pH and Cations Influence Permeability of Marsh Grapefruit Pectinesterase on Polysulfone Ultrafiltration Membrane

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Marsh grapefruit pulp extract was filtered through a 30 000 molecular weight cutoff hollow fiber polysulfone ultrafiltration (UF) membrane in a crossflow mode. At pH values of 3.8 and no added salt, pectinesterase (PE) was retained by the membrane. Permeability increased with increasing pH and/or cation concentration. Flux was not increased at higher pH and/or cation concentration. Divalent cations were more effective than monovalent cations at stimulating activity and permeability of PE. Maximum PE permeability was obtained at pH 8.0 and 0.15 M NaCl, pH 6.0 and 0.15 M NaCl, pH 3.8 and 0.4 M NaCl, or pH 3.8 and 0.1 M $CaCl_2$. The results suggest that PE is bound to soluble pectin and is retained by UF membranes. Adding cations or raising the pH releases PE from the electrostatic binding to pectin and enables the enzyme to permeate UF membranes.

Keywords: *Pectinesterase–pectin interactions; electrostatic interactions; membrane fouling*

INTRODUCTION

De-esterification of high methoxyl pectin by pectinesterase (PE) is a first step in a sequence of enzyme-catalyzed chemical and physical reactions leading to juice clarification and gelation of concentrates (Baker, 1979; Crandall et al., 1983). Clarification, a serious quality defect in orange- and grapefruit-based juices and concentrates, is prevented commercially by heat treatment (Eagerman and Rouse, 1976; Versteeg et al., 1980), which induces flavor deterioration (Nisperos-Carriedo et al., 1990). Reduction in pulp-associated PE can be achieved through removal of the pulp by either centrifugation (Rouse, 1953) or filtration (MacDonnell et al., 1945). Pulp, however, is perceived as an integral component of most orange and grapefruit juice products.

An alternative method utilizing ultrafiltration (UF) for the separation of PE from juice was patented by Köseoglu et al. (1990). Separation of pulp by UF and concentration of clear orange and grapefruit serum by reverse osmosis are utilized to produce stable high-quality juices and concentrates. Pulp is heat treated before being added back to the serum. Separation of pulp and serum streams during processing facilitates optimization of the heat treatment to minimize flavor quality deterioration.

Although the molecular weights reported for citrus PE isozymes range between 32 000 and 54 000 (Versteeg et al., 1980; Seymour et al., 1991), no PE permeability was detected in citrus juice permeates of 50 000 and 100 000 (Köseoglu et al., 1990) or 500 000 (Hernandez et al., 1992) molecular weight cutoff point (MWCO) hollow fiber polysulfone membranes. In a recent study conducted in our laboratory (Snir et al., 1995a), no PE permeability was detected when orange juice, at pH 3.8, was ultrafiltered with hollow fiber membranes of up to 100 000 MWCO. Yet, when extracted from marsh grapefruit (MGF) pulp (0.05 M Tris, pH 8.0, 0.15 M NaCl), less than 40% and 60% of PE was rejected by 100 000 and 30 000 membranes, respectively, and the rejection was correlated to fouling (Snir et al., 1995b). The extracted enzyme was efficiently restricted only by a polysulfone membrane with much smaller (10 000) MWCO.

The mechanisms responsible for the high rejection of PE in juice by large molecular weight cutoff membranes is the theme of this study. The effects of pH, cation concentration, and fouling on PE permeability are studied in a MGF extract model system.

MATERIALS AND METHODS

Chemicals and Enzyme Source. Unheated marsh grapefruit (MGF) pulp was donated by Citrus World, Lake Wales, FL, and stored at −20 °C. All chemicals used were of analytical grade.

PE Extraction Procedure. PE extraction followed the procedure of Wicker et al. (1988) with some modifications. MGF pulp (100 g) was homogenized for 15 s in 500 mL of extraction solution at 4 °C in a Sorvall blender, and the pH was adjusted to 8.0 with NaOH. Homogenization and pH adjustment were repeated three times. The homogenate was stirred for 1 h and centrifuged at 7000*g*, and the supernatant was filtered through two layers of cheesecloth. Throughout the extraction process, pH was monitored and adjusted if required. Extraction was always conducted at pH 8.0, and the adjustment of pH for each run was done immediately before each ultrafiltration run.

To study pH effect on UF at constant ionic strength, a three-buffer mixture was used [0.05 M acetic acid, 0.05 M 2-(*N*-morpholino)ethanesulfonic acid (MES), 0.1 M tris(hydroxymethyl)aminomethane (Tris), and 0.25 M NaCl]. Total sodium ions contributed from MES and NaCl was 0.3 M. The extract was diluted 2-fold with distilled, deionized water before membrane filtration, yielding 0.15 M sodium ions. Tetramethylammonium hydroxide (TMAH) and HCl were used to adjust the pH (Ellis and Morrison, 1982).

Deionized water was used to study the effect of cation concentration at different pH values. MGF extract was initially elevated to pH 8.0 with TMAH. pH was monitored throughout the experimental process, and TMAH and HCl were used for pH adjustments. The pH of the diluted extract used for ultrafiltration was constant (within ±0.1 pH unit) after 4 h of ultrafiltration or several days of storage.

PE Activity Measurement. The amount of enzyme that releases 1 μmol of carboxyl groups/min was defined as 1 unit

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(PEU). Activity was measured by titration (Rouse and Atkins, 1955), with a Brinkman (Westbury, NY) pH stat titrator. Unless otherwise specified, PE activity was measured at pH 7.5 and 30 °C in 25 mL of 1% high methoxyl pectin (Citrus Colloids Ltd., Hereford, U.K.) with 0.1 M NaCl.

In the study of PE activity at different pH values and cation concentrations, the reaction mixture contained the specified concentrations of NaCl and $CaCl_2$, at the specified pH with all other conditions the same. Correction of the pH of pectin solution was done with TMAH and HCl to minimize the effect on cation concentration (Ellis and Morrison, 1982).

UF System. A bench-top polysulfone hollow fiber membrane (0.0325 m^2) with molecular weight cutoff (MWCO) of 30 000 was used in the study (AG Technology Corp., Needham, MA). UF was run in a total recycle mode at a rate of 1.2 L/min (flow speed of 0.73 m/s), cross membrane pressure of 25 psig, and 10 ± 1 °C. Tangential flow of the retentate reservoir to the hollow fiber membrane was followed by collection of the permeate back to the retentate reservoir. Retentate sampling was conducted directly from the reservoir. Permeate flux was measured by timing collection of a fixed volume (50–100 mL) in a 100 mL graduated cylinder. Permeate samples of 3 mL were taken from this volume for further analyses, and the rest was added back into the retentate reservoir.

A full description of the system can be found in Snir et al. (1995b). A 0.5 h treatment with an enzyme-based cleaner, Ultracill P3-53 (Henkel Corp., Burlington, IA) was used for membrane cleaning, with forward and backward flushing. PE permeability is expressed as the fraction of PEU per milliliter in the permeate to PEU per milliliter in the retentate. Pairs of retentate and permeate samples were analyzed successively.

Characterization of Flux and Permeability. The formulas developed by Snir et al. (1995b) were used to relate flux, PE permeability, and time. The flux (J), as described in formula 1, decreases with time (t). Flux is represented by the slope of the regression line (b) and is referred to as the fouling index (Cheryan, 1986). The flux established after 3 h of UF (J_{180}) was used to compare between treatments.

$$\text{flux:} \quad J_t = J_1 - b \log(t) \tag{1}$$

PE permeability (P_t) decreases with time (t) and is represented by the slope (s) in formula 2. Permeability after 3 h (P_{180}) was calculated.

$$\text{permeability:} \quad P_t = P_1 - s \log(t) \tag{2}$$

Data presented are representative of at least duplicate replications.

Total and Full Recycle Experiments. In experiments where permeation of PE was monitored at pH 8.0 followed by pH 3.8, the extract was passed through the hollow fiber membrane and the permeate was collected for 120 min into the retentate reservoir as described above (total recycle). The permeate was then collected into a separate reservoir for approximately 30 min (full recycle). The permeate was adjusted to pH 3.8 and the original starting volume. The membrane was cleaned with enzyme for 30 min, followed by forward and backward flushing. The permeate at pH 3.8 was then passed through the hollow fiber membrane (total recycle) as described above for another 120 min.

Uronic Acid Assay. Uronic acid analyses were conducted according to the procedure of Blumenkrantz and Asboe-Hansen (1973).

RESULTS

PE Permeability—Effect of pH. PE permeability was evaluated with MGF extract at 0.15 M NaCl in a single three-zone buffer. After 3 h (P_{180}), PE permeability at pH 8.0 and 6.0 was near 0.5 but was less than 0.05 at pH 3.8 (Figure 1). Higher permeabilities at the higher pH values were observed regardless of flux values. Higher flux values at pH 3.8 did not result in higher permeability. Although pH did not dramatically affect flux values, permeability of MGF extract was increased 10-fold by an increase in pH from 3.8 to 6.0. Thus, pH is critical in modifying permeation or rejection of PE by the membrane and may elucidate the underlying mechanism and the relevant factors effecting rejection of PE by UF membranes.

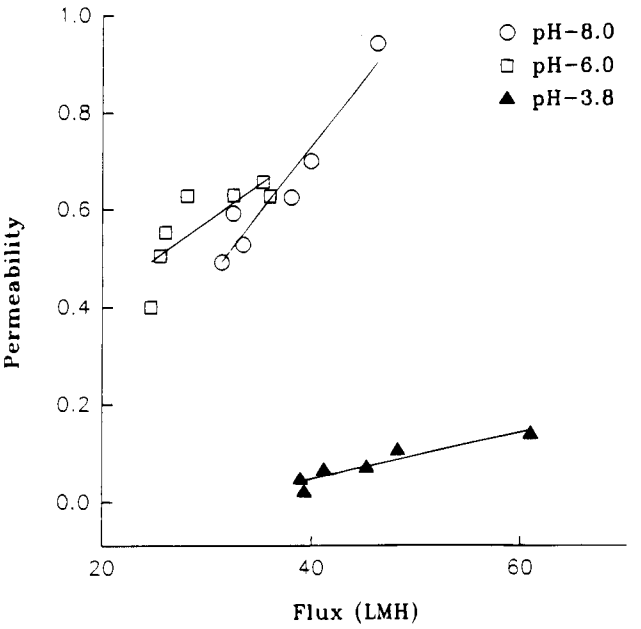

Figure 1. Relationship between PE permeability ($[PEU/mL]_{Permeate}/[PEU/mL]_{Retentate}$) and flux in UF of MGF extract (0.15 M NaCl) at various pH values. $J_{180} = 33 \pm 1.2$; $P_{180} = 0.52 \pm 0.02$; $P_{40LMH} = 0.73 \pm 0.05$. $J_{180} = 25 \pm 1.8$; $P_{180} = 0.46 \pm 0.06$; $P_{40LMH} = 0.72 \pm 0.08$. $J_{180} = 39 \pm 1.3$; $P_{180} = 0.04 \pm 0.01$; $P_{40LMH} = 0.05 \pm 0.02$.

Continuous Studies on PE Permeability—Ion Concentration. A pH-corrected water extract of MGF was used in this phase of experiments. Initially, NaCl was added at intervals to produce the desired total ion concentration. After each addition, pH was adjusted and UF was resumed without cleaning the membrane between runs. The buffering capacity maintained stable pH over the pH range studied during the study.

At pH 8.0, the initial permeability of PE with no NaCl was 0.2, which increased to 0.6 at 0.05 M NaCl (Figure 2). Nearly a 40% decrease in PE permeability was measured at higher (0.6 M) NaCl concentration. At pH 6.0, permeability was zero with no NaCl and close to 0.35 with 0.2 M NaCl (data not shown). At pH 3.8, virtually no PE permeability was observed with 0–0.15 M NaCl. At 0.45 M NaCl and pH 3.8, permeability increased to approximately 0.6–0.7 (data not shown). At all pH values studied, a peak in PE permeability at an optimal NaCl concentration was followed by a decrease in permeability at higher NaCl concentrations.

Permeability at Discrete pH and NaCl. The continuous experimental design efficiently generated information needed to establish the specific combination of cation concentration and pH for permeation and flux evaluation. The isolation of the accumulative effect of fouling with time, the impact of salt concentration and pH on fouling, and the effect of fouling on permeability could not be isolated in such an experimental design. On the basis of the data accumulated in the screening study, specific combinations of NaCl and pH were studied to understand the effect of the individual parameters. Membranes were fully cleaned between runs.

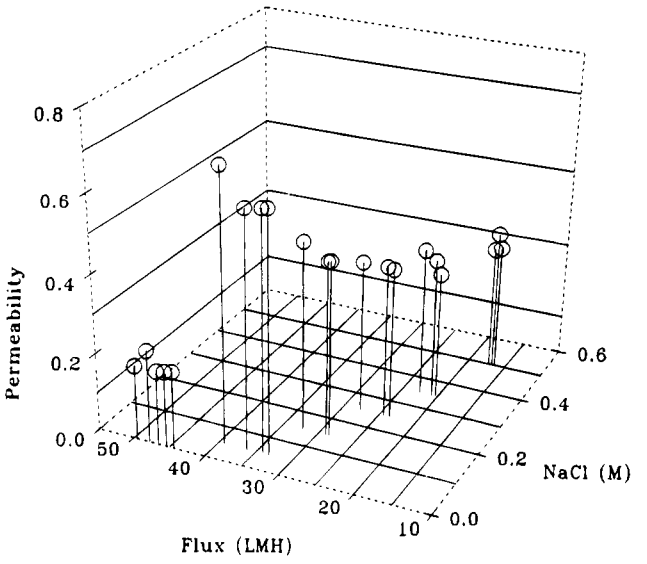

Figure 2. Relationship between permeability ($[PEU/mL]_{Permeate}$/$[PEU/mL]_{Retentate}$) and flux at various levels of NaCl at pH 8.0.

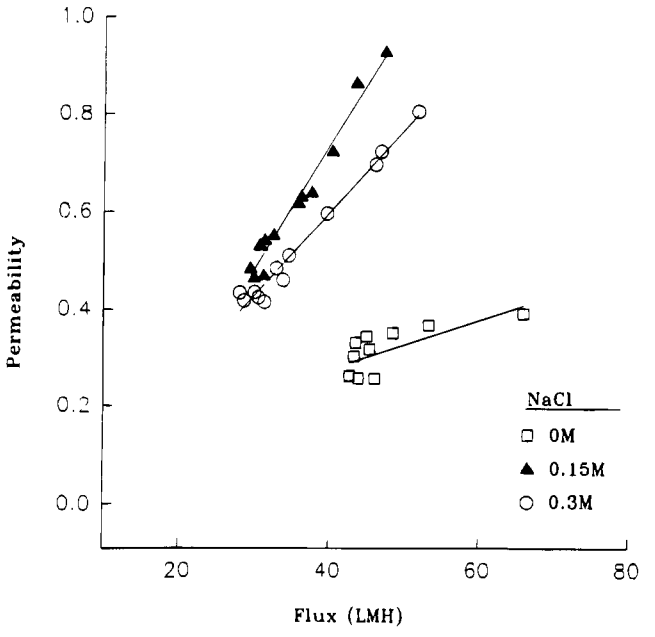

Figure 3. Relationship between permeability ($[PEU/mL]_{Permeate}$/$[PEU/mL]_{Retentate}$) and flux at various levels of NaCl at pH 8.0. 0.0 M: $J_{180} = 41 \pm 4.1$; $P_{180} = 0.25 \pm 0.03$; $P_{40LMH} = 0.26 \pm 0.03$. 0.15 M: $J_{180} = 29 \pm 0.9$; $P_{180} = 0.45 \pm 0.03$; $P_{40LMH} = 0.74 \pm 0.03$. 0.3 M: $J_{180} = 28 \pm 1.2$; $P_{180} = 0.37 \pm 0.04$; $P_{40LMH} = 0.60 \pm 0.04$.

P_{180} was 0.25 at pH 8.0 (Figure 3) without added salt. J_{180} tended to decrease with salt addition at pH 8.0. At pH 6.0, no permeability was observed in the absence of salt but P_{180} increased and J_{180} decreased in 0.15 and 0.3 M NaCl (Figure 4). At pH 3.8, no permeability was observed at salt concentrations below 0.3 M. Permeability was less than 0.1 at 0.3 M NaCl, pH 3.8 (Figure 5), and increased to near 0.35–0.55 in 0.4 M NaCl. Flux was not affected by NaCl at less than 0.4 M NaCl, pH 3.8. P_{180} observed at pH 3.8 and 0.4 M NaCl was about 80% of the P_{180} at pH 8.0 and 0.15 M NaCl.

The concentration of NaCl at discrete pH values that resulted in the highest observed PE permeability was obtained at pH 8.0 and 0.15 M NaCl, pH 6.0 and 0.15 M NaCl, and pH 3.8 and 0.4 M NaCl. An increase of pH and/or NaCl concentration promoted an increase in PE permeability. Conversely, flux decreased with an

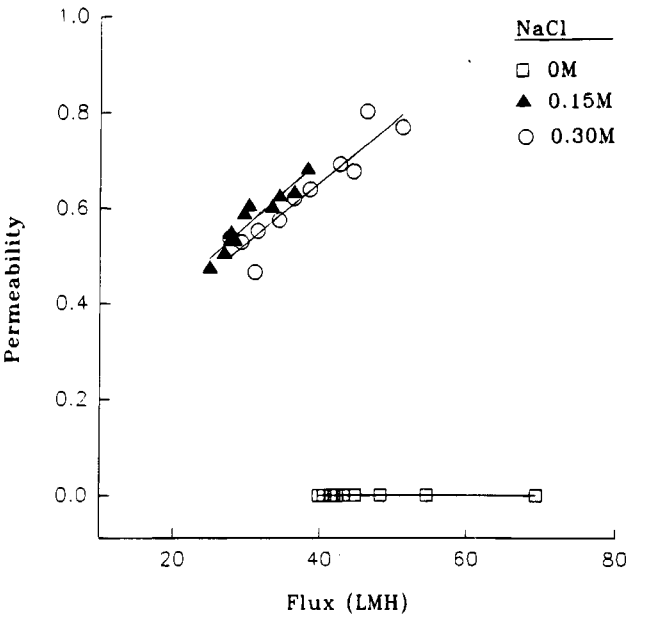

Figure 4. Relationship between permeability ($[PEU/mL]_{Permeate}$/$[PEU/mL]_{Retentate}$) and flux at various levels of NaCl at pH 6.0. 0.0 M: $J_{180} = 36 \pm 5.5$; $P_{180} = 0$; $P_{40LMH} = 0$. 0.15 M: $J_{180} = 26 \pm 0.7$; $P_{180} = 0.50 \pm 0.02$; $P_{40LMH} = 0.70 \pm 0.02$. 0.3 M: $J_{180} = 28 \pm 0.6$; $P_{180} = 0.50 \pm 0.04$; $P_{40LMH} = 0.66 \pm 0.04$.

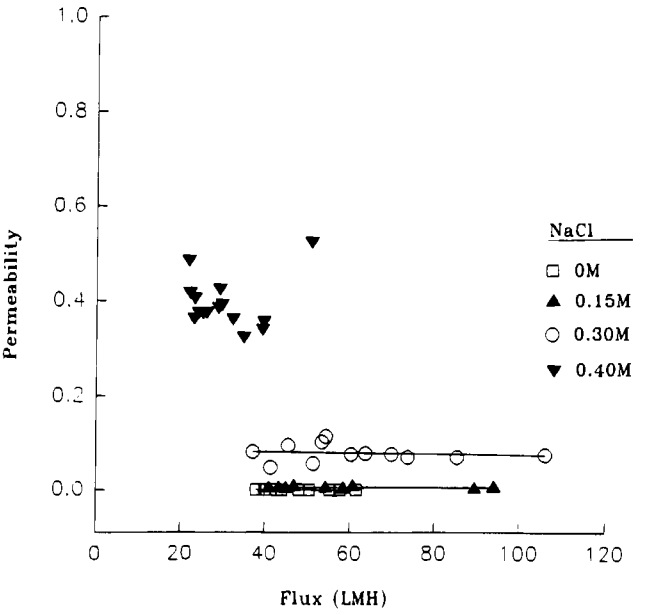

Figure 5. Relationship between permeability ($[PEU/mL]_{Permeate}$/$[PEU/mL]_{Retentate}$) and flux at various levels of NaCl at pH 3.8. 0.0 M: $J_{180} = 39 \pm 3.1$; $P_{180} = 0$; $P_{40LMH} = 0$. 0.15 M: $J_{180} = 36 \pm 8.0$; $P_{180} = 0$; $P_{40LMH} = 0$. 0.3 M: $J_{180} = 43 \pm 4.8$; $P_{180} = 0.08 \pm 0.02$; $P_{40LMH} = 0.08 \pm 0.02$. 0.4 M: $J_{180} = 23 \pm 3.7$; $P_{180} = 0.37 \pm 0.05$; $P_{40LMH} = 0.41 \pm 0.05$.

increase in NaCl concentration, but flux was not influenced as greatly by a change in pH. At all pH values, a slight decline in permeability was observed at higher salt concentrations.

Permeability at Discrete pH and $CaCl_2$. The effect of salt on PE permeability was not specific for NaCl but could be promoted by $CaCl_2$ at 20–25% of the concentrations of NaCl. P_{180} in 0.1 M $CaCl_2$ was approximately 0.33 and similar to the permeability in 0.4 M NaCl at pH 3.8 (Figure 6). The effect does not appear to be related solely to ionic strength. At equivalent ionic strength (0.3 M NaCl and 0.1 M $CaCl_2$), P_{180} differed by 4-fold.

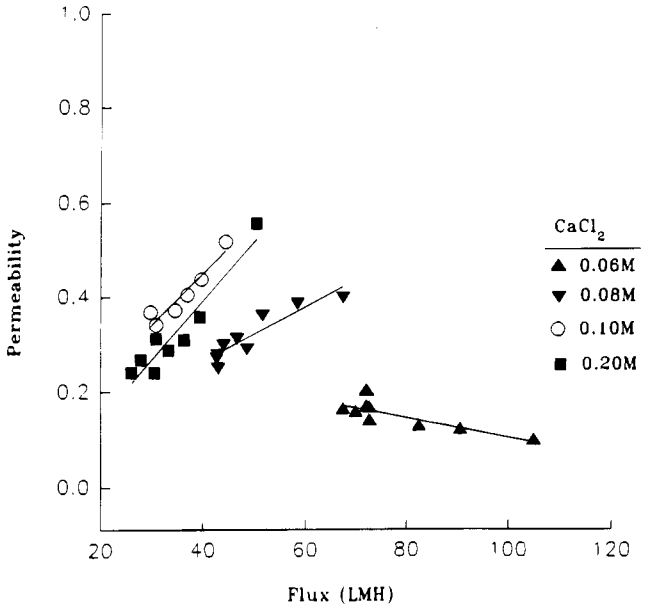

Figure 6. Relationship between permeability ($[PEU/mL]_{Permeate}$/$[PEU/mL]_{Retentate}$) and flux at various levels of $CaCl_2$ at pH 3.8. 0.06 M: $J_{180} = 65 \pm 5.0$; $P_{180} = 0.18 \pm 0.01$; $P_{40LMH} = 0.23 \pm 0.02$. 0.08 M: $J_{180} = 40 \pm 2.5$; $P_{180} = 0.26 \pm 0.02$; $P_{40LMH} = 0.26 \pm 0.02$. 0.1 M: $J_{180} = 29 \pm 0.7$; $P_{180} = 0.33 \pm 0.02$; $P_{40LMH} = 0.45 \pm 0.02$. 0.2 M: $J_{180} = 25 \pm 2.0$; $P_{180} = 0.21 \pm 0.05$; $P_{40LMH} = 0.39 \pm 0.03$.

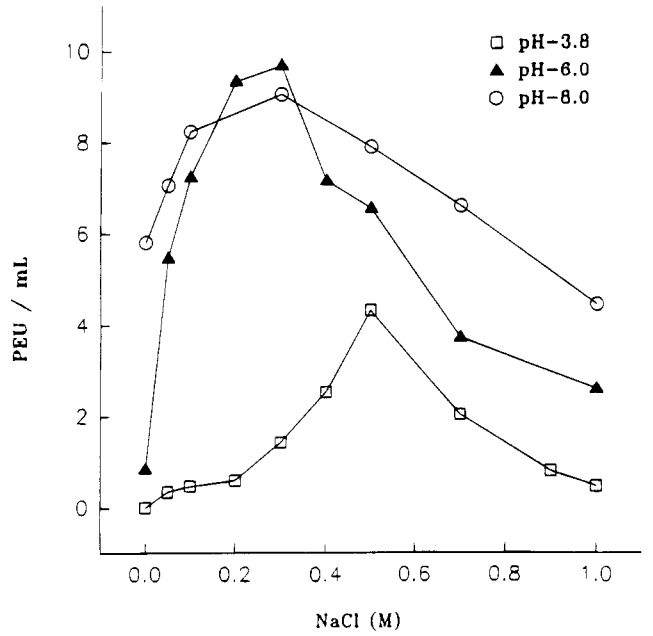

Figure 7. Effect of NaCl concentration on pectinesterase activity (PEU/mL) at various pH levels.

PE Activity Studies. PE activity at pH 8.0 and no NaCl was about 60% of the maximum value. Almost no activity was observed at pH 6.0 or 3.8 with no NaCl. Higher activities were obtained with higher NaCl concentrations, but activity declined at still higher NaCl concentrations (Figure 7). The highest observed activity at pH 3.8 was about 50% of the highest observed activity at either pH 6 or 8.

A similar effect was observed with an approximately 10-fold lower concentration of $CaCl_2$ at pH 3.8 (Figure 8). Five times more chloride anions was required to generate the same activity with added NaCl as compared to $CaCl_2$ at pH 8.0.

PE permeability was measured at pH 3.8 after filtration at pH 8.0. The permeability at pH 8.0 between 5 and 120 min ranged between 0.56 and 0.92 and averaged 0.76 ± 0.16 (Table 1). The permeability at pH 3.8 between 5 and 120 min ranged between 0.60 and 1.18 and averaged 0.90 ± 0.28. The flux was lower (J_{120} = 37 LMH) at pH 8.0 than at pH 3.8 (J_{120} = 114 LMH). At pH 8.0, the uronic acid content in the retentate was about twice that in the permeate. At pH 3.8, the uronic acid content for both the retentate and permeate was about half the content in the pH 8 permeate.

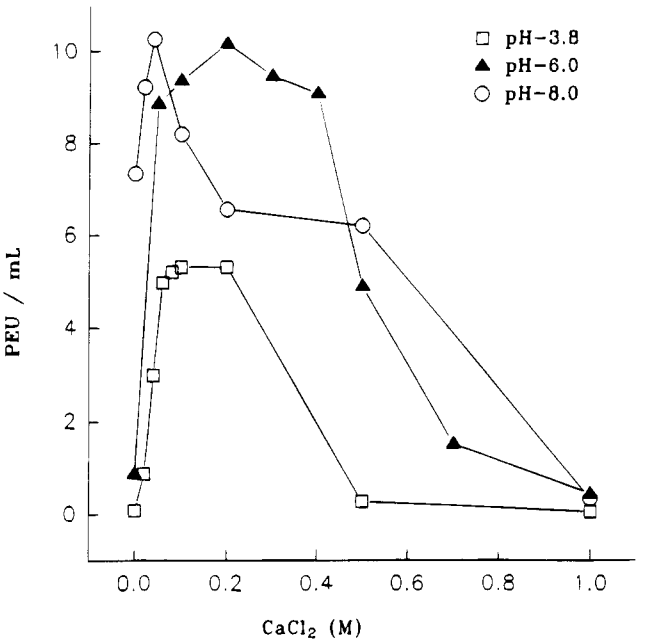

Figure 8. Effect of $CaCl_2$ concentration on pectinesterase activity (PEU/mL) at various pH levels.

Table 1. Permeability, Flux, and Uronic Acid Content of PE Extract Permeated at pH 8.0 followed by Permeation at pH 3.8[a]

pH	time (min)	P/R, PE	flux (LMH)	AGA, R	AGA, P
8.0	0	1.37	127	0.18 ± 0.03	0.09 ± 0.01
	5	0.62	57	0.17 ± 0.02	0.11 ± 0.03
	30	0.92	41	0.08 ± 0.02	0.06 ± 0.01
	60	0.56	39	0.21 ± 0.02	0.07 ± 0.02
	120	0.88	37	0.19 ± 0.02	0.09 ± 0.02
3.8	0	0.58	168	0.06 ± 0.01	0.03 ± 0.01
	5	0.60	137	0.03 ± 0.01	0.04 ± 0.00
	30	0.72	113	0.02 ± 0.01	0.03 ± 0.01
	60	1.18	101	0.02 ± 0.00	0.04 ± 0.01
	120	1.10	114	0.04 ± 0.01	0.06 ± 0.01

[a] P/R, PE = ratio of PE permeability; AGA, R = uronic acid content in mg/mL in retentate; AGA, P = uronic acid content in mg/mL in permeate.

DISCUSSION

The effect of cations and pH on PE activity was described for alfalfa (Lineweaver and Ballou, 1945) and citrus PE (MacDonnell et al., 1945; Jansen et al., 1960). They reported that electrostatic inhibition of PE by pectates was overcome by competitive displacement of PE by cations or neutralization of positive charge on PE by higher pH. Further, at optimum cation concentration, lower activity was reported at low pH than at high pH (Lineweaver and Ballou, 1945; MacDonnell et al., 1945). A 2-fold lower activity was obtained at pH 3.8 compared to pH 6.0 and 8.0 at optimal levels of NaCl or $CaCl_2$. In this study, cation type and concentration and pH also influenced permeability through a 30 000 MWCO hollow fiber membrane. At higher pH values

of pH 6.0 and pH 8.0 and higher concentrations of 0.15–0.3 M NaCl, higher permeabilities suggest that the mechanism involved in restricting permeability of PE through UF membrane is similar to the mechanism affecting PE activity. Binding capacity of pectin for PE was reported to be more than 15 times the natural PE in juice (Jansen et al., 1960). The uronic acid content of the diluted PE extract contained 0.18 ± 0.03 mg/mL. Similar levels of uronic acid were reported for different varieties of single-strength orange juice (Hernandez et al., 1992; Watanabe et al., 1979; Fukutani and Ogawa, 1986). Primarily water soluble pectin was extracted with the extraction procedure used in this study. PE, solubilized by alkaline salt buffers, is likely bound to water soluble pectin, which reduces PE permeability through UF membranes. Once released by high salt and/or high pH, the enzyme is capable of permeating the membrane in larger proportions. The decline in permeability observed at higher salt and/or pH values is likely to be related to the decline in activity observed by Lineweaver and Ballou (1945) and MacDonnell et al. (1945). These authors speculated that calcium released PE from an inactive pectin complex and stimulated activity. Competitive displacement at higher calcium concentrations for PE binding sites on pectin was responsible for PE inhibition. Charnay et al. (1992) later confirmed competition of orange PE binding sites on pectin by calcium using Lineweaver–Burk plots.

Polysulfone membranes are widely used in ultrafiltration due to chemical and physical stability including a wide pH tolerance (Cheryan, 1986). In controls using deionized water, cations up to 0.6 M NaCl did not affect flux. The interactive effects of NaCl, pH, and time on PE permeability and fouling are complex. At high pH and high salt concentrations, higher flux values are reflected in higher permeability (Figures 3 and 4). At low pH and/or in the absence of salt, higher flux values do not correlate with higher PE permeability (Figures 4 and 5). Higher PE permeabilities are obtained with lower fluxes at higher NaCl or $CaCl_2$ concentrations and higher pH.

The incongruity between high membrane fouling and high permeability with increasing salt concentrations may point to a common mechanism effecting the release of PE from pectin and membrane fouling. Membrane fouling in citrus juice processing was previously attributed to aggregation of large pectin polymers on the membrane surface (Watanabe, 1979) in a gel-like boundary layer (Vilker et al., 1981).

Principles of pectin gelation (Burns, 1991; Oakenfull and Scott, 1984) may be applicable to an understanding of membrane fouling. The negative charge of pectin increases as the degree of esterification decreases and/or as the pH increases above the pK_a range of 3.2–4.0. Electrostatic repulsion plays a role in reducing chain aggregation. Increased salt concentration shields electrostatic repulsion, allows hydrophobic interaction and hydrogen bonding, and leads to aggregation of pectin (Oakenfull and Scott, 1984). Aggregation of pectin molecules results in formation of a gel layer on the membrane surface and decreases flux. The cation shielding effect is less pronounced at pH 3.8, where pectin is less negatively charged.

PE is capable of permeating a 30 000 polysulfone membrane at all pH ranges in higher amounts than expected for a 32 000 enzyme. The three-dimensional structure of PE is not known. Two loops in the center one-third portion of the molecule, predicted from disulfide bridges in the amino acid sequence of tomato PE (Markovic and Jornvall, 1992), are the only indication of PE tertiary structure. The pI of MGF PE (Seymour et al., 1991) and other citrus PE (Rombouts et al., 1982) is known to be greater than pH 10. Histidine and cysteine are amino acids in PE with pK_a values, which could conceivably become positively charged in the pH range of study. The combined mole percentage represented by histidine (pK_a of 6.0) and cysteine (pK_a of 8.3) is less than 3 mol % (Seymour et al., 1991). On the basis of the pK_a of the free amino acid and the amino acid composition, it is not likely that a globular enzyme structure would unfold extensively between pH 8.0 or 6.0 and 3.8. If a conformational change did occur between pH 8.0 or 6.0 and 3.8, it is not likely to influence permeability. High permeabilities in high NaCl and lower pH values support the absence of a large pH-driven conformational change in PE and restriction of PE permeability. Although a thorough study would be required and is beyond the scope of this research, an overall high enzyme permeability suggests a native linear rather than a globular structure (Cheryan, 1986) or isozymes of lower molecular weight than that previously reported. High permeability at pH 3.8, 6.0, and 8.0, at optimal cation concentration (Figures 3–6), diminishes the likelihood that pH-induced changes in membrane porosity or chemical nature of the membrane are responsible for the higher permeability of PE at higher pH. High permeability at pH 3.8 after filtration at pH 8.0 and subsequent removal of pectin in the permeate further supports the conclusion that a PE complex with pectin prevents permeation at low pH and/or low salt concentration.

Approximately two-thirds of PE activity was lost in MGF extract during UF at pH 3.8 and 0.3 M or higher NaCl concentration. Similar losses of activity were observed with $CaCl_2$ during UF at low pH. No similar inactivation was observed at higher pH values. The loss of activity over time was affected by circulating the extract in the UF system, with no significant drop in activity over this course of time in an undisturbed stored solution. Inactivation may be related to the loss of the protective effect of pectin to which PE is bound.

SUMMARY

The results obtained clearly show that PE permeability through a 30 000 UF membrane is dependent on both pH and cation concentration. At low pH and/or cation concentration, PE is most likely bound to pectin substances and therefore restricted from permeating the UF membranes. Raising the pH and/or cation concentration is required to release PE from the electrostatic binding to pectin. Once PE is released from pectin, high permeability occurs even at low pH and advanced fouling in UF.

LITERATURE CITED

Baker, R. A. Clarifying properties of pectin fractions separated by ester content. *J. Agric. Food Chem.* **1979**, *27*, 1387–1389.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484–489.

Burns, J. K. The polygalacturonases and the lyases. In *The Chemistry and Technology of Pectin*; Walter, R. H., Ed.; Academic Press: New York, 1991; pp 165–188.

Charnay, D.; Nari, J.; Noat, G. Regulation of Plant Cell-Wall Pectin Methyl Esterase by Polyamines—Interactions with the Effects of Metal Ions. *Eur. J. Biochem.* **1992**, *205*, 711–714.

Cheryan, M. *Ultrafiltration Handbook*; Technomic Publishing: Lancaster, PA, 1986; pp 39–43, 66–68, 73–123.

Crandall, P. G.; Matthews, R. F.; Baker, R. A. Citrus beverage clouding agents—Review and status. *Food Technol.* **1983**, *37*, 106–109.

Eagerman, B. A.; Rouse, A. H. Heat inactivation temperature-time relationships for pectinesterase inactivation in citrus juices. *J. Food Sci.* **1976**, *41*, 1396–1397.

Ellis, K. J.; Morrison, J. F. Buffers of constant ionic strength for studying pH-dependent processes. *Methods Enzymol.* **1982**, *87*, 405–426.

Fukutani, K.; Ogawa, H. Clarification of Satsuma mandarin juice by ultrafiltration. *Nippon Shokuhin Kogyo Gakkaishi* **1986**, *33*, 108–116.

Hernandez, E.; Chen, C. H.; Shaw, P. E.; Carter, R. D.; Barros, S. Ultrafiltration of orange juice: Effect on soluble solids, suspended solids, and aroma. *J. Agric. Food Chem.* **1992**, *40*, 986–988.

Jansen, E. F.; Jang, F.; Bonner, J. Orange pectinesterase binding and activity. *Food Res.* **1960**, *25*, 64–72.

Köseoglu, S. S.; Lawhon, J. T.; Lusas, E. W. Use of membranes in citrus juice processing. *Food Technol.* **1990**, *44*, 124–130.

Lineweaver, H.; Ballou, G. A. The effect of cations on the activity of alfalfa pectinesterase (Pectase). *Arch. Biochem.* **1945**, *6*, 373–387.

MacDonnell, L. R.; Jansen, E. F.; Lineweaver, H. The properties of orange pectinesterase. *Arch. Biochem.* **1945**, *6*, 389–401.

Markovic, O.; Jornvall, H. Disulfide bridges in tomato pectinesterase: Variations from pectinesterase of other species; Conservation of possible active site segments. *Protein Sci.* **1992**, *1*, 1288–1292.

Nisperos-Carriedo, M. O.; Shaw, P. H. Volatile flavor components of fresh and processed orange juice. *Food Technol.* **1990**, *44*, 134–138.

Oakenfull, D.; Scott, A. Hydrophobic interaction in the gelation of high methoxyl pectins. *J. Food Sci.* **1984**, *49*, 1093–1098.

Rombouts, F. M.; Versteeg, C.; Karman, A. H.; Pilnik, W. Pectinesterases in component parts of citrus fruits related to problems of cloud loss and gelation in citrus products. In *Use of Enzymes in Food Technology*; Dupuy, P., Ed.; Technique et Documentation Lavoisier: Paris, 1982; p 483.

Rouse, A. H. Distribution of pectinesterase and pectin in component parts of citrus fruits. *Food Technol.* **1953**, *7*, 360–362.

Rouse, A. H.; Atkins, C. D. Pectinesterase and pectin in commercial orange juice as determined by methods used at the Citrus Experiment Station. *Fla. Agric. Exp. Stn. Bull.* **1955**, *No 570*, 1–19.

Seymour, T. A.; Preston, J. F.; Wicker, L.; Lindsay, J. A.; Marshall, M. R. Purification and properties of pectinesterases of Marsh white grapefruit pulp. *J. Agric. Food Chem.* **1991**, *39*, 1080–1085.

Snir, R.; Sims, K. A.; Koehler, P. E.; Wicker, L. The effect on nominal molecular weight cutoff, cation, pH and fouling on partitioning of pectinesterase in Valencia juice on polysulfone hollow fiber membranes. **1995a**, in preparation.

Snir, R.; Koehler, P. E.; Wicker, L.; Sims, K. A. The effect of nominal molecular weight cutoff and fouling on the partitioning of pectinesterase isozymes on polysulfone hollow fiber membranes. *J. Agric. Food Chem.* **1995b**, submitted for publication.

Versteeg, C.; Rombouts, F. M.; Spaansen, C. H.; Pilnik, W. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. *J. Food Sci.* **1980**, *45*, 969–973.

Vilker, V. L.; Colton, C. K.; Smith, K. A. Concentration polarization in protein ultrafiltration: Part II. Theoretical and experimental study of albumin ultrafiltered in unstirred cell. *AIChE J.* **1981**, *27*, 637–645.

Watanabe, A.; Ohata, Y.; Kimura, S.; Umeda, K.; Kimura, S. Fouling material on the reverse osmosis membrane during concentration of mandarin juice. *Nippon Shokuhin Kogyo Gakkaishi* **1979**, *26*, 260–265.

Wicker, L.; Vassallo, M.; Echeverria, E. Solubilization of cell wall bound, thermostable pectinesterase from Valencia orange. *J. Food Sci.* **1988**, *53*, 1171–1174, 1180.

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