

# EFFECT OF pH AND ASCORBIC ACID ON HIGH HYDROSTATIC PRESSURE-PROCESSED MANGO PUREE

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## ABSTRACT

*Mango puree containing ascorbic acid (AA) (500 ppm) standardized at low pH (3.5) with phosphoric acid and inoculated or noninoculated with Saccharomyces cerevisiae was treated at high pressure (207, 345, 483 and 552 MPa) for selected times. High hydrostatic pressure (HHP)-processed (552 MPa/5 min) standardized mango puree (SMP) was stored at 3C for 1 month and periodically analyzed for color, residual polyphenoloxidase (PPO) activity and microbial load. The remaining PPO activity average in SMP, after HHP processing at 207, 345, 483 and 552 MPa, at all times, was  $35.8 \pm 6$ ,  $21.5 \pm 13.2$ ,  $46.8 \pm 53.2$  and  $61.8 \pm 5.8\%$  PPO activity units, respectively. The  $D_{207}$  values of 8.5 and 7.2 min for total count and yeasts were observed, respectively, after 207 MPa of pressure. A log reduction of 1.62 and 1.35 was observed after applying 345 MPa of pressure (2 s) for total count and yeasts, respectively. However, no microbial growth ( $<10$  cfu/g) was observed after applying 483 or 552 MPa at any time. The addition of AA and the standardization at pH 3.5 reduced the rate of browning during storage.*

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## INTRODUCTION

The increasing consumer demand for minimally processed and additive-free food products has increased the development of high hydrostatic pressure (HHP) processing technology (Mertens 1992). High pressure as a physical technique has the potential for the production of “natural” food products in which heat treatment is reduced (Gould 2001). A wide variety of shelf-stable fruits and vegetables might be supplied at retail in markets. However, studies of sensory, chemical and microbiological characteristics, along with shelf stability, are required. The main concern about quality loss in foods includes microorganisms, enzyme activity, chemical reactions and physical changes (Gould 2001). Minimally processed food products can be produced using HHP along with other food technologies to deliver pasteurized food products maintaining their flavor, texture and color. HHP processing of foods is applied mainly to accomplish: (1) inactivation of microorganisms; (2) modification of biopolymers (protein denaturation, enzyme activation or inactivation or gel formation); (3) quality retention (flavor and color); and (4) product functionality (Knorr 1993). Of those, enzyme activation or inactivation, microbial inactivation and quality retention are of paramount importance in the quality of HHP-processed fruit and vegetable products. The inactivation of enzymes using HHP processing depends on the type of food, temperature, pH and time of treatment. Seyderhelm *et al.* (1996) studied the combination of HHP with mild temperature. They found that HHP processing, for inactivating or inhibiting enzymes, followed the next order: lipoxygenase, lactoperoxidase, pectinesterase, lipase, phosphatase, catalase, polyphenoloxidase (PPO), peroxidase. However, there is a protective effect of food components in enzyme inactivation. Even though peroxidase does not cause detrimental effects on fresh fruits or vegetables, it is used as a quality factor because it is relatively resistant to heat (Whitaker 1996) and pressure (Seyderhelm *et al.* 1996). PPO can be found in moderate concentrations in peaches, apples, bananas, potatoes and lettuce (Whitaker 1996) causing detrimental effects (browning) after foods are cut or damaged. Anese *et al.* (1995) observed enzyme PPO activation in apple extracts after 1 min of HHP treatment (300–500 MPa). However, PPO inactivation occurred at 900 MPa of treatment. The reduction of pH and the addition of antibrowning agents are also desirable as additional hurdles to inhibit or destroy enzymes and/or microorganisms. Antibrowning agents such as reducing agents (e.g., ascorbic acid [AA]) can react with phenol compounds (substrates) to reduce *o*-quinones that polymerize to form brown pigments (melanins) (McEvily *et al.* 1992). Lowering the pH of fruits can reduce the enzyme activity because pH has a marked effect on the activity of most enzymes (Whitaker 1996). Low pH may also reduce the optimum environment for growing of microorganisms. Bacterial vegetative cells are more HHP

sensitive at low pH, whereas yeasts and molds are relatively resistant to low pH and barely affected at pH 4.0 when subjected to pressure (Smelt *et al.* 2001). Microbial growth and reproduction are delayed at moderate pressures (up to 200–300 MPa) and inactivation occurs at very high pressures. However, those growing and inactivating characteristics will depend on the type of microorganisms and species (Barbosa-Cánovas *et al.* 1998). In real food products such as fruits and vegetables, the application of HHP treatment will depend on the objective to be accomplished. Inactivation and/or destruction of microbial load and enzymes are pursued to obtain chemical and microbiologically safe food products. Ogawa *et al.* (1990) studied the effect of HHP on pectinesterase and peroxidase in Satsuma mandarin juice for cloud stability. Cano *et al.* (1997) studied the effect of HHP on strawberry puree and orange juice for inactivating peroxidase, PPO and pectinmethylesterase. López-Malo *et al.* (1998) pointed out that HHP-processed (345–689 MPa) avocado puree (pH 3.9–4.3) lasted up to 100 days of storage between 5 and 25C regarding microbial growth (<10 cfu/g). Avocado puree (<45% PPO) stored at 5C had acceptable color up to 60 days. Palou *et al.* (2000) treated guacamole four times (cycles) at 689 MPa (5 min). Fifty percent of residual PPO activity and nonmicrobial load (<10 cfu/g) were observed after this processing. However, browning during storage was observed and related with decrease in green color. HHP-processed blanched (at selected times) banana puree (pH 3.4; water activity [ $A_w$ ] 0.97) evaluated during 15 days at 25C was not spoiled (<10 cfu/g), but better synergistic effect was observed with longer blanching times and HHP processing (Palou *et al.* 1999). HHP processing is already used for commercial pasteurization of some food products. In 1990, the first HHP-processed pasteurized jam (low acid product) appeared on the Japanese retail market and in 1991 also appeared yogurts, jellies, salad dressings, fruit sausages and citrus juices (Mertens 1992). Avomex, Inc., a U.S. company, has been producing HHP-processed avocado products and salsas since 1996. Guacamole successfully lasting up to 45 days in refrigeration is available. HHP-processed cooked ham has been produced at Espuna S.A., Co. (Olot, Spain) since 1999. The cooked ham has a shelf life of up to 8 weeks (Rovere 2001). The objective of this study was to evaluate the effect of low pH, AA and HHP on the PPO activity and microbial load of mango puree.

## MATERIALS AND METHODS

### Mango Puree

Mature mangoes (*Mangifera indica*) purchased in the local market (Pullman, WA) were washed, maintained in ice water (1–2C), peeled, stoned,

sliced, blended in a domestic blender and maintained at low temperature (1–2C) to avoid browning. Mango puree was added with 500 ppm of L-AA (Sigma-Aldrich, St. Louis, MO) and phosphoric acid (50% w/w) to obtain a pH of 3.5 (standardized mango puree [SMP]). Mango puree was inoculated with *Saccharomyces cerevisiae* (1 mL inoculum/100 g puree) if required. Titratable acidity, pH, total soluble solids (TSS) ( $^{\circ}$ Bx) and microbial load were analyzed in control (fresh mango puree) and SMP before HHP processing. Thirty-five grams of mango puree was weighed and placed in sterile 7.6  $\times$  15.2 cm plastic pouches (Whirl-Pak bags, ~110 mL; Cole-Parmer Instrument Co., Vernon Hills, IL), heat sealed and held in ice water (1–2C) until analysis or HHP processing.

### Pressurization

Plastic pouches containing mango puree were inserted into an outer polyethylene bag (16.3  $\times$  17.8 cm), filled with water and heat sealed prior to HHP treatment. Four batches of mango puree were prepared, one batch to be treated at each pressure. Inoculated SMP was treated at selected pressures (0, 207, 345, 483 and 552 MPa) and times (2 s and 0, 1, 3, 5, 10 and 15 min) for microbial and PPO inactivation. Also, HHP-processed purees were stored at  $3 \pm 1$ C (30 days) to observe a change in color. On the other hand, noninoculated controls, noninoculated and nonpressurized SMP and inoculated and pressurized SMP (552 MPa/5 min) were stored for shelf life at  $3 \pm 1$ C (30 days). An isostatic pressing system (Engineering Pressure System, Inc., Haverhill, MA) was used for pressurization at 25C. 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). All treatments were carried out in duplicate. After HHP processing, all purees were immediately analyzed or stored for 1 month and periodically analyzed. Each sample was analyzed in duplicate as a minimum.

### PPO Activity

PPO activity was assessed spectrophotometrically at 420 nm (25C) in an 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, CA). The enzyme extract was obtained by mixing 5 g of mango puree with 5 mL of McIlvaine buffer (pH 6.6) at 4C, centrifuged at  $4000 \times g$  for 40 min (4C) in a Sorvall RT 6000B centrifuge (DuPont Co., Newtown, CT) and filtered in Whatman paper no. 1. The enzyme extract (0.25 mL) was added to a mixture containing 1 mL of McIlvaine buffer (pH 6.6) and 0.5 mL of catechol (0.175 M). The absorbance was recorded every 15 s for at least 3 min. The linear portion of the curve was used to compute the enzyme activity units (EAU) by plotting absorbance as a function of time. One unit of PPO activity was defined as  $0.001 \Delta A_{420}/\text{min/mL}$ .

## Color Analysis

HHP-processed mango puree and controls were color analyzed. Lightness ( $L^*$ ) and chromaticity coordinates green–red ( $a^*$ ) and blue–yellow ( $b^*$ ) were determined periodically 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 ( $L = 96.66$ ,  $a = -0.23$ ,  $b = -0.3$ ). The color difference ( $\Delta E^*$ ) was computed as described by Marcus (1998).

## General Analysis

The pH was measured using a standardized Orion pH meter model 420 (Orion Research, Inc., Boston, MA). The TSS were measured with an ABBE-3L refractometer (Milton Roy Co., Rochester, NY). For total acidity, 5 g of mango puree diluted with water was titrated with sodium hydroxide solution (0.1 N) using phenolphthalein as acid–base indicator until pH 8.1 was achieved.

## Microbial Growth (Inoculum)

Two milliliters of unfrozen *S. cerevisiae* culture (1 mL of microorganism grown in the early stationary phase plus 1 mL of sterile glycerol [20 mL/100 mL water] stored at  $-21^\circ\text{C}$ ) was added to 100-mL Sabouraud broth. The culture was grown on a rotary platform shaker at  $30^\circ\text{C}$  and 225 rpm. Microorganisms were grown until reaching the early stationary phase (around  $27 \pm 1$  h).

## Microbial Count

Serial dilutions were made of the sample before pour plating. Standard methods agar (BBL, Becton, Dickinson and Co., Cockeysville, MD) for total microbial count or dichloran rose Bengal chloramphenicol agar (Difco, Becton, Dickinson and Co., Sparks, MD) for yeasts and molds was used for counting. Total microbial counts plus yeasts and molds were counted after 48 h or 5 days of storage at  $35^\circ\text{C}$  or  $22^\circ\text{C}$  (room temperature), respectively.

## Statistical Analysis

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

## RESULTS AND DISCUSSION

### HHP-processed Mango Puree

The come-up times (CUTs) for pressures of 207, 345, 483 and 552 MPa were  $2.4 \pm 0.1$ ,  $3.5 \pm 0.1$ ,  $4.3 \pm 0.2$  and  $4.2 \pm 0.06$  min, respectively.

### Initial Physicochemical and Microbial Characteristics

The initial physicochemical and microbiological characteristics of control and SMP are presented in Table 1. The averages presented there were obtained from all data of mango puree to be HHP processed. Averages of 3.63 and 3.22  $\log_{10}$  cycles (cfu/g) of total count plus yeasts and molds, respectively, were estimated in puree just after preparation. The total counts of *S. cerevisiae* added to purees to be treated at 207, 345, 483 and 552 MPa were 5.65, 5.48, 5.71 and 5.48  $\log_{10}$  cycles (cfu/g), respectively. The addition of microorganism was to increase the microbial load in at least 5.0  $\log_{10}$ . The ratio of TSS/acidity was reduced from 46.3 to 23.2 after standardization at pH 3.5. Acidification as an additional hurdle by lowering pH from 4.6 to 3.5 may help in preventing bacterial growth including *Clostridium botulinum* (CCC 1996). No variability regarding pH, TSS or acidity was observed in control mango puree from four different batches of mango (Table 1). However, variability regarding PPO activity was observed in each batch of mangoes for preparing the puree.

### Microbial Load

Figure 1 presents the inactivation of *S. cerevisiae* in SMP treated at 207 MPa. A  $D_{207}$  value of 7.2 min was obtained using the first-order kinetics

TABLE 1.  
PHYSICOCHEMICAL CHARACTERISTICS AND INITIAL MICROBIAL LOAD OF MANGO  
PUREE BEFORE HIGH HYDROSTATIC PRESSURE PROCESSING

	Puree	
	Control	SMP
pH	$4.4 \pm 0.3$	$3.5 \pm 0.0$
TSS ( $^{\circ}\text{Bx}$ )	$13.9 \pm 0.1$	$13.9 \pm 0.1$
Acidity (%)*	$0.3 \pm 0.1$	$0.6 \pm 0.1$
Total count (cfu/g)	$(4.26 \pm 2.4) \times 10^3$	$(3.98 \pm 1.06) \times 10^5$
Yeasts and molds (cfu/g)	$(1.66 \pm 2.7) \times 10^3$	$(4.03 \pm 1.23) \times 10^5$

\* Citric acid.

SMP, standardized mango puree.

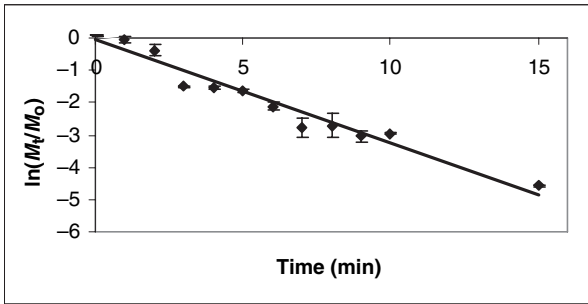


FIG. 1. FIRST-ORDER KINETICS MODELING OF SURVIVAL YEAST AND MOLDS IN HIGH HYDROSTATIC PRESSURE-PROCESSED (207 MPa) STANDARDIZED MANGO PUREE

model  $\ln(M_t/M_0) = -kt$  ( $R^2 = 0.962$ ) where  $M_0$  is the initial microbial load ( $5.1 \pm 1.4 \times 10^5$  cfu/g),  $t$  is time (min) and  $k$  is the inactivation rate constant (1/min) (Goodner *et al.* 1998).  $D_p$  is the time to inactivate or reduce 90% of microbial load at constant pressure and it is obtained from the slope ( $-2.303/k$ ). The CUT for 207 MPa ( $2.4 \pm 0.1$  min) barely reduced the yeast counts ( $0.067 \log_{10}$  reduction). A reduction of  $2.04 \log_{10}$  cycles after applying 15 min of pressure (207 MPa) was observed. On the other hand, a  $D_{207}$  value of 8.5 min ( $R^2 = 0.921$ ) was computed for total microbial count.

The total counts plus yeasts and molds were  $7400 \pm 282$  and  $1350 \pm 1838$  cfu/g, respectively, in mango puree treated for 2 s at 345 MPa, and no microbial growth ( $<10$  cfu/g) was observed after 1 min of treatment. Also, no microbial growth was observed in mango puree treated at 483 or 552 MPa for 2 s or longer times. Palou *et al.* (1997) applied a combination of pressure,  $A_w$  and potassium sorbate on *Zygosaccharomyces bailii* inactivation in model food systems. They pointed out that the lethal effect of pressure on a particular microorganism depends on the composition of the medium. A baroprotective effect on *Z. bailii* was observed at reduced  $A_w$ . No survivals ( $<10$  cfu/mL) were observed after applying 689 MPa for any combination of variables. Ogawa *et al.* (1990) found that the inactivation effect at 200 MPa or higher for inactivating *S. cerevisiae* in mandarin juice decreases as juice concentration increases. Regarding enzymes, Whitaker (1996) assures that "it takes longer to inactivate all the enzyme with higher initial concentration." In this study, the addition of AA and phosphoric acid for lowering pH does not contribute to increase the total solids content. Therefore, the necessity for inhibiting total load of microorganisms may not be higher than 345 MPa for short periods. Nevertheless, PPO activity could be the problem to be faced in obtaining a stable product during storage along with the additional antibrowning effect of AA.

## PPO Activity

The enzyme activities for the four batches of mango puree to be HHP processed were reduced from  $522 \pm 18.7$ ,  $12.1 \pm 3.2$ ,  $6.2 \pm 4.6$  and  $393 \pm 13.8$  to  $186.6 \pm 1.5$ ,  $2.1 \pm 0.3$ ,  $1.7 \pm 0.8$  and  $331 \pm 6.8$  EAU after adding AA and standardization at pH 3.5 (SMP). The variability of EAU probably was mainly because of the date of purchasing the mangoes because the first batch of fruits (to be treated at 552 MPa) was acquired 1 month earlier than the other batches of mangoes. Whitaker (1996) stated that the amount of enzyme activity in raw foods might vary because of cultivar of the same fruit, maturity and environmental conditions of growing. It has been seen in previous studies that mango puree standardized to pH 3.5 (Guerrero-Beltrán *et al.* 2005a) or 4.4 (Guerrero-Beltrán *et al.* 2005b) containing  $11.7 \pm 3.4$  and  $93.8 \pm 2.3$  EAU reduced its activity to 60.7 or 95.6%, respectively, after adding 500 ppm of AA.

PPO activities for control and SMP treated at various pressures and times are represented in Fig. 2. For each pressure, the reduction of PPO activity was barely reduced after treatment at high pressure. Averages of  $186.7 \pm 33$ ,  $2.6 \pm 1.6$ ,  $2.9 \pm 3.3$  and  $343 \pm 23$  were computed for all HHP processing times at 207, 345, 483 and 552 MPa, respectively. The EAUs of mango puree treated at 207 or 552 MPa were significantly different ( $P \leq 0.05$ ) from PPO activities of those treated at 345 and 483 MPa. Also, the PPO activities of mango puree treated during 0, 1 and 5 min were significantly different ( $P \leq 0.05$ ) from PPO activity in mango puree treated for 3, 10 and 15 min. Guerrero-Beltrán *et al.* (2005b) observed an average of  $74.1 \pm 11.3$  EAU of the remaining PPO activity in mango puree (pH 4.4) after applying various pressures (379–586) for various times (2 s to 20 min).

The small effect of HHP on PPO activity in mango puree can also be seen from the data in Fig. 3. In this case, SMP was treated at 207, 276, 345, 414, 483

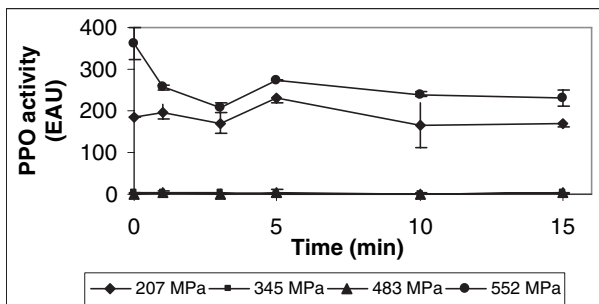


FIG. 2. REMAINING POLYPHENOLOXIDASE (PPO) ACTIVITY IN STANDARDIZED MANGO PUREE AFTER HIGH HYDROSTATIC PRESSURE PROCESSING AT SELECTED TIMES

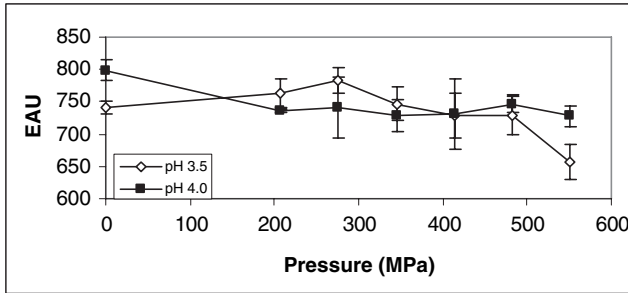


FIG. 3. REMAINING POLYPHENOLOXIDASE ACTIVITY IN CONTROL MANGO PUREE AND HIGH HYDROSTATIC PRESSURE-PROCESSED STANDARDIZED MANGO PUREE

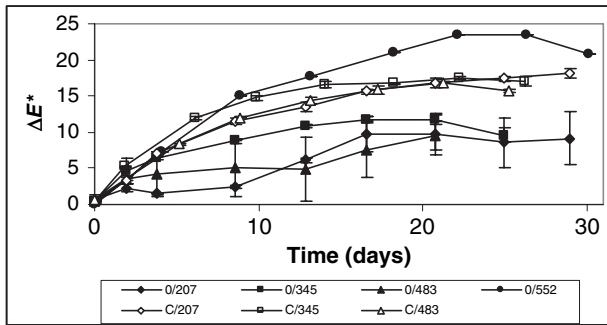


FIG. 4. CHANGE IN COLOR ( $\Delta E^*$ ) FOR CONTROL MANGO PUREE (C/PRESSURE) AND NON-HIGH HYDROSTATIC PRESSURE-PROCESSED STANDARDIZED MANGO PUREE (O/PRESSURE) STORED AT 3°C

and 552 MPa for 18, 15, 12, 9, 6 and 3 min, respectively. Only 7.3% of EAU were reduced after adding AA and adjusting the pH to 3.5. It can be observed that the reduction of PPO activity is no longer improved after pressurization. From these data, the following were observed: (1) there were no significant differences ( $P > 0.05$ ) between PPO activity at both pHs; and (2) only PPO activity after applying 3 min of HHP (552 MPa) was significantly different ( $P \leq 0.05$ ) from the other pressure/times.

## Color

Figure 4 illustrates the change in color ( $\Delta E^*$ ) of controls (C) and non-HHP-processed SMP (0 time) during storage. Numbers below the slash indicate pressure at what samples from those batches that were going to be pressurized. Initially, those purees presented a bright lemon yellow color. The

TABLE 2.  
SIGNIFICANT DIFFERENCES FOR TOTAL COLOR  
CHANGES ( $\Delta E^*$ ) OF CONTROL AND HIGH HYDROSTATIC  
PRESSURE-PROCESSED STANDARDIZED MANGO PUREE  
DURING STORAGE AT 3C (30 DAYS)

Treatment time (min)	$\Delta E^*$ Analysis of variance average <sup>†</sup>			
	Pressure			
	207	345	483	552
Control	11.9 <sup>a</sup>	12.5 <sup>a</sup>	12.0 <sup>a</sup>	–
0	5.5 <sup>d</sup>	8.0 <sup>b</sup>	5.1 <sup>b</sup>	16.1 <sup>a</sup>
1	7.2 <sup>b</sup>	5.0 <sup>cd</sup>	1.5 <sup>d</sup>	12.0 <sup>b</sup>
3	6.5 <sup>c</sup>	4.9 <sup>d</sup>	1.7 <sup>cd</sup>	11.4 <sup>c</sup>
5	5.5 <sup>d</sup>	5.5 <sup>c</sup>	1.3 <sup>d</sup>	10.5 <sup>d</sup>
10	5.4 <sup>d</sup>	5.5 <sup>c</sup>	1.4 <sup>d</sup>	9.7 <sup>c</sup>
15	4.7 <sup>e</sup>	5.2 <sup>cd</sup>	2.6 <sup>c</sup>	9.1 <sup>f</sup>

<sup>†</sup> Equal letters indicate nonsignificant differences ( $\alpha = 0.05$ ) within the same column.

addition of AA and standardization at low pH reduced the rate of browning during storage. The total change in color ( $\Delta E^*$  ANOVA average) of control mango purees (Table 2) was significantly different ( $P \leq 0.05$ ) from  $\Delta E^*$  values of the non-HHP-processed SMP (0 time). Therefore, the reduction of the browning rate was slowing down (Fig. 4). After 22 days of storage, the  $\Delta E^*$  values of control mango purees were around  $17.5 \pm 0.6$ . From this time, the  $\Delta E^*$  values were reduced and the color of the purees faded as the storage increased. This means that the darkened yellow color started to turn to a pale brown–yellow color. The non-HHP-processed SMP changed color no further than 12 total changes in color ( $\Delta E^* < 12$ ) before the yellow color began to fade. However, SMP to be HHP processed at 552 MPa continued to darken until reaching a  $\Delta E^*$  value of 23.5 (22 days). The increase in darkening during storage of this batch of mango purees was mainly because of the remaining PPO activity (Fig. 2) after standardization. Even though the PPO activity was reduced after the addition of AA and lowering the pH, the remaining enzyme can react with substrates to produce browning pigments (Whitaker 1996). Gas bubbles appeared in controls and some non-HHP-processed SMP (from batches to be treated at 207 and 345 MPa) after 14 days of storage.

Figure 5 presents  $\Delta E^*$  average and SD values (for all treatment times) of HHP-processed SMP during storage. Mango purees exhibited a bright lemon yellow color after HHP processing and before storage. The browning process began at different times for mango puree treated at the selected pressures. In general, the higher the pressure applied to the mango puree, the lower the rate

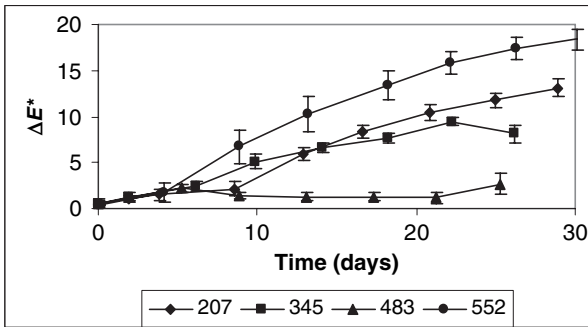


FIG. 5. CHANGE IN COLOR ( $\Delta E^*$ ) OF HIGH HYDROSTATIC PRESSURE-PROCESSED STANDARDIZED MANGO PUREE STORED AT 3°C

of browning (Table 2). However, very probably the amount of initial PPO activity was also involved in retarding the rate of browning. Mango purees treated at 207, 345 and 552 MPa started to fade (pale brown–yellow color) at 29, 22 and 22 days of storage, respectively. The averages of the total change in color ( $\Delta E^*$ ) at those storage times were  $13.1 \pm 1.0$ ,  $9.5 \pm 0.4$  and  $15.6 \pm 1.3$ , respectively. Mango purees treated at 483 MPa started to increase the rate of browning at 22 days of storage and still exhibited a bright lemon yellow color at the end of storage. However, those mango purees presented the lowest initial PPO activity from all batches used for HHP processing.

Table 2 presents the  $\Delta E^*$  ANOVA averages of the stored mango purees. In general, the longer the treatment times, the lower the browning. Even though mango purees treated at 552 MPa for all times were beginning to turn brown, they were still yellow until 22 days of storage. The ANOVA demonstrated that the total changes in color ( $\Delta E^*$  ANOVA averages) for control samples turned browner than the rest of the treatments, and they were also significantly different ( $P \leq 0.05$ ) from non-HHP-processed SMP (0 time) and HHP-processed SMP. Even though  $\Delta E^*$  changes in color for treatment times were different for each pressure, the  $\Delta E^*$  color changes for HHP processing during 5 and 10 min were not significantly different ( $P > 0.05$ ) for pressures of 207, 345 and 483 MPa. However, the inactivation of microorganisms was improved after 2 s of HHP processing, starting at 345 MPa of pressure.

### Storing of HHP-processed (552 MPa) SMP

**Microbial Load.** The SMP inoculated with *S. cerevisiae* contained  $(3.1 \pm 0.2) \times 10^5$  and  $(3.0 \pm 0.7) \times 10^5$  total count plus yeasts and molds (cfu/g), respectively. As already stated, no microbial growth ( $<10$  cfu/g) was

TABLE 3.  
MICROBIAL COUNT IN NONPRESSURIZED  
STANDARDIZED MANGO PUREE STORED AT 3C (27 DAYS)

Storage time (days)	Microbial count	
	Total counts	Yeasts and molds
0	$(2.8 \pm 2.5) \times 10^3$	$(1.7 \pm 6.0) \times 10^3$
5	–	$(2.7 \pm 0.6) \times 10^4$
11	–	$(2.8 \pm 0.4) \times 10^5$
15	–	$(1.3 \pm 0.5) \times 10^6$
20	$(4.4 \pm 0.8) \times 10^6$	$(4.4 \pm 0.5) \times 10^6$
27	$(1.5 \pm 0.4) \times 10^7$	$(1.3 \pm 1.5) \times 10^7$

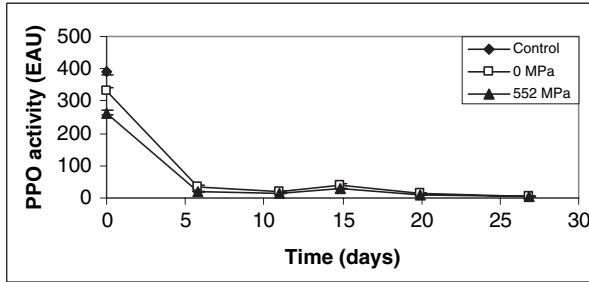


FIG. 6. POLYPHENOLOXIDASE (PPO) ACTIVITY FOR CONTROL, STANDARDIZED MANGO PUREE (SMP) AND HIGH HYDROSTATIC PRESSURE-PROCESSED SMP STORED AT 3C

observed after applying 552 MPa for any time, including 2 s of treatment. No microbial growth ( $<10$  cfu/g) was observed for HHP-processed (552 MPa/5 min) inoculated SMP during the entire storage. Table 3 presents the microbial growth during storage for nonpressurized SMP. More than 7  $\log_{10}$  cycles for total count plus yeast and molds were observed after 27 days of storage. The growth for total count for 5, 11 and 15 days of storage was not observed clearly; however, growth was observed at 22C for 20 and 27 days of storage. This might mean that psychrotrophic microorganisms were probably growing instead of mesophile microorganisms. Montville and Matthews (2001) stated that the growth rate of some types of microorganisms decreases abruptly above the optimal temperature for growing.

**PPO Activity and Color.** Figure 6 presents the remaining PPO activity of control and HHP-processed SMP throughout storage. The main effect on lowering the PPO activity was observed after 5 days of storage. From that

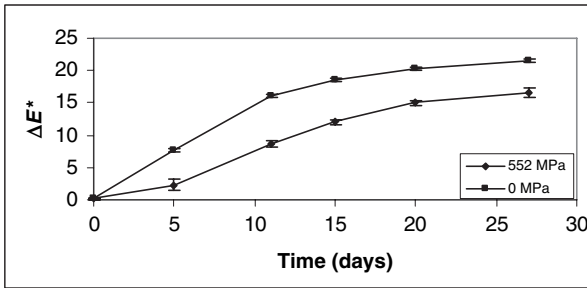


FIG. 7. TOTAL COLOR PARAMETER ( $\Delta E^*$ ) OF STANDARDIZED MANGO PUREE (SMP) AND HIGH HYDROSTATIC PRESSURE-PROCESSED SMP STORED AT 3°C

time, the average remaining PPO activity was  $26.9 \pm 14.1$  and  $17.1 \pm 8.7$  for non-HHP-processed SMP (0 MPa) and HHP-processed SMP (552 MPa), respectively. The PPO activity of control mango puree was statistically different ( $P \leq 0.05$ ) from the PPO activity of non- and HHP-processed SMP products. The  $\Delta E^*$  total change in color (Fig. 7) reached a maximum of  $21.5 \pm 0.7$  and  $16.6 \pm 0.3$  for non- and HHP-processed SMP products, respectively, as a result of the corresponding remaining PPO activity. Therefore, the addition of AA (500 ppm), lowering of pH (3.5) and HHP processing (552 MPa/5 min) may provide microbiologically safe mango puree with good color characteristics for longer times when stored at low temperature.

## CONCLUSIONS

Microbial reduction increased as pressure treatment was increased. The  $D_{207}$  values of 7.2 and 8.5 min were obtained for yeasts and total count, respectively, after pressurization at 207 MPa. No microbial counts were observed after applying more than 2 s of pressure at 345 MPa. Also, no microbial count ( $<10$  cfu/g) was observed after applying 483 or 552 MPa for any treatment time. Every batch of mango for making puree presented different initial concentrations of EAU, which were revealed in color changes during storage. The effect of pressure barely reduced the PPO activity in mango puree independently of the initial concentration of PPO activity. However, an additional hurdle in PPO activity reduction was observed after the addition of AA, standardization of pH and HHP processing of mango puree. SMP with fewer initial EAU and treated at higher pressures exhibited slower browning rate. All mango products began to turn to pale yellow–brown from 22 days of storage. No microbial growth was observed ( $<10$  cfu/g) in SMP

treated at 552 MPa (5 min) stored during 4 weeks at 3C, and its total change in color slowed in comparison with the change in color of non-HHP-processed SMP.

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