High-Pressure Processing of Orange Juice: Combination Treatments and a Shelf Life Study
U. NIENABER AND T.H. SHELLHAMMER

ABSTRACT: Several process alternatives for the stabilization of fresh orange juice at pressures between 500 MPa and 800 MPa and temperatures between 25 and 50 °C were evaluated. Processing at 800 MPa and 25 °C for 1 min and use of thermally pasteurized pulp yielded the lowest level of residual pectinmethylesterase activity (3.9%) and good cloud stability at 4 and 37 °C over a period of more than 2 mo. Ascorbic acid loss was less than 20% after storage for 3 mo at 4 °C or 2 mo at 15 °C. Color values were stable during storage at 4, 15, and 26 °C.

Key Words: high pressure, orange juice, pectinmethylesterase, cloud, shelf life

Introduction

THE SHELF LIFE OF FRESHLY SQUEEZED ORANGE JUICE CAN BE greatly extended through the application of high hydrostatic pressure. Similar to thermal treatment, high pressure processing (HPP) inactivates most of the pectinmethylesterase (PME) isoenzymes responsible for pectin degradation and subsequent cloud loss in citrus juices. As a non-thermal processing technology HPP results in minimal product quality loss, thereby retaining fresh-like flavor. In addition, high pressure also eliminates or reduces the spoilage microflora of yeasts, molds, and lactic acid bacteria commonly found in citrus juice (Ogawa and others 1990; Ogawa and others 1992; Takahashi and others 1993; Donsi and others 1996; Parish 1998a; Goodner and others 1999). Parish (1998a) reported a D-value of 4 s for Saccharomyces cerevisiae ascospores at 500 MPa. 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. Yeasts, molds, lactobacilli, and streptococci were not detected in orange juice after pressure treatment at 350 MPa and 30 °C for 1 min (Donsì and others 1996). Hold times of 1 to 2 min at 500 MPa or 2 to 5 min at 400 MPa were sufficient to eliminate yeasts and molds in Sudachi (Citrus sudachi) juice (Iuchi and others 1996). Inoculation studies with pressure-resistant strains of Escherichia coli O157:H7 in orange juice showed at least a 6-log reduction after treatment at 550 MPa for 5 min at 20 or 30 °C (Linton and others 1999a). High pressure also significantly increased the susceptibility of E. coli O157:H7 to acidity, resulting in accelerated cell death rates during subsequent refrigerated storage (Linton and others 1999b).

The enzyme pectinmethylesterase (PME), which causes the breakdown of citrus juice cloud, is found in all tissues of the fruit, but primarily in the juice sacs (75% of total activity) (Rouse 1953). Some of several PME isoenzymes in orange juice are thermostable (Cameron and others 1994; Cameron and Grohmann 1996; Snir and others 1996; Cameron and others 1997; Cameron and others 1998) and appear to be pressure-resistant as well (Goodner and others 1998). A study on treatment of orange juice at pressures of up to 600 MPa combined with temperatures between 20 and 60 °C concluded that processing at 600 MPa and 20 °C or 400 MPa with additional heating reduced PME activity to less than 10% (Irwe and Olsson 1994). Parish (1998b) found that orange juice stabilized with high pressure received significantly higher sensory scores than heat-pasteurized juice after up the 16 wk storage at 4 °C. 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 first objective of this study was to investigate several processing alternatives within a pressure range of 500 to 800 MPa and hold times between 1 and 5 min. The second objective was to conduct a shelf-life study to monitor a number of quality parameters to assess the feasibility of high pressure pasteurization of orange juice.

Materials and Methods

Juice sample preparation

Freshly squeezed orange juice and pulp were produced at a citrus processing plant in Florida, rapidly frozen, and shipped overnight to Columbus, Ohio. Orange juice and pulp were stored at −25 °C until use. Thawed pulp was milled in a Fitz mill to reduce particle size. Some of the pulp was heat-pasteurized in Nylon-EVA pouches by submersion in a 90 °C waterbath for 2 min. Under these conditions PME was found to be completely inactivated. Samples of 30 mL juice with or without additional pulp (85 g per kg juice) were vacuum-packed in Nylon-EVA pouches (Winpak Ltd., Winnipeg, Manitoba, Canada) and stored at −25 °C until use. For the shelf-life study, orange juice with added pasteurized pulp (85 g per kg juice) was filled into 8-oz PET bottles with screw-cap closures (Novapak Corp., Hazelton, Pa., U.S.A.).

High pressure processing

Pressure treatment was achieved using an ABB Quintus Food Processor QFP-6 Cold Isostatic Press (ABB Flow Pressure Systems, Kent, Washington, U.S.A.). The pressure fluid was a mixture of 1 part distilled water and 1 part Houghto-
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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 or bottles 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 of juice and 40 mL of substrate solution containing 10 g/L of pectin and 0.1 M of 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.

Ascorbic acid
The ascorbic acid concentration was analyzed by a titrimetric method using 2,6-dichloroindophenol (AOAC Official Method 967.21). Samples of 50 mL of juice were extracted with equal volumes of metaphosphoric acid-acetic acid solution and filtered. Aliquots of 5 mL of filtrate were used for quantitative determination of ascorbic acid.

Cloud loss
Cloud loss was determined according to an industrial quality control method (Redd and others 1986). Samples of 50 mL juice were centrifuged at 360 /H11034g for 10 min using a tabletop centrifuge (Dynac II, Becton Dickinson, Sparks, Md., U.S.A.). Transmission at 650 nm was measured in the supernatant. Light transmission values are interpreted as follows: 0% to 24% - no cloud loss; 25% to 35% - slight cloud loss; 36% to 60% - definite cloud loss; 61% to 100% - extreme cloud loss.

Non-enzymatic browning
Non-enzymatic browning was measured spectrophotometrically according to the method by Klim and Nagy (1988). Samples (50 mL) were centrifuged at 1000 × g for 15 min using a tabletop centrifuge (Dynac II, Becton Dickinson, Sparks, Md., U.S.A.). An aliquot (5 mL) of supernatant and 5 mL methanol were mixed and kept on ice for 15 min. After recentrifugation at 1000 × g for 15 min, absorbance was measured at 420 nm with a UV-2401PC spectrophotometer (Shimadzu, Columbia, Md., U.S.A.).

Color
L (lightness), a (redness), and b (yellowness) values were determined directly in juice samples with a HunterLab Ul-

Table 1—Residual PME activities in orange juice with added pulp under different process conditions

<table>
<thead>
<tr>
<th>Process conditions</th>
<th>Juice + unprocessed pulp</th>
<th>Juice + pasteurized pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C) p(MPa) t (min)</td>
<td>Juice pulp</td>
<td>Juice pulp</td>
</tr>
<tr>
<td>25</td>
<td>800</td>
<td>1</td>
</tr>
<tr>
<td>25</td>
<td>600</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>500</td>
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</tr>
</tbody>
</table>

DE = ((L1 - L2)² + (a1 - a2)² + (b1 - b2)²)⁰⁵

Shelf life study design
For the shelf-life study, orange juice with added heat-pasteurized pulp (85 g per kg juice) in PET bottles was processed at 800 MPa with a 1-min hold time at 25 °C. Samples were stored at 4 temperature levels (4, 15, 26, 37 °C). Samples stored at 26 and 37 °C were analyzed at time 0 and every wk for a total of 7 wk; samples stored at 4 and 15 °C at time 0 and every 2 wk for a total of 14 wk. Untreated juice stored at 4 °C was used as a control and sampled every week for a total of 5 wk, at which time it had clearly spoiled. All experiments were performed in triplicate.

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 97. TM

Results and Discussion
A number of process conditions were selected for their commercial feasibility. Since PME is attached to the cell walls of the juice sacs, total PME activity increases with the pulp level. This means that commercial juices with high pulp levels pose a special challenge to achieving sufficient PME inactivation. For these experiments orange juice with 8.5% added unpasteurized or pasteurized pulp was used. This pulp level is found in commercial not-from-concentrate orange juices with high pulp levels.

Processing of juice with unpasteurized pulp at 800 MPa resulted in almost instantaneous PME inactivation with a residual activity of 6.4% (Table 1). This alternative required the shortest processing time, but high pressures above 600 MPa may be commercially undesirable as more expensive equipment is required to continuously operate at these pressures. Processing at 600 MPa or 500 MPa in combination with mild heat required longer processing times of at least 5 mins. Irwe and Olsson (1994) explored combinations of pressure (400 and 600 MPa) and process temperature (20 to 60 °C) and their effect on the reduction of PME activity in orange juice from different varieties. In order to achieve a substantial reduction of PME activity (>90%) at 400 MPa, process temperatures of 40 or 60 °C were required, depending on the vari-
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Table 2—Correlation coefficients of quality parameters in high-pressure processed orange juice during shelf-life study at 4 to 37 °C.

<table>
<thead>
<tr>
<th></th>
<th>T time</th>
<th>T x time</th>
<th>AA loss</th>
<th>Cloud loss</th>
<th>Browning</th>
<th>DE value</th>
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</thead>
<tbody>
<tr>
<td>T</td>
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<td>0.6492</td>
<td>0.8299</td>
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<tr>
<td>time</td>
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<tr>
<td>T x time</td>
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<td>0.9802</td>
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<td>0.9497</td>
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<tr>
<td>AA loss</td>
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<td></td>
<td>0.9557</td>
<td>0.8163</td>
<td>0.7736</td>
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<tr>
<td>Cloud loss</td>
<td>1.0000</td>
<td>1.0000</td>
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<td>Browning</td>
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Shelf-life study

Samples for the shelf-life study were subjected to 800 MPa at 25 °C for 1 min. Under these conditions, PME activity was reduced to a minimum. A correlation analysis using all data from the shelf-life study of high-pressure processed orange juice samples revealed strong correlation between ascorbic acid loss, development of non-enzymatic browning, DE value and the product of storage temperature and storage time (Table 2). Correlation of these quality parameters with either storage temperature or time only was weak. Both DE value and non-enzymatic browning correlated strongly with ascorbic acid loss.

Cloud stability is a very important quality parameter, and in fresh unpasteurized orange juice cloud loss usually occurs within a few days under refrigerated storage (see control in Figure 1). For quality control purposes, a borderline value of 36% light transmission, measured at 650 nm and 1-cm path length, is used as an upper limit for stable cloud in orange juice. HPP orange juice maintained a stable cloud over a period of at least 3 mo not only in refrigerated storage at 4 °C, but also at 37 °C. The extent of cloud loss increased with storage temperature. Parish (1998b) and Goodner and others
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(1998, 1999) observed comparable cloud stability in orange juice treated at 700 MPa for 1 min and stored at 4 °C for up to 4 mo, although residual PME activity was reported to be as high as 18% in one storage study (Goodner and others 1998). Ascorbic acid is an important vitamin in orange juice. At the beginning of the storage period an initial drop in ascorbic acid concentration occurred in all samples (Figure 2). This can be attributed to the fact that the juice was not deaerated; therefore, a portion of the ascorbic acid reacted immediately with dissolved oxygen. During further storage, ascorbic acid concentration gradually decreased in all samples, following pseudo-zero order kinetics (Kaanane and others 1988; Kaanane 1992). This can be attributed to oxygen diffusion through the PET bottles and anaerobic decomposition of ascorbic acid. More than 80% ascorbic acid was retained after 3 mo storage at 4 °C or after 2 mo at 15 °C.

Browning was most severe in samples stored at 37 °C, whereas samples stored at 4, 15, and 26 °C did not brown to an extent that would be objectionable (Figure 3). Color measurement directly in the juice samples with a HunterLab UltraScan revealed that color did not change over time except in the samples stored at 37 °C. In this case lightness (L value) decreased, redness (a value) increased, and yellowness (b value) decreased. This is reflected in the changes of total color differences (DE values) over time (Figure 4). Donsì and others (1996) reported no significant changes of color parameters in orange juice processed at 350 MPa for 1 min during subsequent storage for 2 mo at 8 °C. Nonenzymatic browning in orange juice is primarily caused by degradation of ascorbic acid, which is a precursor of the Maillard reaction. Ascorbic acid loss has been shown to correlate with the extent of nonenzymatic browning (Kaanane and others 1988; Kaanane 1992). Our results confirmed this, but the correlation between absorbance at 420 nm and loss of ascorbic acid was not as good as the correlation between DE values and ascorbic acid loss in samples stored at 37 °C (Figure 5 and Table 2). This is probably due to the fact that direct colorimetric measurements of the original juice give more accurate results than measurements of an extract after sample preparation. The obvious lag-phase in Figure 5 can be explained by the fact that oxidation of ascorbic acid to dehydro-ascorbic acid is only the initial step in a complex Maillard reaction pathway that eventually leads to colored compounds.

Figure 4—Color changes in orange juice during storage: (a) yellowness (b-value), (b) total color difference (DE value). Samples were processed at 800 MPa and 25 °C for 1 min. Error bars represent ± 1 standard deviation, n = 3.

Figure 5—Correlation between ascorbic acid loss and total color difference (DE value) (B) resp. absorbance at 420 nm (C) in orange juice during storage at 37 °C. Samples were processed at 800 MPa and 25 °C for 1 min.

Conclusion

STABILIZATION OF FRESH ORANGE JUICE BY HIGH PRESSURE processing requires pressure levels of at least 500 MPa to sufficiently reduce PME activity. PME inactivation can be further enhanced by combination of pressure treatment with mild heat up to 50 °C and thermal treatment of pulp prior to HPP of juice with added pulp. HPP at 800 MPa and 25 °C for 1 min and use of thermally pasteurized pulp yielded the lowest level of residual PME activity (3.9%). A shelf-life study of juice treated under these conditions revealed that high-pressure processed orange juice retained its fresh-like quality for several mo when stored under refrigeration. After a com-
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Commercially relevant period of 2 mo, cloud was stable despite the presence of about 4% residual PME activity, color changes were minimal and ascorbic acid loss was about 15%. Higher storage temperatures compromised the juice quality substantially and would render the advantages of high pressure processing irrelevant.

References


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