SHELF LIFE OF HHP-PROCESSED PEACH PUREE WITH ANTIBROWNING AGENTS

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ABSTRACT

Natural peach puree and peach puree containing 500 ppm of ascorbic acid (AA) or cysteine (250 ppm) were treated at high pressure (517 MPa/5 min). High hydrostatic processing (HHP)-processed and control purees were stored at 3, 21 and 35 ± 1°C for 30 days. Total count, yeast/molds, color and polyphenoloxidase (PPO) activity were analyzed regularly. PPO activity was reduced to 12.1, 26.4 and 5.5% in both the natural puree and puree containing AA and cysteine after applying HHP. PPO activity of the non-HHP-processed puree containing cysteine was different from the other purees, as well as the PPO activity of HHP-processed purees. Non-HHP-processed natural puree (yellow color) and purees containing AA (yellow color) and cysteine (orange color) maintained their colors individually for 9 days. HHP-processed purees maintained their yellow (natural puree and puree with AA) and orange colors (puree containing cysteine) for 21 to 24 and 30 days, respectively. Less than 10 cfu/g were counted in HHP-processed purees stored at 3°C.

INTRODUCTION

High hydrostatic processing (HHP) as a nonthermal technology can provide high quality pasteurized food products. The use of HHP for delivering

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minimally processed fruit and vegetable products is being used as an alternative processing for thermal pasteurization (Meyer et al. 2000). HHP may provide fruit and vegetable products with suitable shelf life, and maintaining characteristics similar to fresh products that consumers are demanding at present (San Martín et al. 2002). Currently, heat processing is applied to inhibit or destroy microorganisms or deteriorative enzymes. However, HHP can be applied successfully at room temperature, reducing the thermal energy needed by heat processing (Knorr 1993) which gives food products with undesirable changes in nutritional and sensory characteristics (San Martín et al. 2002). HHP as a nonthermal technology can be applied in fruits and vegetables for different purposes. HHP is applied to microbial and/or enzyme inactivation that can deteriorate sensory characteristics.

Color, in real fruit products, which is mainly changed after mixing polyphenol compounds with polyphenoloxidase (PPO) enzyme, has been evaluated in HHP-processed fruit products. Browning because of PPO activity can be used as an indicator of the quality of the HHP in fruit and vegetable products that darken after cutting or processing (Whitaker 1994). Ascorbic acid (AA) and cysteine perform antibrowning properties because of their reducing and complexing properties (McEvily et al. 1992). Palou et al. (1999b) pointed out that microbiologically stable blanched banana puree was obtained after HHP (517 or 689 MPa) for 10 min, and a reduction of browning rate was observed in the blanched banana puree. Cano et al. (1997) observed an activation of PPO activity after HHP of strawberry puree at pressures between 280 and 400 MPa. PPO activity was reduced to 86 and 63% in guava puree after treatment at 400 and 600 MPa, respectively; however, the remaining PPO activity barely decreased after storage of guava puree during 60 days (Yen and Lin 1996). López-Malo et al. (1998) stated that avocado puree containing <40% PPO activity lasted up to 60 days stored at 5C with acceptable color.

Stability of liquid fruit products is pursued by inhibiting pectic enzymes that destroy the cloudy appearance in juices. Cloud in orange juice has been stabilized after high pressure (500 MPa) processing for inhibiting pectin-methylesterase (PME). Orange juice lasted up to 90 days at refrigeration temperature after 900 MPa processing for 1 min (Goodner et al. 1999). They found out that heat labile pectinesterase (PE) was inactivated instantly at 600 MPa or higher in orange and grapefruit, but heat-resistant PE was less sensitive to pressures, grapefruit PE being more sensitive than orange PE (Goodner et al. 1997).

Vegetative cells and ascospores of yeasts have been studied because they can commonly grow in fruit products and spoil them because of fermentation and gas formation. Almost 5 log10 cycles were reduced after applying 300 MPa for 5 min to Zygosaccharomyces bailii vegetative cells suspended in apple,
orange, pineapple, cranberry and grape juices; however, ascospores were much more resistant to the same pressure and time (Raso et al. 1998). Zook et al. (1999) evaluated the inactivation of Saccharomyces cerevisiae ascospores inoculated in orange and apple juice. From their studies, they pointed out that the higher the pressure used, the lower the $D$-values obtained for inactivating ascospores in orange and apple juice.

Other authors have applied HHP in food extracts instead of real food systems to study the resistance of either enzymes or microorganisms. Weemaes et al. (1999) studied the combination of HHP along with ethylene diaminetetraacetic acid (EDTA), NaCl, benzoic acid or 4-hexylresorcinol on PPO avocado extracts as well as pH, benzoic acid, EDTA or glutathione on PPO mushroom extracts (Weemaes et al. 1997). Zook et al. (1999) evaluated S. cerevisiae ascospores inactivation in model juice systems after the application of HHP.

HHP combined with heat has been also applied for microbial and/or enzyme inactivation. Some of these studies have been assessed in crude green bean extracts for inactivating lipoxygenase (Indrawati et al. 2000), PPO in avocado extracts (Weemaes et al. 1998), PPO and microorganisms in potato cubes (Eshtiaghi and Knorr 1993), peroxidase (PO), PPO or pectin methyl-esterase in strawberry puree and orange juice (Cano et al. 1997), PO, PPO and PE in guava puree (Yen and Lin 1996) and kinetic studies regarding PME inactivation were conducted in orange juice (Nienaber and Shellhammer 2001).

Therefore, the application of HHP and holding time will depend on the expected results for the food products. The objective of this study was to evaluate the effect of HHP (517 MPa/5 min) along with AA or cysteine as antibrowning agents on peach puree stored at 3C.

**MATERIALS AND METHODS**

**Peach Puree**

Mature peaches (Prunus persica) (pH 3.75) were washed, maintained at low temperature (1–2°C), peeled, longitudinally sliced in four wedges and pitted. The wedges were maintained in ice water before being blended to obtain puree. Peach puree was placed in glass beakers surrounded with ice water before weighing in plastic bags. The puree was divided in three equal fractions: one fraction was added with 500 ppm of l-AA (Sigma-Aldrich, St. Louis, MO), the second part was added with 250 ppm of l-cysteine hydrochloride (Sigma-Aldrich, St. Louis, MO) and the third fraction was maintained without antibrowning agents (natural). Thirty-five grams of each puree was
weighed into plastic bags (Whirl-Pak, 4 oz, Cole-Parmer Instrument, Co., Vernon Hills, IL), the remaining air evacuated, tabs folded eight times to seal the opening and held in ice until being analyzed or HHP processed. Three replicates of peach puree were obtained to be HHP processed.

**Pressurization**

A set of 15 bags was wrapped with an outer polyethylene bag containing water prior to being treated at high pressure. The bags were high pressure processed at 517 MPa for 5 min. The come up time for reaching 517 MPa was 4.7 ± 0.1 min. An isostatic pressing system (Engineering Pressure System Inc., Haverhill, MA) was used for pressurization at 25°C. A solution of 5% Hydolubic 123-B (Houghton International, Valley Forge, PA) in water was used as the pressure medium in the cylindrical pressure chamber (height = 25.4 cm, diameter = 10.16 cm). After HHP, all purees were immediately analyzed for PPO activity or stored at 3, 21 or 35 ± 1°C. Color, PPO activity and total count plus yeasts and molds were analyzed every 4 days. Nonpressurized purees (controls) were also stored at the same temperatures. All analyses were carried out in triplicate every 4 days.

**Polyphenoloxidase Assay**

PPO activity was evaluated at 30°C in a 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA) at 420 nm. Five grams of puree were mixed with 5 mL of McIlvaine buffer (pH 6.6) was obtained by mixing 27.1 mL of citric acid (Sigma-Aldrich, St. Louis, MO) 0.1M and 72.9 mL of dibasic sodium phosphate (Sigma-Aldrich, St. Louis, MO) 0.2 M. The enzyme extract was obtained by centrifuging for 40 min at 4000 rpm (4°C) and then filtering through a Whatman paper no. 1 (Cole Palmer Instrument Co., Vernon Hills, IL). The reaction mixture consisted of 1 mL of buffer, 0.5 mL of catechol (0.175 M) and 0.25 mL of enzyme extract. The linear portion obtained by plotting reaction time versus absorbance was used for computing the enzyme activity units (EAU). One unit of PPO activity was defined as 0.001 ΔA_{420}/min/mL. All extracts were analyzed in triplicate.

**Color Measurement**

Lightness \((L^*)\) and chromaticity coordinates \(a^*\) (green-red) and \(b^*\) (blue-yellow) were measured using a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Osaka, Japan) in the reflection mode. A white ceramic plate was used for standardizing the instrument \((L^* = 97.5 ± 0.12, \ a^* = 0.23 ± 0.09, \ b^* = 2.66 ± 0.11)\). The total color difference \((ΔE^*)\) was computed as an indicator of the change in color (Marcus 1998).
Microbial Count

Serial dilutions of peach puree were made in peptone water (0.1%) before pour plating. Standard methods agar (BBL: Becton, Dickinson and Co., Cockeysville, MD) for total microbial count or Dichloran-Rose Bengal-Chloranphencicol agar (Difco: Becton, Dickinson and Co., Sparks, MD) for yeasts and molds were used for counting. Total count was counted after 48 h of storage at 35°C and yeasts and molds after 5 days of storage at room temperature (22°C).

General Analysis

Total soluble solids (TSS), pH and TA were measured before pressurization and during storage. TSS (°Brix) were measured with an ABBE-3 L refractometer (Milton Roy Co., Rochester, NY). pH was measured using a standardized Orion pH meter model 420 (Orion Research Inc., Boston, MA). For total acidity (TA), 10 g of peach puree diluted with water was titrated with sodium hydroxide solution (0.1 N) using phenolphthalein as basic-acid indicator or until pH reaches 8.2.

Statistical Analysis

Data were analyzed by lineal regression using a Microsoft Excel program to compute the EAU and its standard deviation (SD). Means were tested by analysis of variance (ANOVA) and least significant difference (LSD) with a predetermined significance of 5% using the SAS System (SAS Institute 1999).

RESULTS AND DISCUSSION

Initial Conditions

No substantial changes were observed regarding TSS (10.42 ± 0.04), pH (3.83 ± 0.19) or TA (0.79 ± 0.02% citric acid) either after the addition of antibrowning agents (AA or cysteine) or pressurization of peach puree. The only significant change was a small change in pH after mixing AA with puree (pH 3.59 ± 0.02). Smelt et al. (2001) pointed out that pH is greatly affected after pressurization of buffer solutions, but no significant changes have been observed in real foods containing organic acids.

PPO activity in natural puree (not containing antibrowning agents) was 476 ± 49 EAU (100%). PPO activity observed after the addition of AA (500 ppm) and cysteine (250 ppm) was 113.1 ± 13.1% or 30.9 ± 4.1%, respectively. Therefore, AA increased PPO activity whereas cysteine reduced
PPO activity in peach puree. Guerrero-Beltrán et al. (2004) observed a reduction of 14.7 or 53.2% of PPO activity after the addition of 1000 or 300 ppm of AA or cysteine, respectively. After applying pressure, the remaining PPO activity was 12.1 ± 1.6, 26.4 ± 9.6 and 5.5 ± 5.0% for natural puree and puree containing AA (500 ppm) or cysteine (250 ppm), respectively. Substantial reduction of PPO activity was observed in puree containing cysteine after HHP. Guerrero-Beltrán et al. (2004) reported a remaining PPO activity of 24.1, 21.3 and 0.5% for natural puree and puree containing AA (1000 ppm) and cysteine (300 ppm), respectively, after applying 517 MPa of pressure for 5 min. The remaining PPO activity of puree containing cysteine (5.5%) was significantly different ($P < 0.05$) from natural puree (12.1%) and puree containing AA (26.3%). PPO activity of non-HHP-processed purees were significantly different ($P < 0.05$) from those of HHP-processed purees. The addition of antibrowning agents reduces the enzyme activity, which depends on the amount and type of antibrowning agent (McEvily et al. 1992) and also on the antibrowning reactions and temperature. However, the application of high pressure can significantly increase the reduction of the enzyme activity because of the denaturation of the enzyme after pressurization (Heremans 2001). As a result, the antibrowning agents very probably will act on the remaining enzyme and improve the color stability for longer periods because fewer enzymes are available for reacting with more concentration of initial antibrowning agent.

Storage of Peach Puree

No significant changes were observed regarding general analysis during the entire storage time for HHP- and non-HHP-processed peach purees stored at various temperatures. The main change was observed in TA (0.78 ± 0.02%) in HHP-processed natural peach puree stored at three temperatures in comparison with nonpressurized peach puree (0.85 ± 0.05%). Similar data regarding pH (3.95 ± 0.08) and TA (0.80 ± 0.02) were observed for HHP-processed purees containing AA and cysteine. The TSS were maintained around 10.41 ± 0.04 during the entire storage of peach purees.

PPO Activity and Color

Table 1 presents the remaining PPO activity of HHP-processed and non-HHP-processed peach puree during storage. The reaction rate of PPO activity decreased rapidly at the beginning of storage. An average of 25.8% of PPO activity for all non-HHP-processed purees was observed at the sixth day of storage. General averages of 15.4 ± 30.3, 15.4 ± 33.6 and 7.6 ± 9.7% were observed for natural puree and purees containing AA and cysteine, respec-
tively, for the entire storage. The initial orange-yellow color of peach puree turned brown until 9 days of storage. The brown color was also revealed in the total color difference ($\Delta E^*$) (Table 2). Natural puree and puree containing AA presented a yellow-brown color after 9 days of storage, but puree containing cysteine presented a yellow-orange brown color at this time. After this time, an average of $3.3 \pm 3.1\%$ of PPO activity for all non-HHP-processed purees was computed. A total average for color differences corresponding to $9.4 \pm 1.8$, $9.3 \pm 1.9$ and $13.8 \pm 0.7$ for natural puree and purees containing AA and cysteine, respectively, was calculated for the range from 9 to 30 days of storage. Even though puree containing cysteine had the higher $\Delta E^*$ average, it maintained a very nice orange-yellow color during the entire storage, which

<table>
<thead>
<tr>
<th>Puree</th>
<th>HPO activity (%)</th>
<th>Non-HHP</th>
<th>HHP</th>
<th>3C</th>
<th>21C</th>
<th>35C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td></td>
<td>15.4 ± 30.3*</td>
<td>16.8 ± 7.3*</td>
<td>6.1 ± 3.3*</td>
<td>1.8 ± 3.6*</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td>15.4 ± 33.6*</td>
<td>20.9 ± 17.7*</td>
<td>7.2 ± 9.5*</td>
<td>3.0 ± 7.9*</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td></td>
<td>7.6 ± 9.7*</td>
<td>9.1 ± 4.2*</td>
<td>3.9 ± 2.7*</td>
<td>1.1 ± 2.1*</td>
<td></td>
</tr>
</tbody>
</table>

* Equal subscripts indicate nonsignificant difference ($P < 0.05$). Each treatment was carried out in triplicate.

Polyphenoloxidase, PPO; high hydrostatic processing, HHP; ascorbic acid, AA.

<table>
<thead>
<tr>
<th>Puree</th>
<th>$\Delta E^*$</th>
<th>Non-HHP</th>
<th>HHP</th>
<th>3C</th>
<th>21C</th>
<th>35C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>8.6 ± 3.9*</td>
<td>3.2 ± 1.5*</td>
<td>5.4 ± 2.8*</td>
<td>7.1 ± 2.8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>9.0 ± 3.5*</td>
<td>4.7 ± 1.7*</td>
<td>7.7 ± 3.3*</td>
<td>9.6 ± 3.3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>12.3 ± 4.9*</td>
<td>3.1 ± 1.8*</td>
<td>9.7 ± 4.5*</td>
<td>12.8 ± 4.6*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Equal subscripts indicate nonsignificant difference ($P < 0.05$). Each treatment was carried out in triplicate.

High hydrostatic processing, HHP; ascorbic acid, AA.
may be because of the lower remaining PPO activity (7.6 ± 9.7%) and its antibrowning properties by forming o-diphenol cysteine adducts (non-substrates for PPO) (McEvily et al. 1992) or by reducing o-quinines to o-dihydroxiphenols (Kahn 1985) and consequently delaying the browning reaction. No significant differences were observed between natural puree and purees containing AA (P > 0.05), either for PPO activity (Table 1) or total color differences (Table 2). PPO activity and ΔE* color were significantly different (P < 0.05) in puree containing cysteine and it maintained a nicer yellow color than natural puree and puree containing AA.

Figure 1 presents the remaining PPO activity of HHP-processed peach puree stored at 3°C. PPO activity of puree containing AA increased until reaching a maximum of 58.5 ± 13% after 6 days of storage. From this time the rate of the remaining PPO activity was slowed until reaching 4.1 ± 1.1% at 15 days of storage. A general average of 9.3 ± 2.9% of remaining PPO activity for all purees was obtained for the period from 15 to 30 days of storage. An average of 16.8, 20.9 and 9.11% of remaining PPO activity for natural puree and purees containing AA and cysteine, respectively, was observed during the entire storage time. The three types of purees were significantly different (P < 0.05) regarding PPO activity (Table 1) during the entire storage time. Averages of total color differences (ΔE*) (Table 2) of 3.2 ± 1.5, 4.7 ± 1.7 and 3.1 ± 1.8 were observed during the entire storage time for natural puree and purees containing AA and cysteine, respectively. No significant difference (P > 0.05) was observed between natural puree and puree containing cysteine (Table 2), but puree containing AA was significantly different (P < 0.05) from the other purees. The initial orange-yellow color was turning less orange, but remained yellow up to 21 and 24 days of storage for natural puree and purees containing AA, respectively. On the other hand, puree containing cysteine maintained its
orange-yellow color up to 30 days of storage. After these times, the purees started to brown or to turn pale. High pressure, low temperature and the addition of antibrowning agents can be an alternative for maintaining peach puree with adequate color for considerable periods before it starts to darken (Guerrero-Beltrán et al. 2004). The browning effect, along with inactivation of microbial load, can also be delayed with heat (blanching), but heat can change the flavor and color of fresh products (Knorr 1993). Therefore, high pressure as a nonthermal process can deliver pasteurized fresh-like fruit products that will last for some time before altering characteristics such as flavor and increased microbial load.

The remaining PPO activity for HHP-processed peach puree stored at 21 and 35°C is presented in Table 1. It is observed that the general average for the remaining PPO activity for purees stored at 21 and 35°C was smaller than for purees stored at refrigeration temperature. It is very probable that the reduced PPO activity was because of temperature during storage because the higher the temperature, the lesser the PPO activity during storage. Even though pH of purees (~4.0) was far off from the optimal pH for peach (pH 6) PPO activity (Whitaker 1994), the remaining enzyme activity still reacted with polyphenol compounds (McEvily et al. 1992) because browning or brown-reddish color was increasingly faster during storage of purees at 21 and 35°C. In general, enzyme activity of puree containing cysteine was significantly different (P < 0.05) from natural puree and puree containing AA (Table 1). The initial orange-yellow color of purees was not maintained beyond 3 days of storage at 21 and 35°C. The initial orange-yellow color started to brown or to turn pale after 3 days of storage in natural purees and purees containing AA, but puree containing cysteine started to turn brown-reddish and then pale. Three types of purees were significantly different (P < 0.05) in color (Table 2). Browning is recognized as a reaction of PPO with polyphenols (Whitaker 1994), but paling has been observed in apple products (Walker and Reddish 1964) and peach puree (Guerrero-Beltrán et al. 2004).

**Microbial Load**

The initial total count increased from 5.1, 5.2 and 5.4 log\textsubscript{10} cycles to 6.0, 5.8 and 6.4 log\textsubscript{10} cycles for non-HHP-processed natural puree and puree containing AA and cysteine, respectively. However, a decrease in total count was observed throughout the first 10 days of storage. In general, puree containing cysteine presented the higher total count throughout the storage time (P < 0.05). No significant difference was observed between total count of natural puree and puree containing AA (P > 0.05). Similar outcomes were observed for yeasts during the storage, but the initial count was 4.0, 3.6 and 3.8 log\textsubscript{10} cycles for natural puree and purees containing AA and cysteine, respec-
atively. The average count for yeasts during the complete storage in purees containing cysteine (1.8 × 10⁶ cfu/g = 6.3 log₁₀ cycles) was significantly different (P < 0.05) from total yeasts in natural puree (4.5 × 10⁵ cfu/g = 5.7 log₁₀ cycles) or puree containing AA (4.4 × 10⁵ cfu/g = 5.6 log₁₀ cycles). Therefore, total count was mostly yeasts rather than bacteria.

No microbial growth of total count or yeasts and molds (<10 cfu/g) was observed in HHP-processed natural puree and purees containing AA and cysteine throughout the entire storage at 3C. Palou et al. (1999a) pointed out that in general vegetative cells are inactivated between 400 and 600 MPa. However, pressures up to 1200 MPa can destroy spores (San Martín et al. 2002). Raso et al. (1998) observed a reduction of almost 5 log₁₀ cycles of *Z. bailii* pressurized at 300 MPa (5 min) in apple, orange, pineapple, cranberry and grape juice.

Table 3 presents averages for microbial growth of HHP-processed peach puree during storage at different temperatures.

<table>
<thead>
<tr>
<th>Puree</th>
<th>Microbial count (cfu/g) × 10⁻³</th>
<th>Yeast and molds</th>
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<tbody>
<tr>
<td></td>
<td>Total count</td>
<td>Yeast and molds</td>
</tr>
<tr>
<td></td>
<td>21C</td>
<td>35C</td>
</tr>
<tr>
<td>Natural</td>
<td>7.8 ± 16.0</td>
<td>19 ± 41</td>
</tr>
<tr>
<td>AA</td>
<td>2.2 ± 4.90</td>
<td>45 ± 81</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.86 ± 2.70</td>
<td>14 ± 36</td>
</tr>
</tbody>
</table>

Each treatment was carried out in triplicate. High hydrostatic processing, HHP; ascorbic acid, AA.

Table 3 presents averages for microbial growth of HHP-processed puree from complete storage at 21 and 35C. Growth of yeasts and molds at total count was observed at occasional times during the storage of purees at temperatures of 21 and 35C. For some dates no microbial count was observed (<10 cfu/g) and for other dates the microbial count was high enough to be counted because samples for analysis were taken randomly throughout the storage. The sensitivity of different types of microorganisms to pressure is dependent on media composition, time of treatment, temperature and growth stage (San Martín et al. 2002). Thus, the synergistic effect of pressure and low temperature can be desirable for reducing the risk of growth of microorganisms in real food such as peach purees. In addition, low temperature and antibrowning agents can inhibit browning of HHP-processed peach purees because of the remaining PPO activity.
CONCLUSIONS

PPO activity of non- and HHP-processed purees was reduced substantially at three temperatures during storage. No microbial growth (<10 cfu/g) was observed in purees after the application of HHP and during storage at 3C. Sporadic growth was observed during the storage of peach purees at 21 and 35C. PPO activity was reduced at room and mild temperature during storage, but all purees turned from their original orange-yellow color to a brown (natural puree and puree containing AA) or brown-reddish color (puree containing cysteine). Therefore, even though the content of PPO activity was reduced, the temperature increased the PPO activity, turning purees dark after 3 days of storage. HHP-processed natural puree and purees containing AA and cysteine maintained a very nice yellow (natural puree and puree containing AA) or orange-yellow (puree containing cysteine) color up to 21–30 days of storage at 3C. The use of high pressure along with antibrowning agents such as cysteine and AA in addition to storage at refrigeration temperature may be an alternative to reduce the microbial load and increase shelf life of peach puree and it also maintains a very good color quality because of the reduction of discoloration effect by PPO activity.

REFERENCES


