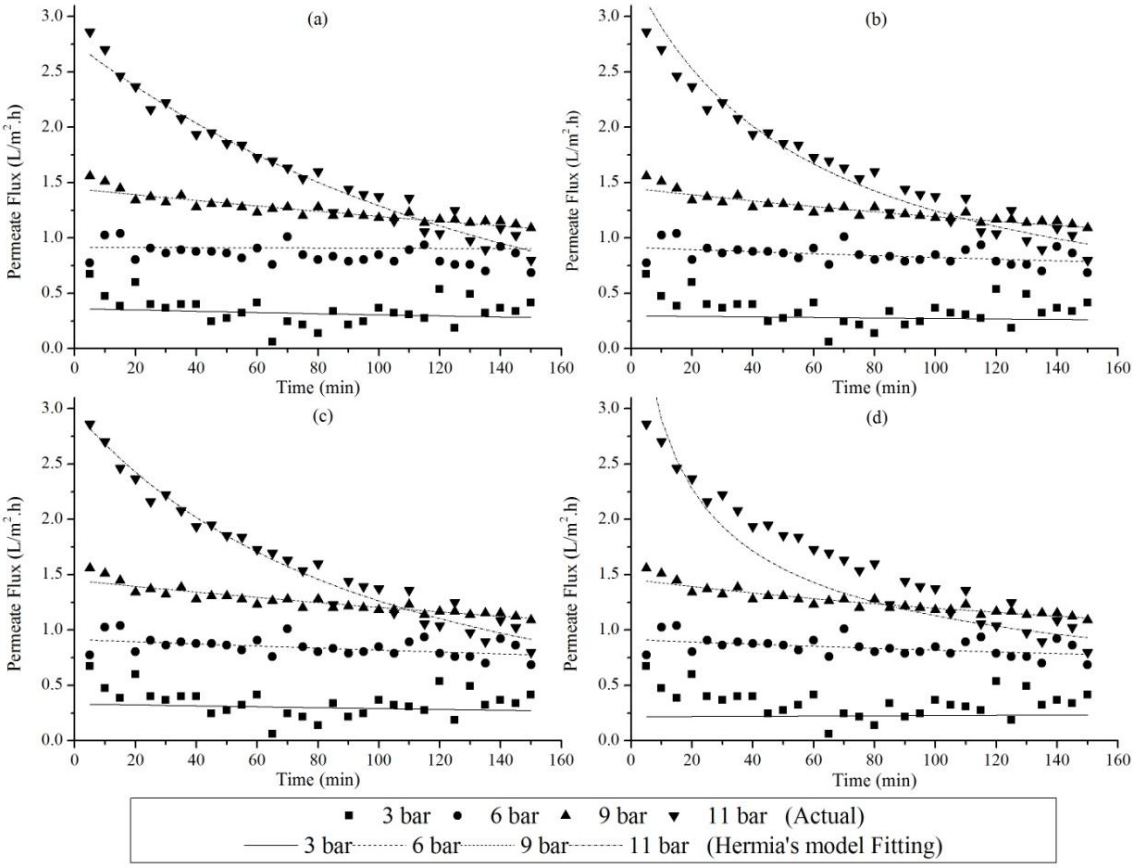
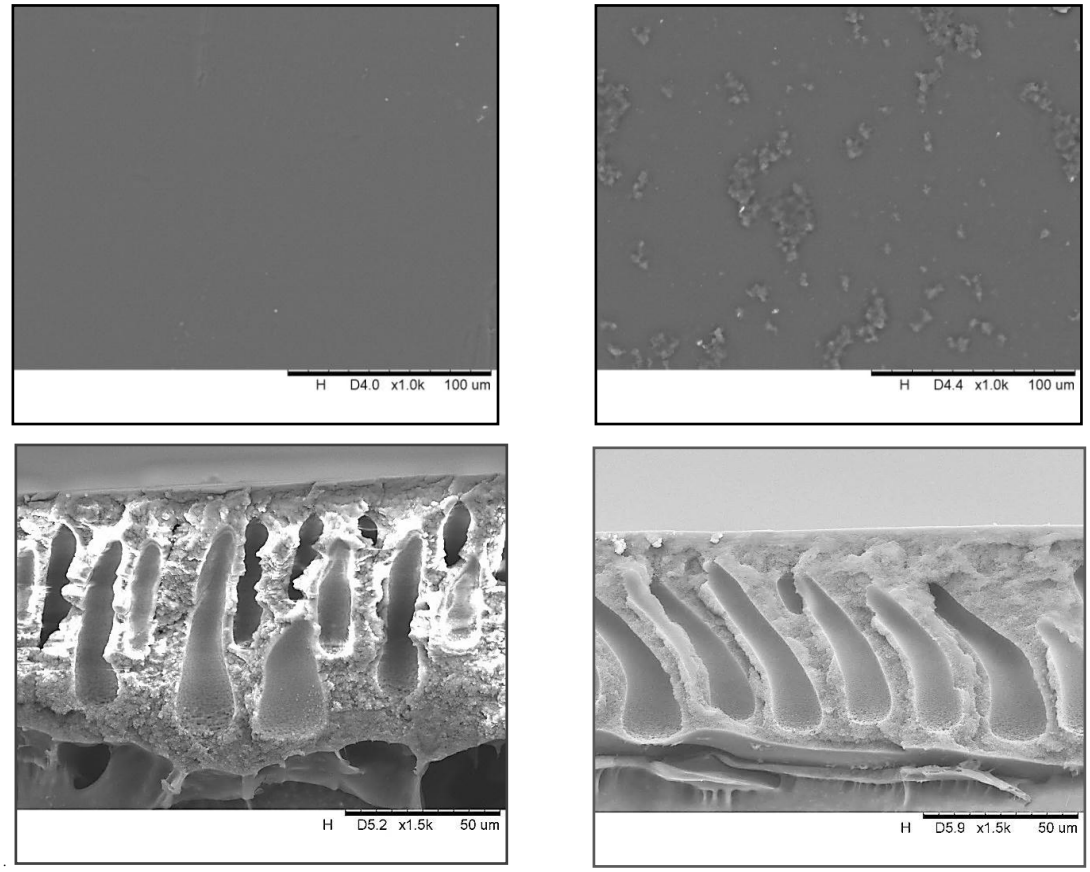


Fig. 4 Hermia's model fitting for NF 270 membrane: (a) complete blocking; (b) intermediate blocking; (c) standard blocking; and (d) cake formation

including chrysin, pinocembrin, pinobanksin-acetate and galangin (Gardana *et al*. 2007). The major foulants for hydrophobic Starmem™ 122 membrane were expected to be lipophilic compounds with high MW such as artepillin C (300.40 g/mol) and galangin (270.24 g/mol). Hence, cake formation instead of pore blockage dominated in the fouling on Starmem™ 122 membrane. Meanwhile, more hydrophilic compounds such as ρ-coumaric acid (164.16 g/mol) and chrysin (254.24 g/mol) were predicted to be adsorbed on the hydrophilic NF270 membrane. The adsorption of hydrophilic compounds with small MW eventually caused pore blockage.

Table 4 The coefficient of determination for NF270 membrane

Pressure (bar)	R^2			
	Complete blocking	Intermediate blocking	Standard blocking	Cake layer formation
3	0.2841	0.2837	0.2875	0.2793
6	0.4478	0.4470	0.4472	0.4463
9	0.9250	0.9326	0.9283	0.9390
11	0.9836	0.9806	0.9856	0.9419

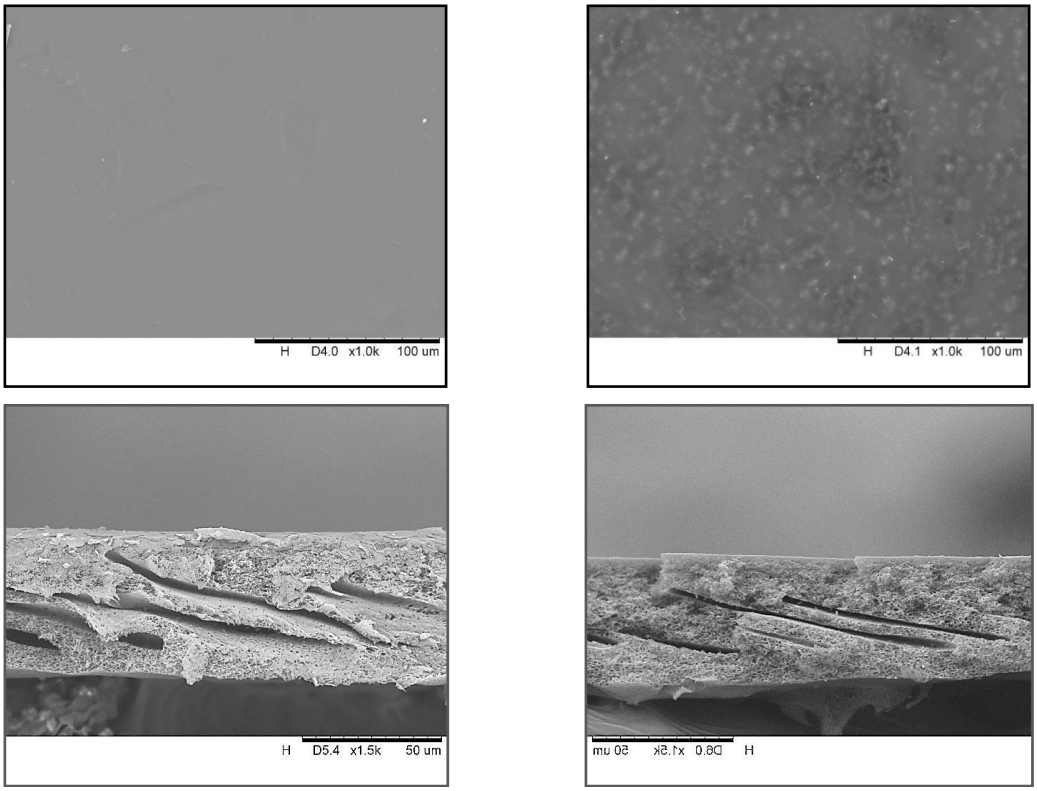


(a) Before nanofiltration (b) After nanofiltration.

Fig. 5 SEM micrographs of the surface and cross-section of Starmem™122 membrane

3.4 Membrane characterization for the verification of Hermia's model study

The surface morphology of Starmem™ 122 and NF270 membrane before and after the filtration process was shown in Figs. 5 and 6 respectively. The deposition of small particles on membrane surface could be observed after the filtration. More foulant deposited on NF270 membrane, explaining the dominant of pore blockage as the fouling mechanism. From cross-sectional view of Starmem™ 122 membrane (Fig. 5), the finger-like macropores seemed to be



(a) Before nanofiltration (b) After nanofiltration.

Fig. 6 SEM micrographs of the surface and cross-section of NF 270 membrane

shorter after filtration. The cake layer actually formed on the top section of asymmetric Starmem™ 122 membrane. Meanwhile, the diameter of long macropores in NF270 membrane reduced after filtration as shown in Fig. 6. The reduction could be related to pore blockage as estimated using Hermia's Model.

In order to verify the foulant on the membranes surface, FT-IR analyses of the fresh and used membranes were conducted. The presence of new peaks for the fouled membrane indicates the presence of new components. The spectra of Starmem™ 122 membrane before and after filtration were shown in Fig. 7. The peaks at 2966.66 cm^{-1}, 2921.94 cm^{-1} and 2852.81 cm^{-1} appeared in the spectrum of Starmem™ 122 membrane. These peaks were most probably contributed by the C-H stretching of lipophilic compounds such as artephilin C. Fig. 8 showed the spectra of NF270 membrane before and after filtration. The peaks 3324.66 cm^{-1}, 2966.66 cm^{-1} and 2968.01 cm^{-1} were observed from the spectrum of fouled NF270 membrane. The peak at 3324.66 cm^{-1} may indicate the O-H stretching due to the presence of hydrophilic phenolic or flavonoid compounds such as p-coumaric.

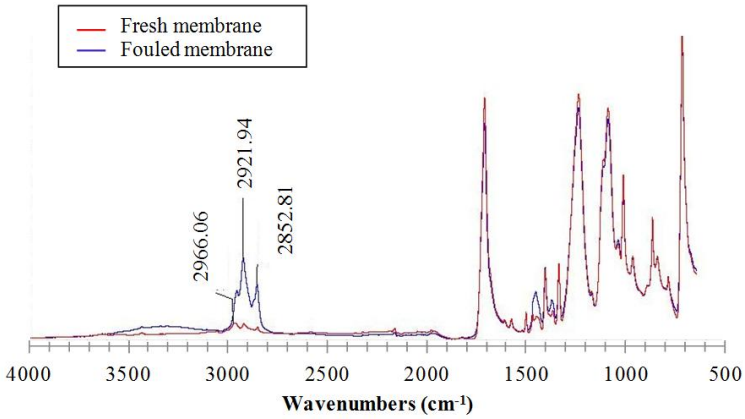


Fig. 7 FT-IR spectra of Starmem™122 membrane before and after filtration

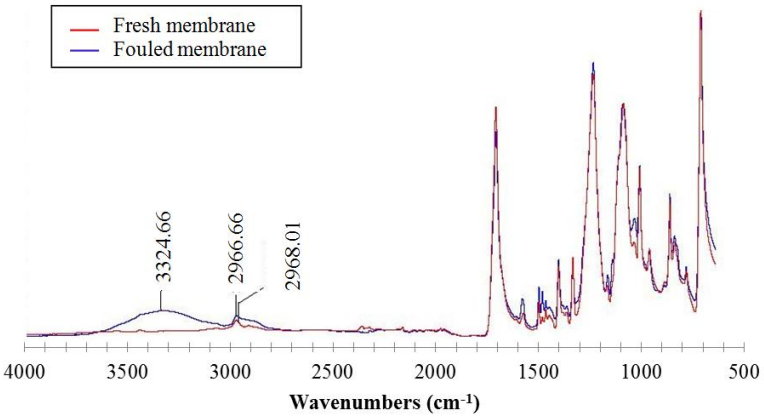


Fig. 8 FT-IR spectra of NF270 membrane before and after filtration

The difference of fouling on Starmem™ 122 and NF270 membranes could be explained by the surface hydrophilicity. Hydrophilic NF270 membrane would tend to adsorb hydrophilic phenolic and flavonoid compounds which are similar to its pore size, resulting pore blockage. Meanwhile, large lipophilic compounds formed a cake layer on the hydrophobic surface of Starmem™ 122 membrane. Chemical cleaning is generally recommended for organic fouling. Alkaline and acid can be used for the solubilization of both hydrophilic and lipophilic foulant, but extra surfactant was required to remove lipophilic foulant (Schäfer *et al*. 2004).

4. Conclusions

The effects of operating pressure on the concentration of ethanol extracted propolis using Starmem™ 122 and NF270 membranes were studied. For NF270 membrane, a higher operating pressure led to a higher initial flux. However, a greater flux decline was observed after 120 min at higher pressure. Compared to Starmem™ 122 membrane, NF270 membrane showed better rejection of bioactive compounds in propolis extract, more than 85%. NF270 membrane not only

retained more bioactive compounds in propolis extract, but it also allow solvent to be removed at higher permeate flux than Starmem™ 122 membrane. Thus, NF270 membrane is a better option for the concentration of propolis extract.

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