High-Pressure Processing of Orange Juice: Kinetics of Pectinmethylesterase Inactivation

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ABSTRACT: A kinetic study of pectinmethylesterase (PME) inactivation in orange juice was conducted. Juice samples were subjected to combinations of high pressure (400, 500, 600 MPa) and thermal (25, 37.5, 50 °C) treatments for various time periods. PME inactivation followed a first-order kinetic model with a residual activity of pressure-resistant enzyme remaining. Calculated D-values ranged from 4.6 min to 117.5 min at 600 MPa/50 °C and 400 MPa/25 °C, respectively. Pressures in excess of 500 MPa resulted in sufficiently fast inactivation rates for economic viability of the process.

Keywords: high pressure, orange juice, pectinmethylesterase, citrus, kinetics

Introduction

HIGH PRESSURE PROCESSING (HPP) IS AN EFFECTIVE NON-thermal processing technique for the stabilization of freshly squeezed orange juice. The shelf life of fresh orange juice is limited by cloud loss and microbial growth. The former is caused by the enzymatic activity of several pectinmethylesterase (PME) isoenzymes that are highly associated with the pulp; while the latter is primarily due to yeasts and lactic acid bacteria. HPP has the potential to reduce spoilage microflora and PME activity without the use of heat, thereby retaining fresh-like flavor. Microbiological stability of orange juice can be achieved, as yeasts, molds, and lactic acid bacteria are only mildly resistant against high pressure (Ogawa and others 1990; Ogawa and others 1992; Takahashi and others 1993; Donsì and others 1996; Parish 1998; Goodner and others 1999). Parish (1998) calculated a D-value of 4 s for *Saccharomyces cerevisiae* ascospores at 500 MPa. Temperature was not controlled in their experiments, but reported as not exceeding 40 °C with an average of 25 °C. Goodner and others (1999) estimated plate counts on orange serum agar of 5 to 10 colonies per mL orange juice processed at 800 MPa for 1 min. Temperatures at pressure were not reported. According to Donsì and others (1996) yeasts, molds, lactobacilli, and streptococci were undetectable after pressure-treatment at 350 MPa and 30 °C for 1 min.

A bigger challenge than microbial inactivation is the reduction of PME activity, an enzyme causing the breakdown of citrus juice cloud. The enzyme is found in all tissues of the fruit, with juice sacs containing around 75% of the total activity (Rouse 1953). PME is usually inactivated by pasteurization of orange juice at 90 °C for 1 min. PME in orange juice consists of several isoenzymes of which some are thermally tolerant (Cameron and others 1994; Cameron and Grohmann 1996; Snir and others 1996; Cameron and others 1997; Cameron and others 1998). Recently, kinetic models for the thermal inactivation of PME in citrus juices were reviewed (Chen and Wu 1998). The same heat-tolerant isoenzyme also appears to be pressure-resistant (Goodner and others 1998). Irwe and Olsson (1994) studied PME inactivation in orange juice by applying pressures of up to 600 MPa combined with temperatures of 20 to 60 °C. They concluded that processing at 400 MPa required additional heating, whereas 600 MPa was effective at 20 °C in reducing PME activity to less than 10%. They also noted that the degree of PME inactivation was dependent on the orange variety used. Kinetic studies on the inactivation of orange PME by high pressure have been done in the range of 100 to 400 MPa at unspecified temperatures in orange juice (Basak and Ramaswamy 1996) and in the range of 50 to 900 MPa combined with temperatures from 15 to 67 °C in model solutions with added orange peel PME (Van den Broeck and others 1999, 2000).

The costs associated with pressure processing are directly related to the treatment pressure and dwell time necessary to achieve the desired effect. Pressures lower than 400 MPa are of no commercial interest because of excessively long processing times. At pressures higher than 600 MPa kinetic studies are practically impossible because of nearly instantaneous PME inactivation during the pressure come-up time. Improvements in HPP efficacy will result in a reduction of processing costs through optimized operating pressures and dwell times. At the same time residual PME activity needs to be minimized to extend the shelf life of HPP orange juice. The objective of this research was to determine the inactivation kinetics for PME in orange juice in the pressure range of 400 to 600 MPa combined with temperatures between 25 and 50 °C.

Materials and Methods

Juice sample preparation

For initial experiments, Florida juice oranges were purchased at a local supermarket. Juice was extracted using a household centrifugal juice extractor (Kenwood JE500) and filtered through cheese cloth (grade 80). For the kinetic study, freshly squeezed orange juice was produced at a citrus processing plant in Florida, rapidly frozen and shipped overnight to Columbus, Ohio. Samples of 30 mL juice were vacuum-packed in Nylon-EVA pouches (Winpak Ltd., Winnipeg, Manitoba, Canada) and stored at −25 °C until use.

High-pressure processing

Pressure treatment was achieved using an ABB Quintus Food Processor QFP-6 Cold Isostatic Press (ABB Autoclave Systems, Columbus, Ohio, U.S.A.). The pressure fluid was a mixture of 1 part distilled water and 1 part Houghto-Safe

620-TY (Houghton International, Valley Forge, Pa., U.S.A.). Houghto-Safe 620-TY contains glycol derivatives and is used because of its anti-corrosive properties. Pressure fluid and samples in pouches were pre-chilled and pressurization started at defined temperatures to compensate for adiabatic heating increase during pressure come-up. Constant process temperatures were achieved by setting the temperature of the water-jacketed pressure chamber to the desired process temperature. Reported temperatures are actual process temperatures during hold time at reported pressure levels. Pressure, product temperature, and water-jacket temperature were monitored and recorded in 3 s intervals using a 21X Micrologger (Campbell Scientific Inc., Logan, Utah, U.S.A.) connected to a computer running PC208W datalogger support software (Campbell Scientific Inc.).

Pectinmethylesterase assay

PME activity was determined titrimetrically at pH 8.0 and 30 °C, using the method of Rouse and Atkins (1955) with modifications. The pH was maintained using a Computer-Aided Titrimeter (CAT) titration system (Fisher-Scientific, Pittsburgh, Pa., U.S.A.) by addition of 0.05 N NaOH from an automatic buret. The reaction mixture consisted of 10 mL juice and 40 mL substrate solution containing 10 g/L pectin (P 9135, Sigma, St. Louis, Mo., U.S.A.) and 0.1 M NaCl. The rate of NaOH consumption over time was linear and proportional to PME activity, expressed as microequivalents (meq) per min and mL juice. Each sample was analyzed in duplicate. Analysis run times ranged from 5 min to 30 min depending on the level of PME activity.

Kinetic study design

The inactivation of PME in orange juice by combined pressure and thermal treatments was studied using a blocked full-factorial design with 3 pressure levels (400, 500, 600 MPa), temperature levels (25, 37.5, 50 °C) and 5 process time levels (0 min to 30 min, different for each of the 9 pressure/temperature combinations). The process time levels were chosen appropriately depending on the expected inactivation rates. For practical reasons, experiments at the same temperature level had to be executed within 1 block, since conditioning of the equipment to different temperatures took considerable time. All experiments were performed in triplicate.

Kinetic calculations

A typical sample plot of pressure and temperature data is shown in Figure1. Pressure build-up occurred at a rate of about 5 MPa per s. This means that a pressure come-up time of 2 min is required for pressurization at 600 MPa. Process temperatures remained constant throughout the pressure hold interval. The start time of kinetic experiments was defined as the time when the set pressure was reached. Depressurization at the end of the pressure hold interval occurred practically instantaneously within 2 to 3 s. Inactivation of PME follows a fractional conversion model that is a special case of a 1st-order kinetic model (Van den Broeck and others 1999). Inactivation rate constants k were obtained from the slope of the regression of $\ln(A_t/A_0)$ versus time:

$$\ln(A_t/A_0) = -kt \quad (1)$$

Decimal reduction times D were calculated using the formula: $D = 2.303/k$. Activation energies E_a were obtained from the slope of the regression of ln k versus 1/T:

$$\ln k = -E_a/R_T \cdot 1/T \quad (2)$$

Activation volumes V_a were calculated from the slope of the regression of ln k versus p:

$$\ln k = -V_a/R_pT \cdot p \quad (3)$$

z_T values were calculated from the negative reciprocal slope of the regression of log D versus T:

$$z_T = (T_2 - T_1)/(\log D_1 - \log D_2) \quad (4)$$

z_p values were derived from the negative reciprocal slope of the regression of log D versus p:

$$z_p = (p_2 - p_1)/(\log D_1 - \log D_2) \quad (5)$$

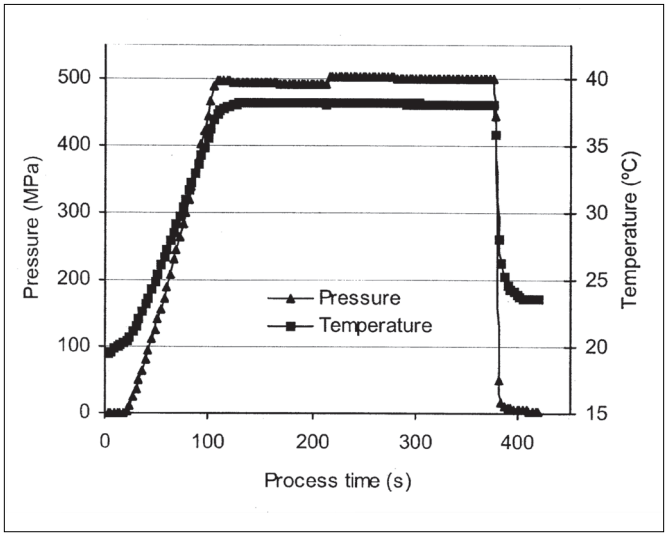

Figure 1—Sample plot of experimental run at 500 MPa and 37.5 °C for 4.5 min.

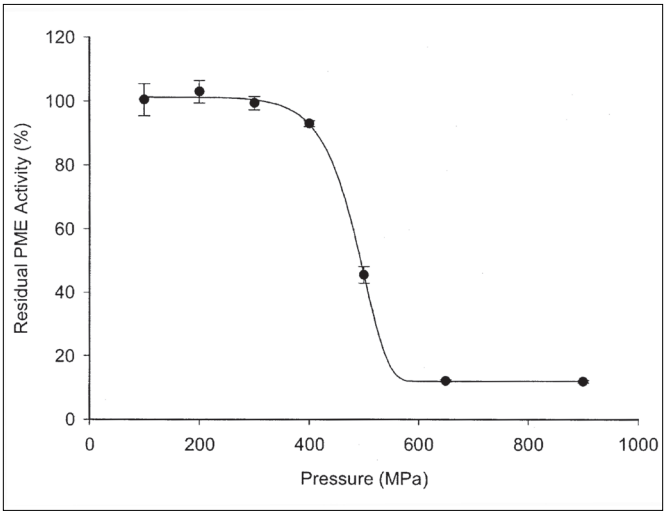

Figure 2—Inactivation of orange juice PME at various pressures applied for 3 min at 25 °C. Error bars represent ± 1 standard deviation, n=3.

Table 1—Residual PME activities in orange juice, processed at 400 MPa and atmospheric conditions.

T (ºC)	p (MPa)	t (min)	residual PME activity (%)	PME inactivated (%)
37.5	0.1	15	89.9	10.1
25	400	15	74.9	25.1
37.5	400	15	64.9	35.1
50	0.1	12	65.0	35.0
25	400	12	78.3	21.7
50	400	12	50.1	49.9

Table 2—Kinetic parameters for isobaric-isothermal PME inactivation in orange juice.

p (MPa)	T (ºC)	k (1/min)	D (min)
400	25.0	0.0197	116.9
	37.5	0.0292	78.9
	50.0	0.0505	45.6
500	25.0	0.0907	25.4
	37.5	0.1363	16.9
	50.0	0.1786	12.9
600	25.0	0.3308	7.0
	37.5	0.3890	5.9
	50.0	0.5048	4.6

Statistical analyses

Analysis of variance, correlation analysis, and general linear modeling were performed with the SAS system for Windows, release 6.12 (SAS Institute Inc., Cary, N.C., U.S.A.) and data analysis functions in Microsoft Excel. ™

Results and Discussion

In order to establish a feasible range for the inactivation of PME in orange juice, samples were initially subjected to pressures ranging from 100 to 900 MPa for 1 min to 30 min. The temperature at operating pressure was 25 °C. A sample plot for treatment times of 3 min is shown in Figure 2. A minimum pressure around 400 MPa was necessary to inactivate PME in fresh orange juice. However, the long hold time necessary at this pressure level to achieve significant reduction in PME activity, renders this processing condition commercially unfeasible. From an economical standpoint, a pressure of 650 to 700 MPa appears more favorable, as inactivation occurs sufficiently fast in less than 3 min. Further increasing the pressure did not show significantly greater PME inactivation. At all pressure-time combinations, residual PME activity remained due to pressure-resistant isoenzymes. Goodner and others (1998) found similar inactivation rates in fresh Valencia orange juice and proved the hypothesis that the heat stable fraction of orange PME is also pressure-resistant.

Process pressure and temperature control

Data for all process pressure and temperature combinations (135 runs) were analyzed for non-variance using a general linear models procedure. The mean values for the 3 pressure levels were 399, 498, and 597 MPa with coefficients of variance of 0.5%, 0.4%, and 0.4%, respectively. The mean values for the 3 temperature levels were 25.6, 37.6, and 50.0 °C with coefficients of variance of 4.3%, 1.4%, and 1.5%, respectively. The values showed that pressure could be controlled very accurately. Precise temperature control was somewhat more difficult to achieve, especially at lower temperatures.

PME inactivation kinetics

Results of the kinetic study showed that PME inactivation rates increased with increasing pressure and temperature (Figures 3 and 4). Increasing the process temperature from 25 to 37.5 °C at constant pressure resulted in higher inactivation rates for all pressures applied. The application of 400 MPa pressure for 15 min at 25 °C inactivated 25.1% of the total PME activity (Table 1). Holding the juice at 37.5 °C for 15 min without the application of pressure, reduced PME activity by 10.1%. The combination of 400 MPa and heating to 37.5 °C produced 35.1% enzyme reduction. These results and the ones obtained from the combination of 400 MPa pressure and heating to 50 °C for 12 min showed no evidence of a synergistic effect for PME inactivation through combination of pressure and thermal treatments. Nevertheless, mild heat can be used to accelerate the process if relatively low pressures are used.

Tables 2 and 3 summarize kinetic parameters for the inactivation of PME in orange juice. The D value is defined as the time necessary to reduce the activity by a factor of 10 at a given pressure and temperature. D values indicated that only

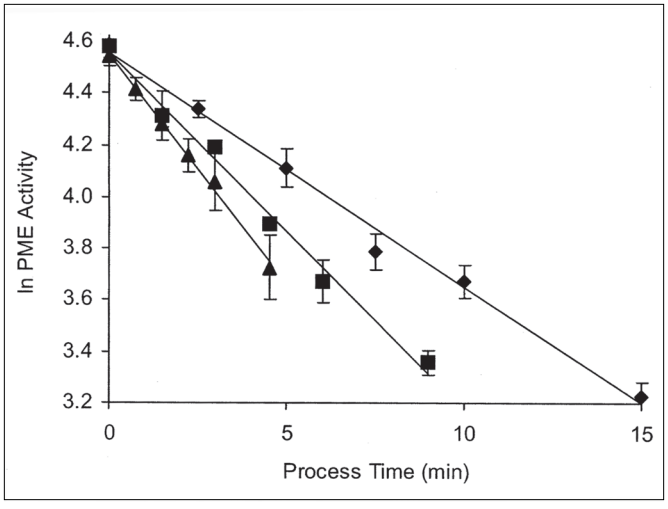

Figure 3—Inactivation of orange juice PME at 500 MPa and 25.0 °C (●), 37.5 °C (■) and 50.0 °C (▲). Error bars represent ± one standard deviation, n=3.

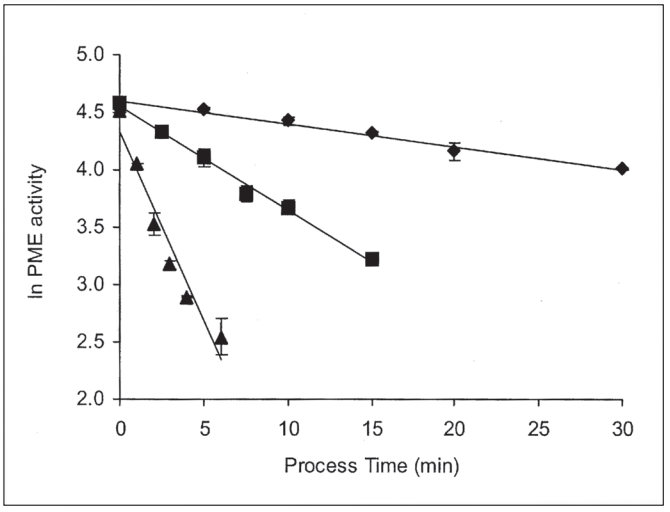

Figure 4—Inactivation of orange juice PME at 25 °C and 400 MPa (●), 500 MPa (■) and 600 MPa (▲). Error bars represent ± one standard deviation, n=3.

Table 3—Kinetic parameters for isobaric-isothermal PME inactivation in orange juice.

p (MPa)	E_a (kJ/mol)	Z_T (K)	T (ºC)	V_a (cm 3 /mol)	Z_p (MPa)
400	30.1	61	25.0	-35.0	164
500	21.8	85	37.5	-33.3	179
600	13.5	135	50.0	-30.9	200

pressures beyond 500 MPa resulted in sufficiently rapid inactivation of PME. For a pressure of 600 MPa, D values ranged from 7.0 min at 25 °C to 4.6 min at 50 °C. Goodner and others (1998) found a D value of 2.4 min for 600 MPa, but reported temperature only as being within the range of 20 to 50 ºC. On the other hand, their D value for 500 MPa was with 83.3 min considerably higher than ours. These differences can be attributed to natural variations between orange varieties, soluble solids content, and the juice pH. Irwe and Olsson (1994) pressure-treated juice from several orange varieties and reported substantial differences in PME sensitivity to pressure and temperature depending on the variety. Basak and Ramaswamy (1996) observed a decrease in PME inactivation rates with increased soluble solids content when applying pressures of 200 and 300 MPa to orange juice samples with 10 to 40 °Brix and attributed this to a protective effect of sugars on proteins. Several authors found that the inactivation of the heat labile PME was strongly enhanced by lower pH values (Basak and Ramaswamy 1996; Sun and Wicker 1996; Goodner and others 1998). For a pressure of 400 MPa at unspecified temperatures, Basak and Ramaswamy (1996) reported D values of 260 min at pH 3.7 and 145 min at pH 3.2. It is also apparent from the curve in Figure 2 that slight variations in the PME inactivation profile of a particular juice in the pressure range between 400 and 600 MPa will greatly affect D values.

Activation energies, E_a, decreased substantially with increasing pressure. Negative activation volumes, Va, indicated that PME inactivation was favored by pressure. Increasing temperatures resulted in slightly reduced activation volumes. With increasing pressure, z_T values increased, indicating that the enzyme inactivation rate became less temperature-dependant at higher pressures. z_P values increased with increasing temperature, as enzyme inactivation rates became less pressure-dependant. The z value responses are hypothesized to be due to competitive action of pressure and temperature on PME inactivation.

Conclusions

HIGH PRESSURE PROCESSING CONSTITUTES AN EFFECTIVE technology to stabilize fresh orange juice through reduction of PME activity. Preferably pressures higher than 500 MPa should be applied for economic and commercial viability of the process. High pressures in the range 400 to 600 MPa can be combined with mild heat (<50 °C) to accelerate PME inactivation.

Abbreviations

- A_0 enzyme activity at time 0 (meq/min·mL)
- A_t enzyme activity at time t (meq/min·mL)
- D decimal reduction time at constant pressure and temperature (min)
- E_a activation energy (kJ/mol)
- k inactivation rate constant (min^{-1})
- R_t universal gas constant (8.314 J/K·mol)
- R_p universal gas constant (8.314 cm^3·MPa/K·mol)
- V_a activation volume (cm^3 /mol)
- z_p z value at constant temperature (MPa)
- z_T z value at constant pressure (K)

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