Separation and Concentration of Health Compounds by Membrane Filtration

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Abstract

The performance of nano-filtration (NF) for separating phenolic compounds from sugar in apple juice was studied using 1 and 0.25 kDa molecular weight cut-off (MWCO) spiral wound membranes. If these phenolic compounds could be recovered, they could stabilize the juice from haze formation or be added as antioxidants to foods and beverages in order to increase their health properties. Batch experiments were conducted on a pilot scale rig using a diluted clear apple juice concentrate. For the 1 kDa MWCO membrane, the research determined the effect of operating conditions on process efficiency and membrane fouling. The concentration of polyphenolics on the retentate side increased by a factor of up to 4 and the sugar concentration increased by 1.5 times under optimum conditions of lower temperature (30°C), acidic pH (2), lower trans-membrane pressure (5 Bar) and higher initial sugar concentration (20 oBrix). Despite the increase in polyphenolics in the retentate, there was little difference in the phenolic composition between retentate and permeate solutions. As the molecular mass of the rejected phenolics was smaller than the membrane cut-off, this indicated that the rejection was related to the formation of a secondary membrane formed as a result of fouling. A mass balance of polyphenolics in the final retentate and permeate compared with the initial feed solution indicated that up to 4.3 gm of polyphenolics were bound per m² of membrane. The permeate solutions collected from the 1 kDa MWCO membrane were then filtered using a 0.25 kDa MWCO membrane. Most phenolic compounds were retained by the membrane and the concentration increased by a factor of up to 2. Catechin, rutin, phloridzin and quercetin derivatives were concentrated on the retentate side. However, around 20 - 40% of chlorogenic acid and epicatechin was observed on the permeate side. It is concluded that membrane separation represents a potentially efficient and cost-effective technology to separate the phenolic fraction of fruit juice in a form suitable for use as a functional ingredient.

KEYWORDS: nano-filtration, membrane, separation, apple juice, fouling, polyphenolics

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1. Introduction

Apples synthesize a wide diversity of polyphenolic compounds, which can be classified into five major groups: flavan-3-ols, hydroxycinnamic acids, dihydrochalcones, anthocyanins and flavonoid polymers such as the proanthocyanidins (Alonso-Salces et al., 2004). In apple juice, polyphenols influence flavor, color, bitterness, astringency and storage stability. They are also reported as the source of many health benefits (Boyer and Liu, 2004). However standard processes for the production of clear apple juice have steps to lower or remove apple phenolics in order to limit color changes and prevent the formation of precipitate (Spanos et al., 1990). With increasing consumer desire to improve health through food, technologies are needed to return the phenolic compounds for use as functional ingredients. Similarly the various waste streams from apple juice production processes are good sources of polyphenolic phytochemicals. Potentially these could be recovered by similar means for addition back into foods and beverages (Kammerer et al., 2005).

The standard extraction methods for separating polyphenols from fruit waste involve organic solvents, such as ethanol, methanol and hexane. However solvent processes are more expensive than aqueous processing and the extracts may contain residual solvents which make them less desirable for food use (Nawaz et al., 2006). Additionally, extractions with hydrophilic solvents also result in the co-extraction of sugar from the fruit. Therefore, cost-effective technology is needed to separate sugar from the polyphenolic compounds in order to concentrate the polyphenolic fraction for nutraceutical use (Nawaz et al., 2006).

Membrane filtration is desirable as a processing technology because it does not require the addition of chemicals to the process stream (Nawaz et al., 2006). Additional advantages include easy automation (Mondor and Girard, 2000) and scale up (Nawaz et al., 2006), shorter process time (Mondor and Girard, 2000), lower labor and energy costs, less waste disposal (De Bruijn et al., 2003), no additives and mild operation conditions (Nawaz et al., 2006). However, the main disadvantage of membrane filtration is membrane fouling resulting in decline in permeate flux. This is caused by deposition and accumulation of solute or colloidal particles at the membrane surface (concentration polarization/gel layer) or inside the pores (De Bruijn et al., 2002). Membrane performance is strongly affected by trans-membrane pressure, feed velocity and operating temperature while juice quality may be influenced by membrane pore size or molecular weight cut-off (MWCO) (De Bruijn et al., 2003). For processing apple juice, components potentially causing membrane fouling may be pectins, proteins, starch, hemicelluloses and cellulose as well as tannins and other polyphenolics (Mondor and Girard, 2000). As a result, flux declines with time, reducing process efficiency (De Bruijn et al., 2003).
Flavonoid monomers have molecular weights less than 400 Da. In this present work, membrane filtration has been applied to the separation and concentration of polyphenolics from diluted apple juice concentrate in a 2-step fractionation process. Polymerized proanthocyanidins and oxidized phenolic fractions have molecular weights greater than 1000 Da and therefore would be expected to be retained on the retentate side of the membrane. The permeate was then filtered using a 0.25 kDa cut-off membrane to concentrate the smaller monomer and dimer from the smaller sugars and organic acids. The investigation therefore involved studying the effects of various operating parameters such as initial sugar concentration, feed pH, feed flow-rate and trans-membrane pressure on the separation of polyphenols from fruit juices using nano-filtration processes.

2. Materials and Methods

2.1 Materials

Clear apple juice concentrate, with an enhanced level of polyphenols (EPAJC) and 75 °Brix sugar, was supplied by ENZAFOODS New Zealand Ltd. Catechin and Folin Ciocalteu’s phenol reagent (2N) came from Sigma (Mo., USA), anhydrous sodium carbonate (GR grade) from Merck (Germany), hydrochloric acid (35%, reagent grade) from Scharlau Chemicals (Spain) and sodium hydroxide pellets were from BDH (UK). The SelRO® spiral wound membrane (MPS-36) with 1 and 0.25 kDa MWCO, and 1.2 m² area was purchased from KOCH membrane systems (USA). Nitric acid-phosphoric acid, potassium hydroxide and sodium meta bi-sulfite were obtained from Orica-Chemnet, New Zealand.

2.2 Analyses

Total phenolics in the sugar solutions were measured using the Folin method by the procedure of Singleton et al. (1998), with minor modifications using a catechin reference standard and a SpectraMax micro plate reader (Molecular Devices, Ca., USA). The phenolic profiles were determined by reverse phase HPLC chromatographic analyses using a Shimadzu HPLC system with an SPD M10A diode array detector and a Phenomenex (NZ) Synergi 4μ Hydro RP 80 Å column (250X4.6 mm) at 35°C using a 40 μl injection volume. The binary mobile phase consisted of (A) acetonitrile: water 5:95 v/v containing 0.1% v/v formic acid and (B) acetonitrile containing 0.1% v/v formic acid. Separations were developed at a flow rate of 1 ml/min using gradient series from 100% to 5% A over 65 minutes. Individual compounds were quantified at 280 nm using calibration curves of known standards. The sugar content was determined as °Brix equivalents using a digital refractometer (ATAGO, PR-101).

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2.3 Experimental Procedures

Membrane filtration runs were performed using diluted apple juice concentrate. The membrane apparatus (Pilot Plant Model L from GEA Process Engineering (NZ) Ltd) consisted of a re-circulation loop containing a 20 L feed tank and a pump that could generate a pressure up to 70 bar. Orifice plates with digital flow meters were used to measure the flow rate for feed, retentate and permeate. Each sample was poured into the feed tank, heated and pumped through the membrane vessel. The temperature was maintained constant by an online heat exchanger. The inlet and outlet pressures were measured by differential pressure meters and the solution temperature was measured via a digital thermocouple. The data-logger and the PC were activated to record system variables at a scanning interval of 1 minute. The experiment was run until the retentate volume was ~2 L with run periods varying from 20 – 75 minutes due to different rates of flux decline caused by fouling.

Following an experiment, collected samples were analyzed for total phenolic concentration, phenolic profile and sugar content. The flux (L m\(^{-2}\)h\(^{-1}\)) was calculated from the permeate flow rate per unit membrane area and the fouling deposits were calculated from the difference in mass balance of polyphenolic material recovered in the retentate and permeate compared with the initial feed solution. The membrane rig was cleaned by 3% nitric - phosphoric acid solution at 50ºC for 20 minutes followed by 2% potassium hydroxide for 20 minutes at a temperature of 50ºC. Recovery of water flux after cleaning was 100%.

3. Results and Discussion

3.1 Effect of Operating Conditions on Separation and Fouling of Polyphenolics Using 1 KDa MWCO Membrane

The roles of fluid temperature, fluid concentration, fluid pH and trans-membrane pressure on separation and concentration of polyphenolics from apple juice were investigated using a nano-filtration process. Operating variables tested were: temperatures from 30 to 50ºC, pH from 2 to 5, trans-membrane pressures from 5 to 30 bar and initial sugar concentrations from 8 to 20 °Brix.

Membrane filtration runs were carried out for periods of up to 75 minutes using 1 kDa MWCO membrane cartridge. Results are presented in Table 1. An example of typical results is shown in Figure 1, in which temperature, trans-membrane pressure, feed flow rate, retentate flow rate, and permeate flow rate are plotted against time.
Table 1: Results from all membrane separation experiments using 1 kDa MWCO.

<table>
<thead>
<tr>
<th>Run</th>
<th>T(°C), P(bar), F°Brix</th>
<th>Process Conditions</th>
<th>Phenolics Content</th>
<th>Sugar Concentration</th>
<th>Fouling deposits (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F (mg/ml)</td>
<td>Ret. (mg/ml)</td>
<td>Per. (mg/ml)</td>
</tr>
<tr>
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<td>0.08</td>
</tr>
<tr>
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<td>0.14</td>
</tr>
<tr>
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</tr>
<tr>
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<td>6</td>
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<td>0.39</td>
<td>0.78</td>
<td>0.14</td>
</tr>
<tr>
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<tr>
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<td>0.68</td>
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<tr>
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<td>0.78</td>
<td>0.14</td>
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<tr>
<td>15</td>
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<td></td>
<td>0.36</td>
<td>1.20</td>
<td>0.07</td>
</tr>
</tbody>
</table>

T = temperature, P = trans-membrane pressure, F = feed, Ret. = retentate, Per. = permeate

Figure 1. Typical results of a membrane run using 1 kDa cut-off, initial sugar concentration = 8 °Brix, pH = 3, temperature = 40°C and trans-membrane pressure = 10 bar.

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3.1.1 Effect of Temperature

Temperature is an important variable in membrane fouling. Most fouling involving organic systems has shown a strong dependence on temperature (Watkinson and Wilson, 1997) because of changes to the fluid characteristics which produce deposits. In cases where fouling is due to chemical reaction or solubility effects in the bulk solution, bulk temperatures can play a major role in the formation of fouling precursors (Eaton and Lux, 1984). The bulk temperature and viscosity of fruit juice are inversely related. Therefore, the effect of temperature on viscosity, and hence flow regime through the membrane, is very important.

The effect of temperature on separation and concentration of polyphenolics are shown in Figure 2. The maximum concentration of phenolics in the retentate decreased by a factor of 1.7 as temperature increased from 30 to 50°C. This is consistent with the findings of (Goosen et al., 2002), that the porosity of polysulfone membranes can be very sensitive to changes in the feed temperature. The lower temperatures could also increase the formation of insoluble aggregates, increasing the secondary membrane that restricted the flow and reduced the pore size of the membrane.

The change in flux with time at different temperatures is shown in Figure 3. The flux declined immediately after the start of each run and the decrease was faster at lower temperatures. The improvement of flux with increasing temperature was primarily due to viscosity effects on the water (Jackson et al., 2004). Higher temperatures may also change the flow regime by increasing the turbulence and the cross flow velocity removing fouling material with higher shear forces (Tanada-Palma and Matta, 1999).

![Figure 2](image-url)

**Figure 2.** Effect of fluid temperature on membrane performance, pH = 4, trans-membrane pressure = 10 bar and initial sugar concentration = 8 °Brix.
Increasing the temperature from 30 to 50°C reduced the amount of polyphenolics fouled on the membrane by a factor of 1.5, from 3.2 to 2.1 gm. Results are presented in Table 1. The experiments performed at lower temperatures were longer because of the additional fouling resistance to the flow across the membrane.

![Graph](image)

**Figure 3.** Decline of flux with time at three temperatures, trans-membrane pressure = 10 bar, initial sugar concentration = 8 °Brix and pH 4.

HPLC analyses (Figure 4) revealed that, although there was a considerable increase in the concentration of phenolic compounds on the retentate side, the composition of the retentate and permeate fractions were similar.
Figure 4. HPLC chromatograms (A280nm) for feed, retentate and permeate from 1 kDa MWCO membrane.

3.1.2 Effect of pH

The effects of pH on the separation and concentration of polyphenolics are shown in Figure 5. The pH value of the retentate solutions during filtration had a significant effect on membrane efficiency. The concentration on the retentate side decreased by a factor of 2.4 with increasing feed pH from 2 to 5. Increasing the acidity of the solution improved the separation behavior of the phenolic compounds. This is in contrast to previous research which found that juice filterability is poor at acidic pH but improves dramatically at pH 7.5 (Balakrishnan et al., 2000).

The lower pH may have tightened the polysulfone membrane pores and resulted in higher concentrations of phenolics on the retentate side. The increase in retentate concentration at lower pH values was partly due to the formation of a secondary membrane that restricted the flow and reduced the pore size or the cut-off of the membrane.

It was found that the phenolic material deposited on the membrane decreased by 38 wt% from 3.2 to 2.1 gm when increasing feed pH from 2 to 5 (Table 1). Over the course of the run, the sugar concentration on the retentate side increased by up to 1.5 times and the extent of the increase paralleled the decline in flux and the increase in amount of fouling material on the membrane surface. Around 80 to 90% of the sugar was recovered on the permeate side.
The flux greatly reduced over the run duration from 27 to 12 L m⁻²h⁻¹ and the decrease was more rapid at lower pH values.

![Graph showing Retentate Concentration vs pH](image)

**Figure 5.** Effect of feed pH on membrane performance, temperature = 40°C, trans-membrane pressure = 10 bar, and initial sugar concentration = 8 °Brix.

### 3.1.3 Effect of Feed Concentration

The effect of concentration is very important in membrane fouling. Membrane processing is very sensitive to a critical concentration of moderately high molecular weight molecules (Karode et al., 2000).

The effect of the initial sugar concentration on separation of polyphenolics is shown in Figure 6. The polyphenolics concentration on the retentate side increased linearly by a factor of 2.1 with increasing initial sugar concentration from 8 to 20 °Brix. The increase in polyphenolics concentration observed on the retentate side may have been due to the formation of a secondary membrane that plugs the pores or reduces the pore size and restricts the flow across the membrane.

The amount of polyphenolics fouled on the membrane was increased by a factor of 2.4, from 2.4 to 5.8 gm with increasing initial sugar concentration from 8 to 20 °Brix (Table 1). Increases in the amount of fouling material paralleled increases in sugar rejection or sugar concentration on retentate side. Around 70 – 80% of the sugar was recovered on the permeate side. The flux declined rapidly from the start of the experiment and the decline was more severe at higher concentrations.
There was a significant increase in the concentration of phenolic compounds with increasing initial sugar concentration from 8 to 20 °Brix.

![Figure 6](image_url)

**Figure 6.** Effect of initial sugar concentration on membrane performance, temperature = 40°C, trans-membrane pressure = 10 bar and pH = 4.

### 3.1.4 Effect of Trans-Membrane Pressure

The effect of trans-membrane pressure on separation and concentration of polyphenolics is shown in Figure 7. The concentration on the retentate side increased by a factor of 1.8 as the trans-membrane pressure increased from 5 to 30 bar. This increased fouling at higher trans-membrane pressures is in agreement with the literature (Tanada-Palmu and Matta, 1999). It can be explained by higher pressure compressing the rejected solutes into a thicker and denser fouling layer and lower cross flow velocity removing retained materials because of lower shear forces.

The amount of polyphenolics fouled on the membrane was increased by a factor of 2, from 1.9 to 3.8 gm with increasing trans-membrane pressure from 5 to 30 bar. Almost 67% to 93% of the sugar was recovered on the permeate side depending on the operating trans-membrane pressure (Table 1). The decline in the flux was rapid at the early stages of the experiment but was gradual and limited afterwards.
Concentration of phenolic compounds on the retentate side increased significantly with increasing trans-membrane pressure.

**Figure 7.** Effect of trans-membrane pressure on membrane performance, temperature = 40°C, initial sugar concentration = 8 °Brix and pH = 4.

Based on the experimental data from this study, a strategy for mitigation of membrane fouling can be formulated for this fruit juice. Mitigation can be achieved by controlling the operating conditions such as increasing the temperature, feed pH and decreasing initial sugar concentration and trans-membrane pressure. Also, it is very important to eliminate the insoluble solids in the feed and larger molecular weight phenolics by pre-treating the feed stream using a higher MWCO membrane.

### 3.2 Separation and Concentration of Polyphenolics from Permeate Collected from 1 kDa MWCO Experiments Using 0.25 kDa MWCO.

Membrane filtration runs were carried out on permeate solutions collected from previous runs with 1 kDa MWCO for periods of up to 50 minutes using a 0.25 kDa MWCO membrane cartridge. Results are presented in Table 2. The following set of condition was used: temperature 40°C, pH 4, and trans-membrane pressure 10 bar.

Decline in flux was minimal compared with previous runs using the 1 kDa MWCO membrane. This was because of the removal of larger molecular weight phenolic compounds during earlier trials which had caused the
formation of the secondary membrane and fouling. The results showed that the concentration of polyphenolics on the retentate side increased by a factor of up to 2.4 and decreased on the permeate side by a factor of up to 3 compared with the initial feed solution. The amount of fouling material was found to be minimal compared with previous runs. This proves that fouling was caused by the larger molecular weight phenolic compounds in the feed that were removed by the 1 kDa MWCO membrane.

**Table 2:** Results from all membrane separation experiments using a 0.25 kDa MWCO and permeate samples collected from 1 kDa experiments. Temperature 40°C, pH 4, and trans-membrane pressure 10 bar.

<table>
<thead>
<tr>
<th>Run</th>
<th>Phenolics Content</th>
<th>Sugar Concentration</th>
<th>Fouling deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F (mg/ml)</td>
<td>Ret. (mg/ml)</td>
<td>Per. (mg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>0.08</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
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<td>0.14</td>
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<td>0.05</td>
</tr>
<tr>
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<td>0.20</td>
<td>0.37</td>
<td>0.07</td>
</tr>
<tr>
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<td>0.21</td>
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<td>0.07</td>
<td>0.17</td>
<td>0.02</td>
</tr>
</tbody>
</table>

F = feed, Ret. = retentate, Per. = permeate

Around 60 - 70% of the sugar was collected on the permeate side of the membrane, depending on initial sugar concentration used in the earlier trials. HPLC (Figure 8) and total phenolics analyses revealed a significant increase in the concentration of phenolic compounds on the retentate side. The composition of the retentate and permeate fractions were different and in most cases catechin (M.wt = 290), rutin (M.wt = 610), phloridzin (M.wt = 472) and quercetin (M.wt = 338) derivatives were fully separated/concentrated on the retentate side. However, around 20 - 40% of chlorogenic acid (M.wt = 352) and epicatechin (M.wt = 290) was observed on the permeate side. These findings are consistent with the literature and proved that the separation is based on phenolic compounds molecular weight and membrane cut-off.
Figure 8. HPLC chromatograms (A280nm) for feed, retentate and permeate from 0.25 kDa MWCO membrane.

4. Conclusions

A study of separation/concentration of polyphenolics from apple juice by membrane filtration led to the following conclusions:

- Potentially, membrane filtration can be used to recover and enrich polyphenols from apple juice and, by extension, for other clear juices.
- The 1 kDa nano-filtration membrane partially retained both polyphenolics and sugar, indicating that the formation of a secondary membrane controlled the separation process.
- The membrane filtration process was shown to be significantly affected by temperature, with the phenolics concentration on the retentate side decreasing by a factor of 2 with increasing temperature from 30 to 50°C.
- The concentration of polyphenolics on the retentate side increased with lowering feed pH and increasing initial sugar concentration.
- Increasing the trans-membrane pressure increased fouling and indicated an attachment-controlled mechanism caused by the formation of a secondary membrane.
- The increase in fouling paralleled decreases in the amount of polyphenols found in the permeate solution.
- The composition of phenolic compounds on retentate and permeate sides was the same using 1 kDa MWCO suggesting a concentration step, and
different using 0.25 kDa MWCO, indicating a separation/fractionation step. 

- Most of the phenolic compounds were separated from sugar using 0.25 kDa membrane and around 70% of the sugar was recovered on the permeate side. 
- Minimal fouling was observed using a 0.25 kDa membrane which proved that fouling was caused by larger molecular weight phenolic compounds and pre-treatment or removal of these compounds is essential in such processes. 
- The importance of particulate fouling was proven by a significant decrease in flux and increase of fouling material over each run. Therefore, higher molecular weight polyphenolics in feed are expected to have a significant role in membrane fouling.

5. References


