

Polyphenoloxidase Activity and Color of Blanched and High Hydrostatic Pressure Treated Banana Puree

E. Palou, A. López-Malo, G. V. Barbosa-Cánovas, J. Welte-Chanes, and B. G. Swanson

ABSTRACT

The effects of blanching and high hydrostatic pressure (HHP) treatments on natural flora evolution, polyphenoloxidase (PPO) activity and color of banana puree adjusted to pH 3.4 and water activity (a_w) of 0.97 were evaluated during 15 days storage at 25°C. Standard plate as well as yeast and mold counts of HHP treated purees were <10 CFU/g throughout storage. Blanching time was found to affect ($p < 0.05$) puree color. HHP treatments retained the initial color of the banana purees. Longer browning induction times and slower browning rates were observed when a longer blanching time was combined with a 689 MPa pressure treatment. A residual PPO activity < 5% was observed in the puree when a 7 min blanch was followed by HHP treatment at 689 MPa for 10 min.

Key Words: banana, blanching, color, high hydrostatic pressure, polyphenoloxidase

INTRODUCTION

THE USE OF HIGH HYDROSTATIC PRESSURE (HHP) is a preservation technology which can provide fresh-like quality to heat sensitive foods (Hayashi, 1989). HHP reduces microbial counts (Knorr, 1995a; Palou et al., 1997) and inactivates enzymes (Knorr, 1995b; Seyderhelm et al., 1996; López-Malo et al., 1998), while retaining the flavor and color quality of foods (Yen and Lin, 1996). However, enzymatic reactions are key problems in HHP processing of fruits. The pressure required to inactivate polyphenoloxidase (PPO) surpasses that needed to inactivate microbial vegetative cells. HHP effects on the enzymes depend on type of enzyme, nature of substrates, pressure, temperature and time of processing. Weemaes et al. (1997) reported an extreme pressure resistance of PPO from mushrooms. Knorr (1995b) reported that PPO activity after HHP treatments depended on the specific type of fruit or vegetable. Eshtiaghi and Knorr (1993) and López-Malo et al. (1998) reported notable reductions in initial PPO activity when HHP was combined with heat or low pH. PPO activity was enhanced after pressurization of several other foods (Asaka and Hayashi, 1991; Anese et al., 1995; Cano et al., 1997).

Flavor and color deterioration in pressure treated foods during storage were attributed to residual enzyme activity by Horie et al. (1991) and Cano et al. (1997). Hoover (1997) indicated blanching as a prerequisite for pressure treatment of fruits and vegetables to minimize enzymatic and oxidative reactions. Consequently, for the development of HHP processes for fruit products, it is essential to understand the influence of pressure on deteriorative enzymes. The purpose of this research was to determine the effects of blanching and HHP treatments on natural flora evolution, PPO activity, and color during storage of banana puree adjusted to pH 3.4 and water activity of a_w 0.97.

MATERIALS & METHODS

Preparation of banana puree

Bananas (*Musa sapientum*) were washed, peeled, cut transversally into slices (1 cm thick) and blanched in saturated steam for 1, 3, 5 or 7 min. Immediately after, the slices were sprinkled with a 1% w/v ascorbic acid solution (0.2 mL/g banana) and aseptically processed with a hand blender to produce a puree. Phosphoric acid solution (10% w/v) and sucrose were added to adjust the pH to 3.4 and reduce the initial a_w of 0.986 to 0.97, respectively. Control (unblanched) banana puree was also prepared in the same way. Each banana puree was divided into four equal parts with one stored as a microbial control without any treatment, another maintained as a color control with 1000 ppm potassium sorbate added and without HHP treatment, and the other two parts subjected to selected HHP treatments. Ten bags (ea

100g) were filled with each puree for each HHP treatment or control.

High hydrostatic pressure treatment

The bags with banana puree were wrapped with an outer polyethylene bag containing water, heat sealed, and exposed to HHP treatments of 517 or 689 MPa for 10 min (holding time) at 21°C. The HHP treatments were applied in a warm isostatic pressing system (Engineered Pressure Systems, Inc., Andover, MA) with a cylindrical pressure chamber (height = 0.25m, diameter = 0.10m). A 5% Mobil Hydrasol 78 water solution was used as the pressure medium. The pressure build-up velocity was 2.4 MPa/s after an initial delay of 45s. The come-up time required to reach 517 and 689 MPa was 4.4 or 5.6 min, respectively. The decompression time was <15s. The slow pressure build-up velocity obtained in our work assured minimum adiabatic heating (De Cordt et al., 1995) and temperature variations were limited to within a few °C.

Unblanched (control), blanched, and blanched-HHP treated banana purees were stored at 25±0.5°C for 15 days for color and microbiological analyses. Survival of microorganisms and color of the banana purees were determined daily during storage.

Microbiological analysis

Banana puree (10g) was serially diluted with sterilized peptone water (0.1%). For the standard plate counts, 1 mL of each dilution was pour plated in triplicate using plate count agar (Difco Laboratories, Detroit, MI) and incubated for 48h at 37°C. Yeast and mold counts were determined in triplicate on potato dextrose agar (Difco Laboratories, Detroit, MI), acidified with a 10% tartaric acid solution, and incubated for 5 days at 25°C.

Polyphenoloxidase activity

Banana purees were immediately analyzed after blanching and HHP, or stored at -40°C until analysis, but not longer than 12h. A 10g aliquot of puree was mixed for 60s with 10 mL of McIlvaine citric-phosphate buffer, pH 6.5, using a vortex mixer. The homogenate was centrifuged at 6000 rpm and 4°C for 30 min in a Sorvall RT 6000B centrifuge (Du Pont Co., Newtown, CT). The supernatant was filtered with Whatman pa-

Authors Palou and Barbosa-Cánovas are with the Biological Systems Engineering Dept. and author Swanson is with the Food Science & Human Nutrition Dept., Washington State Univ., Pullman, WA 99164-6120. Authors López-Malo, and Welte-Chanes are with Depto. de Ingeniería Química y Alimentos, Universidad de las Américas-Puebla, Sta. Catarina Mártir, Puebla, 72820, México. Author Address inquiries to Dr. G.V. Barbosa-Cánovas.

per No. 1 and analyzed for PPO activity at 420 nm and 25°C as described by Pizzocaro et al. (1993). Catechol solution (1 mL 0.175M) and 2 mL of McIlvaine buffer pH 6.5 were added to 0.5 mL of PPO extract. PPO activity was assayed in a 8452A Diode Array Spectrophotometer (Hewlett-Packard, Palo Alto, CA) and calculated on the basis of the slope from the linear portion of the curve of ΔA_{420} vs time up to 3 min. One unit of PPO activity was defined as 0.001 ΔA_{420} min⁻¹ (mL of extract)⁻¹. Residual PPO activity was expressed as the ratio between the blanched or blanched-HHP treated and unblanched (control) banana purees.

Color measurement

Hunter a, b and L parameters of banana purees were determined with a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Osaka, Japan) in the reflection mode. The instrument was standardized with a white ceramic plate (L=96.55, a=-0.35, b=-0.16). The hue (H), chroma (C) and browning index (BI) were calculated as follows:

$$H = \tan^{-1}[b/a] \quad C = \sqrt{a^2 + b^2}$$

$$BI = [100(x - 0.31)]/0.172$$

where:

$$x = (a + 1.75L)/(5.645L + a - 3.012b)$$

The browning index (BI) represents the purity of brown color and is reported as an important parameter in processes where enzymatic or nonenzymatic browning takes place (Buera et al., 1986; Guerrero et al., 1996; Castañón et al., 1998).

Statistical analysis

Analysis of variance (ANOVA) of the effects of pressure and blanching as well as their interactions on residual PPO activity, color parameters, and browning rates of banana purees was performed (Gacula and Singh, 1984). Statistica™ software (Statsoft™, Tulsa, OK) was used to analyze the experimental results. Significance of differences was defined at $p \leq 0.05$.

RESULTS & DISCUSSION

STANDARD PLATE AS WELL AS YEAST and mold counts of HHP treated purees were <10 CFU/g throughout 15 days of storage; equivalent results were observed for the color control purees containing potassium sorbate. However, the purees without HHP treatment or preservative spoiled within the first 3 days of storage at 25°C and before changes in color were noticed. Castañón et al. (1998) reported that banana puree with a_w 0.97 and pH 3.4 prepared with 1000 ppm of potassium sorbate was sensory evaluated after formulation with an overall acceptability of 6.4 in a 9 point hedonic scale. Also, banana puree microbial stability was demon-

Table 1 - Initial color parameter values of banana puree pretreated for selected blanching times and subjected or not to high pressure treatments

Blanching time (min)	L	a	b	Hue	Chroma	Browning Index
Without pressure treatment						
0	67.61	0.76	17.40	87.50	17.42	30.04
1	65.49	1.35	18.18	85.75	18.23	32.20
3	62.93	0.94	18.05	87.02	18.07	32.94
5	61.19	-0.58	15.06	92.21	15.07	27.53
7	58.11	-1.29	10.13	97.26	10.21	18.14
Pressure treated at 517 MPa for 10 min						
0	66.16	0.70	17.21	87.69	17.22	29.86
1	65.71	1.40	17.82	85.51	17.87	32.13
3	62.97	0.81	17.55	87.36	17.56	32.47
5	60.91	-0.53	14.43	92.10	14.44	25.42
7	57.30	-1.39	10.41	97.58	10.50	17.53
Pressure treated at 689 MPa for 10 min						
0	66.99	0.63	17.20	87.90	17.21	29.33
1	65.97	1.30	18.00	85.87	18.05	32.22
3	63.20	0.86	17.74	87.22	17.76	32.80
5	60.62	-0.41	14.76	91.59	14.77	26.40
7	57.22	-1.34	10.57	97.23	10.65	17.94

^aMean values of six replicates.

strated during 60 days of storage at 25 and 35°C.

During blanching (0.1 MPa) the PPO activity of banana puree was reduced (Fig. 1) from 416 to 390, 272, 141 or 63 units when the blanching time was 1, 3, 5 or 7 min; this represented a residual PPO activity of 93.8, 65.4, 33.9 and 15.1%, respectively. PPO activity increased after HHP treatment at 517 MPa for 10 min in the puree prepared without a blanching pretreatment. However, a 79% residual activity was observed for unblanched puree when HHP was applied at 689 MPa for 10 min. PPO activity was reduced during steam blanching and further reduced after HHP treatments.

Seyderhelm et al. (1996) reported important differences in the barostability of enzymes. Peroxidase, catalase, phosphatase and PPO are resistant to pressures of 600-700

MPa at 25°C. Anese et al. (1995) reported a reduction ($p < 0.05$) in PPO activity of a pH 4.5 apple cell free extract after pressurization at 700 MPa. Weemaes et al. (1997) observed that HHP treatments between 600 and 900 MPa induced structural changes in mushroom PPO conformation and a HHP treatment at 800 MPa inactivated PPO. López-Malo et al. (1998) reported a reduction ($p < 0.05$) in PPO activity in avocado puree after pressure treatments at 689 MPa, which was greater as the avocado puree pH was reduced from 4.3 to 4.1 or 3.9 with phosphoric acid.

Appreciable differences in PPO pressure resistance are dependent on the plant source. However, it is recognized that pressures higher than 700 MPa are needed to completely inactivate PPO. In our case, the low pH (3.4) and steam blanching aided in reducing PPO

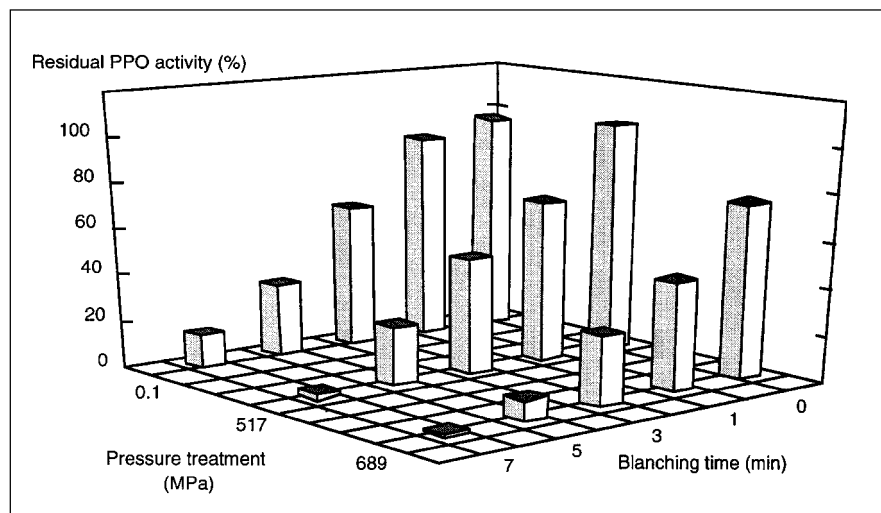


Fig. 1—Blanching time, high hydrostatic pressure (HHP) treatments (10 min holding time) and residual polyphenoloxidase (PPO) activity in banana puree.

Table 2—Zero order kinetic analysis during storage at 25°C, for the normalized browning index (DBI) of banana purees subjected or not to high pressure treatments

Blanching time (min)	Induction period (day)	Browning rate (DBI/day)	Correlation coefficient
Without pressure treatment			
0	1.4	11.4	0.98
1	1.7	10.6	0.97
3	2.5	6.4	0.99
5	4.7	4.2	0.99
7	6.2	2.0	0.98
Pressure treated at 517 MPa for 10 min			
0	2.0	9.1	0.97
1	2.1	7.1	0.98
3	3.1	5.7	0.99
5	5.0	2.7	0.98
7	6.9	1.7	0.99
Pressure treated at 689 MPa for 10 min			
0	2.4	8.0	0.97
1	3.2	5.0	0.97
3	5.4	3.7	0.98
5	5.9	1.6	0.99
7	6.8	0.8	0.99

activity in banana puree during HHP treatments. However, only when a 7 min steam blanch was followed by a 689 MPa pressure treatment, did the residual PPO activity in banana puree reach <5%.

Blanching time affected ($p < 0.05$) the color parameters of the banana purees (Table 1). For each blanching time, the initial color parameters were not different ($p > 0.05$) among treatments, including the purees without HHP. High pressure treatment preserved the initial color of the banana purees; however, during storage several color changes occurred. Since the banana purees initial col-

or depended on blanching time, a normalized browning index (ΔBI) was calculated as the difference between the initial BI and the BI at selected times (t) during storage for each treatment. For ΔBI changes in the blanched and blanched-HHP treated banana purees during storage at 25°C, an induction period ($\Delta BI > 0$) was found dependent on blanching time and application of HHP treatment. After the induction period, ΔBI was linear with a positive slope, until ΔBI was 46-47. Considering the linear portion of increasing ΔBI , a linear regression of ΔBI vs time was performed for each treatment to calculate brown-

ing rates ($\Delta BI/\text{day}$).

The color changes during storage of banana purees confirmed that residual PPO activity results in enzymatic browning of banana puree. The induction period to observe appreciable ($\Delta BI > 0$) changes in color as well as rate of browning thus depends on residual PPO activity. Longer induction times and slower browning rates were found with decreasing residual PPO activity in the banana purees (Fig. 2). Long blanching time followed by a 689 MPa pressure treatment, resulted in lengthy browning induction times and reduced browning rates (Table 2). A 3 min or longer blanching time reduced ($p < 0.05$) the browning rate. Also, HHP treatment at 689 MPa for 10 min reduced ($p < 0.05$) banana puree browning rates compared to HHP treatments at 517 MPa for 10 min, or no pressure treatment. Although a notable reduction of banana puree PPO activity was observed when a 7 min steam blanch was combined with a 689 MPa treatment for 10 min, the residual PPO activity (< 5%) was sufficient to initiate browning, which was noticeable after 6 days of storage at 25°C.

CONCLUSIONS

MICROBIOLOGICALLY STABLE BANANA purees were obtained by the addition of 1000 ppm potassium sorbate or the application of HHP at 517 or 689 MPa for 10 min. Reduced PPO activity in banana puree was obtained with HHP treatment and a short steam blanching pretreatment. Thus, browning of banana puree during storage can be diminished to extend the acceptable shelf life during storage at 25°C. The resistance of PPO to HHP at ambient temperatures was also confirmed. Consequently, applications of high pressures to control enzymatic browning reactions in fruit products must be accompanied by other factors effective in controlling enzyme activity. These include blanching, a_w , pH, certain additives, elevated temperatures during pressure treatment and/or refrigerated storage.

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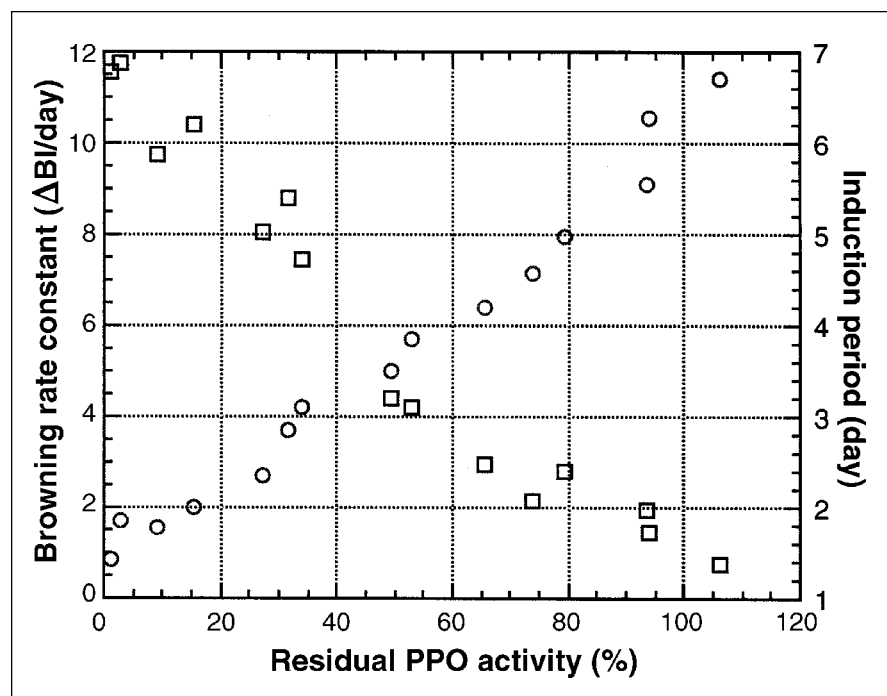


Fig. 2 —Effect of residual polyphenoloxidase (PPO) activity on banana puree browning rate (○) and induction period (□).

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