Inactivation of Pectinesterase in Orange and Grapefruit Juices by High Pressure†

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The enzyme pectinesterase (PE) reduces the quality of citrus juices. Current inactivation of the enzyme is accomplished by heat, resulting in a loss of fresh fruit flavor in the juice. We explored the use of pressurized treatments of orange and grapefruit juices to bypass the use of extreme heat during processing. PE inactivation using isostatic high pressure in the range of 500−900 MPa was accomplished in orange and grapefruit juices. The higher pressures (>600 MPa) caused instantaneous inactivation of the heat labile form of the enzyme but did not inactivate the heat stable form of PE. Treatment times caused significantly different ($\alpha = 0.05$) total PE activity losses in orange but not in grapefruit juices, and PE inactivation at different pressures was significantly different in both juices. Heat labile grapefruit PE was also more sensitive than orange to pressure. D_p values for orange PE inactivation at 500 and 600 MPa were 83.3 and 2.4 min, respectively; while the z_p value between 500 and 600 MPa was 65 MPa. Orange juice pressurized at 700 MPa for 1 min had no cloud loss for >50 days.

Keywords: *Orange; grapefruit; juice; pectinesterase; high pressure; citrus*

INTRODUCTION

As an alternative to heat pasteurization, high pressure has been shown to reduce the microbial count (Takahashi et al., 1993; Ogawa et al., 1992), affect properties and functionalities of proteins (Messens et al., 1997; Masson, 1992), and influence enzyme activity (Seyderhelm et al., 1996; Basak and Ramaswamy, 1996). As such, it is rapidly gaining interest as a tool for food processing. The ability to cause the abovementioned changes in food products without the introduction of extreme heat, which can be deleterious to flavors and nutrients, is the main benefit of high-pressure processing. Orange and grapefruit juices are prime candidates for high-pressure processing.

Current processing of citrus juice employs a pasteurization step, which has the purpose of reducing the microbial load as well as inactivating pectinesterase (PE), the enzyme responsible for cloud loss during storage. Severe commercial pasteurization treatments are necessary to inactivate PE, and these are in excess of what is necessary to make the product microbially stable. Our purpose was to determine the effectiveness of high pressure for PE inactivation. Since it has been generally observed that constituents of food can have a protective effect on the enzyme against inactivation by heat or pressure (Seyderhelm et al., 1996; Ogawa et al., 1990), we chose to investigate PE in two of its biochemical environments, orange and grapefruit juice.

MATERIALS AND METHODS

Juice Preparation. Samples of orange and grapefruit juice were extracted in the pilot plant of the Citrus Research and Education Center in Lake Alfred, FL. The juice was not subjected to a finishing step. Juice not immediately used for PE inactivation studies was stored frozen at −23 °C and thawed before use. Additional fresh frozen finisher pulp from previous juice runs was added after thawing on a weight basis at 10.7% for orange and 8.7% for grapefruit, for added PE activity. The juices were then homogenized with a blender for 2 min to ensure small, relatively uniform particle size and distribution. The resulting pulpy juice was stirred before packaging samples (30 mL) into sterile polyethylene bags (Fisher Scientific, Pittsburgh, PA) and impulse sealed, retaining as little headspace as possible.

Cloud loss was monitored to determine the effectiveness of residual PE after treatment. Fresh squeezed, late season Valencia orange juice subjected to 700 MPa for 1 min was used for the cloud loss test. Extraction was accomplished by hand reaming. The method for determining cloud loss is described by Cameron et al. (1997). Samples (50 mL) were periodically drawn for analysis and were stored at 4 °C between analysis times. Sodium bisulfite was added at 1000 ppm to repress microbial growth and preserve pressurized and control juices.

Pressurization. Juice was pressurized using an isostatic high-pressure unit (Stansted Fluid Power, Stansted, England) at 600, 700, 800, or 900 MPa for 1, 15, or 30 s dwell time. Runs at 500 MPa were from 1 s to 1 h. Dwell time is defined as the time spent at the set point pressure. The packaged 30 mL samples were kept in an ice bath until they were pressurized. The pressure unit was at 5−10° C before pressurization began. A mixture of ethanol and castor oil (85/15 (v/v)) constituted the pressure medium. The time to reach the desired pressure was 12−15 s, while decompression was approximately 10 s. The use of a chiller to cool the pressure vessel jacket and the pressure medium ensured that samples remained in the temperature range of 20−50 °C during processing. All runs were done in duplicate. After pressurization, samples were kept at 0 °C until PE activity could be determined.

PE Activity Determination. PE was assayed using the titration method of Rouse and Atkins (1955) using 100 mL of a 1% pectin solution in 10% 1 M NaCl. Pectin from citrus fruits was obtained from Sigma (St. Louis, MO) and had an

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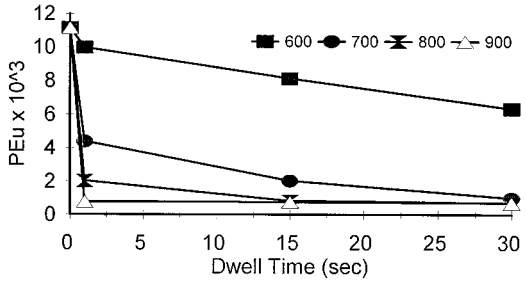

Figure 1. Inactivation of orange juice PE at 600, 700, 800, and 900 MPa pressure for three dwell times. Values are the average of two experiments.

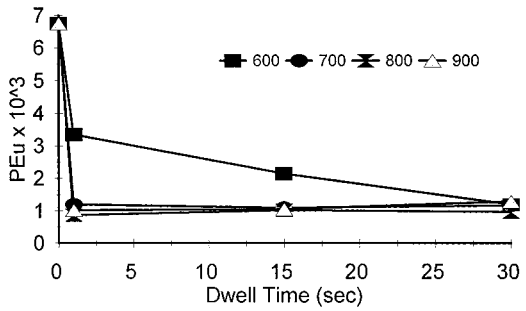

Figure 2. Inactivation of grapefruit juice PE at 600, 700, 800, and 900 MPa pressure for three dwell times. Values are the average of two experiments.

8% methoxy content. Pectin solutions were kept at a constant temperature of 28 °C. Results were reported in the conventional manner for citrus PE as pectinesterase units $\times 10^3$ min^{-1} g^{-1} juice (PEu $\times 10^3$). All samples were titrated in duplicate. Average %RSD of titrations was 8.0 for orange juice and 7.4 for grapefruit.

Data Analysis. Data were analyzed using the ANOVA\MANOVA function in Statistica (StatSoft, Tulsa, OK) to determine the significance of pressure and time treatments at the 95% level.

D_p values were calculated from the regression equation of the plot of log (PE activity) $\times 10^3$ versus time pressurized at each pressure indicated. The negative reciprocal of the slope of this plot is the D_p value. The z_p value is obtained from the negative reciprocal of the slope of log D_p versus pressure and is an indication of the pressure increase necessary to lower the D_p value by one log cycle.

RESULTS AND DISCUSSION

Fresh Valencia juice has PE activity in the range of 2−6 PEu $\times 10^3$ (Snir et al., 1996). Blending pulp into the sample juices increased juice PE activity to 10−12 PEu $\times 10^3$, the point that at least a log cycle reduction resulting from pressure treatment could be measured by the assay. Juice pulp was chosen over prepared enzyme to approximate the natural food system, since Pollard and Kierser (1951) found that enzyme inactivation in a raw juice was distinctly different from the results of a purified enzyme preparation. Also, commercial citrus PE is prepared from the citrus peel and may not have the same ratio of isozymes found in the internal parts of the fruit.

Kinetics. Figures 1 and 2 show remaining PE activity versus the dwell time at four different pressures. Inactivation of PE with higher isostatic pressure was biphasic, in accordance with the different forms of the enzyme, which was reported for thermal inactivation (Versteeg et al., 1980; Wicker and Temelli, 1988). The first drop in activity after pressurization has been described as an "instantaneous pressure kill" by Basak and Ramaswamy (1996). These researchers investigated pressure effects on PE in the range of 100−400 MPa and observed a much less pronounced initial drop. The time to reach the set point (come-up time) was longer on their pressure equipment, taking up to 3 min. They pointed out the come-up time at the lower pressures should not have much effect. Dwell times in their study were as long as 720 min. Since a hold time of this duration is commercially impractical, the need for information at higher pressures is clearly indicated. Seyderhelm et al. (1996) reported the effect of higher pressures on PE, but the data given were for commercial PE in pH 7 Tris buffer at 45 °C. The shortest processing time shown, 2 min, was sufficient to completely inactivate PE at 900 MPa. An approximately 45 °C increase in temperature can be expected at 900 MPa (Morild, 1992), so it is possible that the complete inactivation was augmented by heat. At 600, 700, and 800 MPa, less inactivation of PE was experienced in buffer (Seyderhelm et al., 1996) compared to our 15 s results in orange juice (Figure 1). This stresses the need for empirical data using the natural enzyme in the appropriate biochemical model for applicability to real food systems.

Table 1. Inactivation of PE by Pressure and Heat Treatments in Orange and Grapefruit Juice

	% inactivation	
treatment, MPa (time, s)	orange	grapefruit
600 (1 s)	10	50
700 (1 s)	61	82
800 (1 s)	82	87
900 (1 s)	93	85
acid treatment[a]	91	71

[a] pH adjusted to 2 with 0.5 M HCl for 5 min and then returned to the initial pH with 0.5 M NaOH.

It was suspected the initial drop in activity was due to an inactivation of the heat labile form of PE, while the remaining activity illustrated the effect of pressure on the heat stable form (Figures 1 and 2). The heat labile form of PE comprises from 86 to 94.4% of the total enzyme in Valencia juice (Snir et al., 1996), and at the higher pressures the rapid inactivation is very close to this percentage. Sun and Wicker (1996) confirmed that exposing juice to pH extremes (pH 2 for 5 min) can also inactivate the heat labile form, but this treatment was ineffective against the heat stable form. Subjecting the orange and grapefruit juice to a pH of 2 for 5 min resulted in 91% inactivation of the total PE activity in orange juice. The residual PE activity following higher pressure treatments of juice was similar to the activities reported after low-pH treatment, suggesting that the remaining activity represented heat stable PE. Table 1 is a summary of the inactivation percentages for orange and grapefruit juice at varying pressures and acid treatments. Subsequent heating of a pressurized orange juice sample (1 min at 700 MPa) for 2 min at 70 °C only reduced PE activity from 0.21 to 0.18 PEu, while heating for 2 min at 90 °C resulted in a marked decline in PE activity, from 0.21 to 0.08 PEu, substantiating that only the heat stable form remained after pressurization. These temperatures were chosen because they represent two levels of heating that can distinguish the two isozymes (Versteeg et al., 1980).

The question of whether the heat generated by pressurization was sufficient to inactivate PE was considered. Samples were placed in the unit at 5−10 °C and reached temperatures between 20 and 50 °C

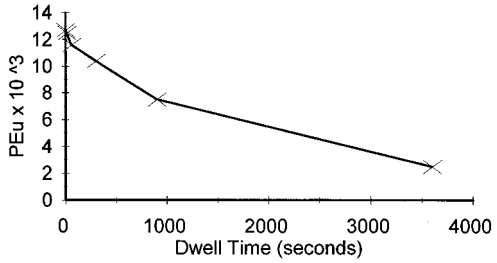

Figure 3. Inactivation of orange juice PE pressurized at 500 MPa versus dwell time.

(measured by a thermocouple) depending on set point pressure. Immediate cooling occurred upon decompression. Morild (1992) described the temperature change due to pressure changes as 1.86×10^{-3} K bar^{-1}. After adjusting the equation to the heat capacity of our pressure medium and converting to MPa, the conversion factor becomes 4.8×10^{-2} K MPa^{1-}. At the highest pressure used in this study, 900 MPa, the maximum theoretical temperature increase is 43.2 °C. This suggests that temperatures generated by pressures used in this study were not sufficient to thermally inactivate PE.

Figure 3 shows pressure treatment at 500 MPa, illustrating the difference in curve shape between lower (<600 MPa) and higher (600−900 MPa) pressures. At ≤600 MPa, it is possible to observe the first order inactivation of heat labile PE. At 700 MPa and above, pressure application inactivates this fraction more rapidly than the 1 s minimum dwell limitation of the equipment, leaving the heat stable form active (see Figures 1 and 2). The time required to reach the set point pressure was approximately 15 s, so the enzyme spent some time at the lower pressures before starting the dwell time counter, contributing to the inactivation of the enzyme. Higher pressures inactivate the heat labile form too quickly to measure this decline. Longer processing times at >600 MPa did not indicate any inactivation of the remaining heat stable form. Samples held at 700 and 800 MPa for as long as 1 min had little decrease in activity over a 15 s dwell time. These results showed that dwell times of 15 s or less were sufficient to reduce PE activity in orange juice, with inactivation increasing significantly with increasing pressures in both juices. ANOVA designates the probability of difference between PE inactivation pressure levels as 100% for both juices. This analysis does not include the control, to avoid skewing the results. Varying dwell times at the higher pressures did not cause significantly different PE inactivation in grapefruit juice (80% probability of difference) but caused significant differences in orange juice PE at a probability level of 100%.

The time necessary to reduce activity one log cycle, or 90% at a given pressure, is defined as the D_p value. The D_p value of PE in orange juice for 600 MPa was 143 s (2.4 min), while the D_p value for 500 MPa was 5000 s (83.3 min). This represents inactivation of the heat (and pressure) labile forms of PE. The corresponding z_p value was 65 MPa. Basak and Ramaswamy (1996) report a D_p value of 260 min at pH 3.7 and 14 min at pH 3.2 at 400 MPA. Our juice was in the middle of this pH range at 3.45. For comparison, the temperature necessary to accomplish 90% PE inactivation in less than a minute was reported as 85 °C by Rouse and Atkins (1952).

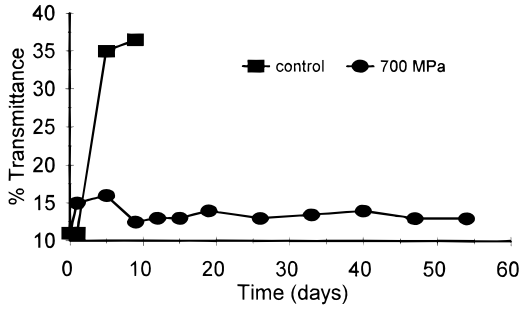

Figure 4. Cloud loss in pressurized (700 MPa for 1 min) and untreated orange juice stored at 4 °C.

Comparison of Figures 1 and 2 show that grapefruit PE was initially more sensitive to pressure treatment than orange. The same observation was made for the sensitivity of grapefruit PE to thermal inactivation (Rouse and Atkins, 1952). Due to the high percentage of grapefruit PE rapidly inactivated at 600 MPa, no D_p value was calculable from the pressure data of this study. Table 1 shows that grapefruit PE inactivation is not greater than ~85%, even at the highest pressures used. Comparing this to the values presented for orange, one may initially form a contrary conclusion, but our hypothesis is that since grapefruit PE has a percentage of heat stable enzyme as high as 33% (Rombouts et al., 1982), it will not experience as much total inactivation as orange PE by high pressure. Thus, we propose the heat labile form of grapefruit PE was more sensitive to pressure treatment. To substantiate this hypothesis, pressurized grapefruit juice samples (700 MPa for 1 min) were heated to either 70 or 90 °C and then assayed for PE activity after the sample was brought down to 4 °C. The 70 °C treated sample showed no change in activity, while the 90 °C sample decreased in PE activity from 1.5 PEu to 0, showing complete inactivation of both forms.

Cloud Loss. Juice cloud loss is the result of demethylated pectin interacting with calcium ions, causing a precipitation into a clear serum layer on top of a viscous layer of settled pectin and insoluble solids. Cloud loss is considered a quality defect in citrus juice, and it is one of the main reasons for the level of heating in commercial pasteurization. Since some PE activity remained in the juice, it was of interest to determine the stability of the cloud after pressure treatment.

The cloud is considered "definitely" broken, or lost, when the percent transmittance reaches 36% (Redd et al., 1986). Figure 4 summarizes the cloud loss over time in pressurized as well as untreated orange juice. PE activity was 1.3 PEu $\times 10^3$ (g^{-1} min^{-1}) before pressure treatment and 0.24 PEu $\times 10^3$ (g^{-1} min^{-1}) after. High pressure was very effective in preventing cloud loss for >50 days and is associated with inactivation of PE in orange juice, even though 18% of the initial activity remains. PE inactivation is the major reason for cloud preservation (Rouse and Atkins, 1952).

CONCLUSIONS

High pressure was shown to be useful for the inactivation of PE in orange and grapefruit juice. As such, it is a potentially useful tool for extending the shelf life of fresh juice, while preserving its fresh taste and appearance. Results indicate inactivation of the heat labile form of PE while having little or no effect on the heat stable form. For most efficient inactivation of PE in

these juices, pressures greater than 600 MPa should be used. Pressurization also stabilized orange juice cloud for an extended period, despite the remaining PE activity from the heat stable PE isozyme.

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