Membrane Fouling and Molecular Weight Cutoff Effects on the Partitioning of Pectinesterase

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The effects of membrane fouling and molecular weight cutoff (MWCO) on the partitioning of pectinesterase (PE) isolated from Marsh grapefruit were evaluated. Statistically higher fouling rates were observed for Marsh grapefruit extracts on 30K and 100K poly(sulfone) UF membranes than on a similar membrane with a MWCO of 10K. A simple logarithmic expression adequately described the fouling phenomena for all three UF membranes. The permeability of PE was also determined by using the same membranes and found to decrease 30−60% during processing regardless of the MWCO of the membrane. Permeability was found to decrease in a fashion similar to permeate flux. A separate logarithmic expression was developed to evaluate the rate of change of permeability on each of the membranes. About 40−50% of the total activity in the permeate of the 30K UF membrane was thermostable PE.

Keywords: *Polysulfone membrane; thermostable pectinesterase; citrus processing*

INTRODUCTION

Pectinesterase (EC 3.1.1.11) is synthesized by plants as well as some molds and bacteria (Sajjaanantakul and Pitifer, 1991). In citrus and other fruit juices, pectinesterase (PE) de-esterifies high-methoxyl pectin to the extent that a series of events leading to clarification of the juice is initiated (Stevens et al., 1950; Baker, 1979; Crandall et al., 1983; Shomer, 1988). Clarification is considered a major defect in the citrus-processing industry that affects the rheological properties, appearance, and flavor of both citrus juices and concentrates (Bruemmer, 1980).

Traditional methods of processing orange juice employ heat to destroy spoilage bacteria, inactivate enzymes, and produce concentrates (Varsel, 1980). Heat treatment, however, induces severe flavor deterioration (Nisperos-Carriedo and Shaw, 1990). Since PE is associated with juice pulp, separation of the pulp and serum by filtration or centrifugation prior to heat treatment helps to minimize flavor loss and deterioration. The flavor-rich serum stream is not heat treated, while the pulp stream undergoes heating to inactivate PE (Köseoglu et al., 1990).

The molecular weights of most PE isozymes vary from 22 000 to 37 000, depending on the source and methods of purification and analysis (Sajjaanantakul and Pitifer, 1991). A much larger PE with a molecular weight of 54 000 was first isolated from navel oranges by Versteeg et al. (1980). This high molecular weight isozyme was relatively thermostable and responsible for most of the clarification occurring in orange juice at temperatures below 5 °C and at low pH.

Seymour et al. (1991a) isolated thermostable and thermolabile pectinesterase isozymes from Marsh grapefruit by using a selective inactivation procedure at 70 °C. Both isozymes consisted of a single polypeptide. The thermostable (TS-PE) isozyme had a molecular weight of 51 000, and the thermolabile (TL-PE) isozyme had a molecular weight of 36 000. In addition to its enhanced thermostability, the TS-PE isozyme also exhibited greater stability at low pH and was more resistant to freeze−thaw cycles (Seymour et al., 1991b).

Recently, Köseoglu et al. (1990) and Hernandez et al. (1992) reported that no PE activity was detected in the permeate during UF of orange juice using hollow-fiber poly(sulfone) membranes with molecular weight cutoffs of 100K and 500K, respectively. On the basis of the reported molecular weight, it was expected that PE would permeate high-MWCO UF membranes. Since the MWCOs of the membranes were considerably larger than the reported molecular weights of PE, Hernandez et al. (1992) speculated that fouling of the membrane by the pulp and the association of PE with the pulp was responsible for PE rejection. In peel extracts, Shomer and Merin (1984) reported that membrane fouling and loss of flux were caused by soluble high molecular weight complexes, including pectins.

Ultrafiltration of citrus juice is complicated by the complex nature of citrus juices, which include pectins, proteins, and other cell wall constituents. The difficulty is compounded by the limit of detection of low PE activity levels in citrus juices. In this study, we used a model system of Marsh grapefruit extracts with higher PE activity and lower pectin than in whole juice or peel extracts. PE extracts were used as model systems to determine the permeability of PE in UF membranes with MWCOs of 10K, 30K, and 100K. The effects of membrane fouling on PE permeability were also determined. This research will facilitate the development of a process to inactivate, inhibit, or separate PE from citrus juice to improve product quality and maintain cloud stability.

MATERIALS AND METHODS

Extraction of Pectinesterase from Marsh Grapefruit. Unstabilized Marsh grapefruit (MGF) pulp was donated by Citrus World (Lake Wales, FL) and stored at −20 °C. Extraction of PE was accomplished by using a modified procedure of

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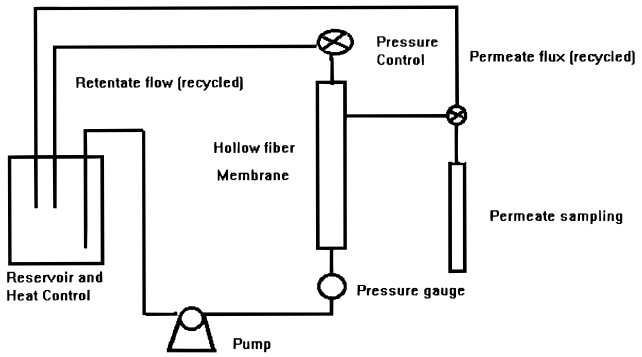

Figure 1. Schematic diagram of the apparatus used for the study of ultrafiltration.

Wicker et al. (1988). One hundred grams of MGF pulp was homogenized in 500 mL of 0.1 M Tris [tris(hydroxymethyl)-aminomethane] and 0.3 M NaCl in a Sorvall blender. After homogenization for 15 s, the pH was adjusted to 8.0 using 2 N NaOH. The MGF pulp was further homogenized for an additional 45 s (3 × 15 s). The homogenate was stirred for 1 h at 4 °C, centrifuged (7000*g*; 20 min, 4 °C), and filtered through two layers of cheesecloth. The extract was diluted 2-fold with deionized water, giving a final NaCl concentration of 0.15 M. During the UF studies, the pH was maintained at 8.0 using either 2 N NaOH or 2 N HCl.

Determination of Pectinesterase Activity. Total pectinesterase (PE) activity was measured by using the method of Rouse and Atkins (1955). One pectinesterase unit (PEU) was defined as the amount of enzyme required to release 1 μequiv of carboxyl groups per minute at pH 7.5 and 30 °C. The substrate solution (25 mL) contained 1% (w/w) high-methoxyl pectin (donated by Citrus Colloids, Ltd., Hereford, U.K.) in 0.1 M NaCl. PE activity was expressed as PEU/mL of extract.

Thermostable pectinesterase (TS-PE) activity was defined as the activity that remained after heating 3 mL of extract for 5 min at 70 °C and pH 8.0. Samples were analyzed within 1 h.

Ultrafiltration Membranes and Description of the UF System. The membranes used in this study were laboratory-scale hollow-fiber ultrafiltration cartridges obtained from A/G Technology Corporation (Needham, MA). The hollow-fiber internal diameter was 1 mm, and all membranes were constructed of poly(sulfone). The membranes had molecular weight cutoffs of 10K, 30K, and 100K.

The ultrafiltration system (Figure 1) consisted of a 2 L reservoir, a variable speed peristaltic pump (Cole Parmer, Chicago, IL), an inlet pressure gauge, a clamp placed at the outlet of the membrane cartridge to provide back-pressure, and a single hollow-fiber-membrane cartridge. Due to the small differences in pressure between the inlet and outlet of the membrane cartridge, only an inlet pressure gauge was employed for monitoring system pressure. For the purposes of this study, the driving force for permeation was taken as the inlet pressure to the membrane cartridge and not the trans-membrane pressure often reported for larger membrane systems.

The membrane system was operated in two modes. In the total recycle mode, both the retentate and the permeate were recirculated back to the reservoir to minimize changes in the chemical and physical properties in the MGF extract. In the concentration mode, permeate was collected in a separate reservoir.

Retentate and permeate flow rates were measured by using a stop watch and graduated cylinder. Retentate samples (3 mL) were taken directly from the reservoir. Permeate samples (3 mL) were collected from the graduated cylinder after measuring permeate flux. If the system was operating in the total recycle mode, the permeate remaining after sampling was added back to the reservoir.

Cleaning UF Membranes. The UF membranes were cleaned immediately after each use. The system was initially flushed with water (60 °C) for 10 min. After flushing, the membranes were cleaned using either a 1% w/w solution of P3 Ultracil 25 or a 0.5% w/w solution of P3 Ultracil 53. Both cleaners were obtained from the Henkel Corp. (Burlington, IA). The cleaning solutions were circulated through the system for approximately 30 min followed by rinsing with distilled water. Both forward and backward water flushing was employed after each cleaning step to aid in removing the cleaning solution from the membrane system. After cleaning and rinsing, the membrane's water flux was checked to evaluate the efficacy of the cleaning procedure. Either circulation of 1% (w/w) P3 Ultracil 25 for 30 min followed by overnight storage in 0.1 N NaOH or circulation of 0.5% (w/w) P3 Ultracil 53, an enzyme-based cleaning solution, for 30 min and storage in distilled water was sufficient to clean the membrane.

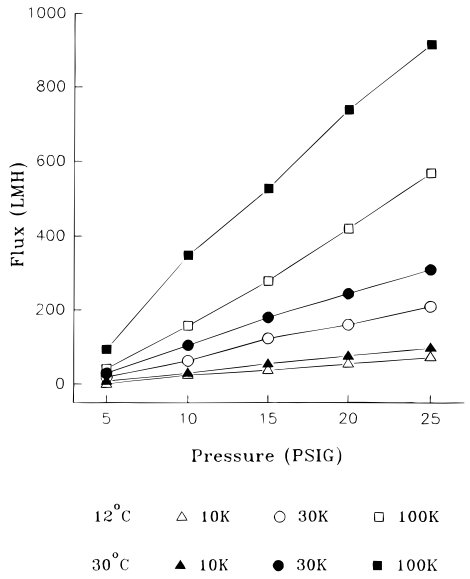

Figure 2. Effect of temperature and pressure on water flux for each of the three membranes used in this study. The membranes were hollow-fiber poly(sulfone) ultrafiltration (UF) membranes with MWCOs of 10K, 30K, and 100K.

Statistical Analysis. All experiments were run at least in duplicate. Correlations between system variables were evaluated to attain the best model fit using PC-SAS. The slopes and intercepts of correlation lines rather than the averages were used to present the results from individual runs. Slopes or intercepts were compared with a *t*-test. A confidence level of 0.05 was used.

RESULTS AND DISCUSSION

Characterization of Ultrafiltration Membranes. The flux characteristics of each of the three membranes were initially evaluated with distilled water and a buffer solution (0.1 M Tris in 0.15 M NaCl, pH 8.0). The effects of temperature and pressure on permeate flux were determined. Data in Figure 2 illustrate water flux as a function of temperature and pressure for each of the three membranes. As would be expected, water flux increased significantly with an increase in MWCO, temperature, and pressure for all three membranes. As the MWCO of the membrane increases, the greater the increase in water flux at 30 °C, than that at 12 °C. For the 10K, 30K, and 100K membranes, water flux increased 33%, 47%, and 61%, respectively, at the higher temperature. Figure 2 also illustrates that temperature effects were less pronounced at lower pressures than at higher pressures. Flux of the buffer solution was approximately 30% lower than that obtained with distilled water on the 30K membrane. Water flux measurements served as a reference point for comparing all experimental results obtained throughout the re-

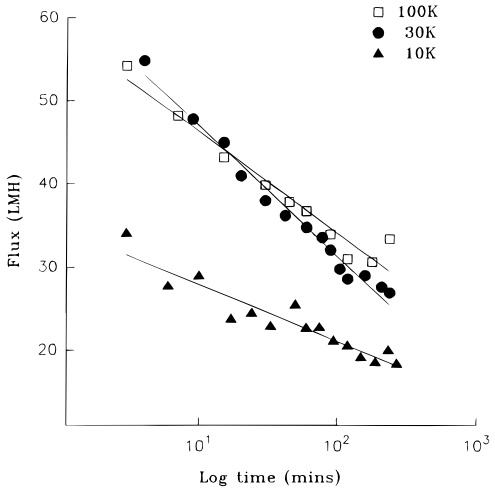

Figure 3. Fouling of UF membranes (25 psig, 10 °C) by MGF extract (pH 8.0, 0.15 M NaCl). Fouling could be described by eq 1. See Table 1.

Table 1. Fouling Attributes of UF Membranes by Marsh Grapefruit Extract[a]

MWCO	fouling index	J_{180}[b] (LMH)	R^2
10 K	6.84 ± 0.68^A	19.34 ± 1.51^A	0.89
30 K	15.64 ± 0.62^B	27.42 ± 1.20^B	0.98
100 K	13.11 ± 1.22^B	30.35 ± 1.83^B	0.94

[a] Means within columns with the same upper case letter are not statistically significant at $p = 0.05$. [b] J_{180} is permeate flux at 180 min.

mainder of this study and also as a baseline for evaluating the various cleaning procedures used.

The flux characteristics of the membrane system were also evaluated by using MGF extract to establish a common operating point that could be used throughout the fouling and permeability studies. On the basis of these studies, the membrane system was operated at an inlet pressure of 25 psig and a retentate flow rate of 1.2 LPM.

Because PE can be partially inactivated at 30 °C after extended periods, all fouling and permeability studies were conducted at 10 ± 1 °C. The contribution of a ±1 °C temperature fluctuation to the variability in flux and permeability was assessed to be less than 2% and was therefore neglected.

Effect of MWCO on Fouling of the UF Membrane by MGF Extract. Data in Figure 3 illustrate the results obtained from the fouling studies. The rate of fouling is significantly greater for the 30K and 100K membranes than for the 10K membrane. In an attempt to quantify the fouling process, we developed the following empirical model

$$J_t = J_1 - b \log[t] \quad (1)$$

where J_t is the permeate flux at time t, J_1 is the initial permeate flux, t is time, and b is the fouling index. Several other empirical models exist that utilize a logarithmic or exponential expression to describe the fouling process. All of these models are based on the assumption that the buildup of the fouling layer follows first-order kinetics (Cheryan, 1986). The value of these empirical models is that they provide insight into the fouling phenomena and potential methods to minimize or control fouling. The fouling index, b, which is simply the slope of the individual graphs in Figure 3, indicates the rate of fouling. The larger the fouling index, the greater the rate of fouling. The fouling index as well

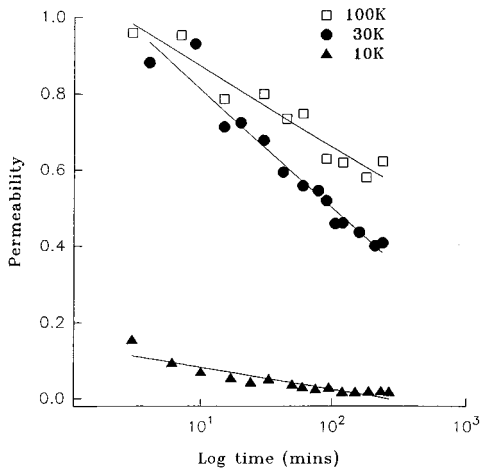

Figure 4. Change in PE permeability as a function of processing time and MWCO. PE permeability could be described by eq 2. See Table 2.

Table 2. Permeability on UF Membranes of Marsh Grapefruit Extract[a]

MWCO	permeability index, s	P_{180}[b]	R^2
10 K	0.06 ± 0.01^A	0.01 ± 0.003^A	0.84
30 K	0.31 ± 0.02^B	0.42 ± 0.040^B	0.95
100 K	0.19 ± 0.02^C	0.56 ± 0.031^C	0.91

[a] Means within columns with the same upper case letter are not statistically significant at $p = 0.05$. [b] P_{180} is permeability of PE at 180 min.

as the initial and final (J_{180}) permeate flux values were significantly lower on the 10K membrane than on the 30K and 100K membranes. No statistical difference was observed between the 30K and 100K membranes with respect to the fouling indices and the final permeate flux values. Additional fouling studies conducted using washed pulp with lower pectin concentrations did not significantly reduce fouling (Snir and Chen, 1993).

On the basis of the results obtained from the fouling studies, MGF extract, with its high PE activity, was found to be an acceptable model system for the study of PE permeability and the effects of fouling on PE permeability in an ultrafiltration membrane.

Permeability of Pectinesterase during UF. Figure 4 illustrates the effect of processing time and MWCO on the permeability of pectinesterase during ultrafiltration. We expressed the data in terms of solute "permeability" rather than the more traditional "solute rejection". The permeability of a solute is a direct indication of how much solute is passing through the membrane. Solute rejection can be easily determined by subtracting the solute's permeability value from 1.

As indicated in Figure 4, the initial permeabilities of PE in the 30K and 100K membranes are nearly 1 or 100%, while the permeability on the 10K membrane is approximately 0.15 or 15%. The permeabilities were all measured at an inlet pressure of 25 psig and 10 °C. As ultrafiltration continues, the permeability of PE decreases in all three membranes. In the 10K membrane, the permeability approaches 0 or the membrane becomes capable of nearly 100% rejection of the enzyme. PE permeabilities also decreased with time on the 30K and 100K membranes. After 180 min, PE permeability on the 100K membrane had decreased to approximately 0.60 (60%), while on the 30K membrane it had decreased to 0.40 (40%).

The loss of PE permeability was found to closely coincide with the loss of permeate flux due to membrane

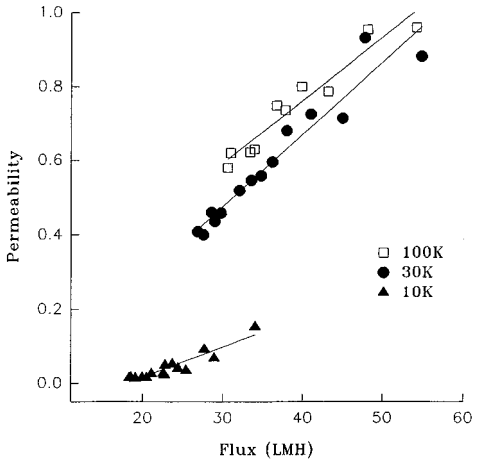

Figure 5. Effect of permeate flux and MWCO on MGF PE permeability (permeate/retentate). The UF system and Marsh grapefruit extract were identical to those described for Figure 3.

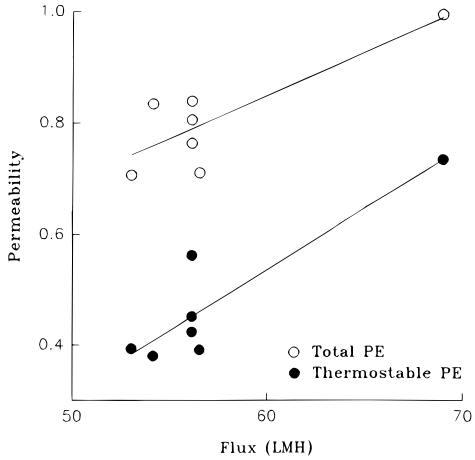

Figure 6. Permeability of the thermostable isozyme of PE on a 30K poly(sulfone) membrane. UF system operating conditions and Marsh grapefruit extract were identical to those for Figure 3.

fouling. Therefore, it would appear that an empirical equation similar to eq 1 could be used to help predict the effects of operating parameters on a solute's permeability. The following equation was used to determine the effects of operating variables on PE permeability in various MWCO membranes

$$P_t = P_1 - s \log[t] \quad (2)$$

where P_t is the permeability at time t, P_1 is the initial permeability, t is time, and s is the permeability index. In eq 2, the permeability index has the same physical meaning as the fouling index in eq 1. Thus, at a given set of operating conditions (e.g., 25 psig, 10 °C, 1.2 LPM), a larger permeability index indicates that solute permeability is changing faster than at another set of operating conditions, in this case on a different MWCO membrane. Figure 5 illustrates the effect of flux on PE permeability on each of the three membranes. At equivalent fluxes, the larger MWCO membranes have greater PE permeabilities than the lower MWCO membranes. At a flux of 30 LMH, the difference in permeabilities for all three membranes is statistically significant at $p = 0.05$. It is also interesting to note that PE permeability increases with an increase in flux for all three membranes regardless of MWCO. On the basis of the data presented, it is obvious that PE permeability is dependent on both the MWCO of the membrane and the extent to which the membrane is fouled. Interaction between the enzyme and pectin in the MGF extract was reported to influence permeation and flux (Snir et al., 1995). Flux was higher, but less permeation was observed due to higher interaction at pH 3.8 than at pH 6.0 or 8.0.

Permeability of Thermostable PE Isozyme during UF. By using the procedure reported here, both thermolabile PE (TL-PE) and thermostable PE (TS-PE) are extracted from the Marsh grapefruit. As previously discussed, the TS-PE isozyme is a high molecular weight isozyme and is most responsible for the clarification that occurs in orange juice at temperatures below 5 °C (Versteeg et al., 1980). In Marsh grapefruit, TS-PE has been previously isolated and characterized by Seymour et al. (1991a,b). They reported that TS-PE from Marsh grapefruit possessed a molecular weight of 51 000, while TL-PE has a molecular weight of 36 000.

Elimination of TS-PE isozyme activity thus would appear to provide the most benefits to the juice processor. As a general rule, molecular weights of any macromolecules to be separated by ultrafiltration should differ by at least a factor of 10. Because of the relatively small difference in the molecular weights of the PE isozymes, membrane processing is not likely to effectively separate the isozymes. However, we wanted to determine whether membrane processing selectively affected either of the isozymes. Figure 6 illustrates the results obtained by using only the 30K membrane. The data indicate that approximately 40−50% of the total PE activity observed in the permeate is due to the thermostable isozyme. Extracts of citrus PE typically contain 10−20% thermostable PE (Rombouts et al., 1982). Thermostable PE from Marsh grapefruit, extracted under similar conditions, accounted for 17% of the total activity (Wicker, 1992). The higher ratio of TS-PE to TL-PE in the permeate could be due to a number of factors, including selective retention of TL-PE in the foulant, selective inactivation of TL-PE under the high shear, and/or pressure of UF processing or selective permeation of TS-PE due to differences in the shape of the two isozymes. These possibilities are under investigation in another study.

Conclusions. The results detailed here clearly indicate that MWCO classification of UF membranes should not be the only factor used to determine which membrane could be used for a particular application. PE permeabilities were much greater than anticipated solely on the basis of the MWCO of the UF membrane. Further, the high shear environment encountered in the hollow-fiber cartridge may affect enzyme permeability. Commercial classification of UF membranes is not well defined and is usually based on the rejection of a globular macromolecule. The membrane must be tested in the actual environment in which it is expected to perform to fully evaluate its potential.

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