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Effect of high hydrostatic pressure on antioxidant content of 'Ataulfo' mango during postharvest maturation

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Abstract

The objective of this study was to evaluate the effect of pressurization on the concentration of some antioxidant compounds and the antiradical efficiency during the ripening process of 'Ataulfo' mango. The fruits at physiological maturity stage were pressurized at 15, 30, or 60 MPa for 10 or 20 min. Control fruits were not pressurized. The fruits were stored at 25 °C and changes in the concentration of ascorbic acid, total phenols, total flavonoids, total carotenoids, and antiradical efficiency were evaluated. It was demonstrated that in 'Ataulfo' mango high hydrostatic pressure treatments at 60 and 30 MPa for 20 minutes induced the synthesis of ascorbic acid during storage maybe as a consequence of physiological changes and possible structural modification of the cells, while the fruits pressurized at 15 MPa showed no effect on this parameter. On the other hand, the use of 15 MPa for 10 minutes increased the synthesis of phenols, flavonoids, carotenoids, and antiradical efficiency in 'Ataulfo' mango compared to that of the control fruit. In conclusion, this behavior seemed to be due to the low hydrostatic pressure treatments (15 Mpa), which stimulated the synthesis of antioxidants in the mango fruit and ripening was not inhibited.

Keywords: *Mangifera indica L*; high hydrostatic pressure; antioxidants.

1 Introduction

High hydrostatic pressure (HHP) is a relatively new, non-thermal food preservation process relatively new, which can inactivate or reduce the load of pathogenic microorganisms and inactivate deteriorative enzymes with a minimal effect on the overall quality of nutrients, color, and flavor in liquid or solid foods (GUERRERO; SWASON; BARBOSA, 2005). HHP is considered a non-thermal technology in the food industry because pressurization can be carried out at room temperature (KORHONEN et al., 1998).

HHP has been reported as an appropriated method to improve the extractability of bioactive compounds from vegetables and fruits. It has been demonstrated that the availability of lutein was higher in green beans pressurized at 600 MPa than that of control samples or samples pressurized at 400 MPa. The effect seemed to be associated with the disruption of cellular structures during the pressure treatment. However, the use of HHP from 400 to 600 MPa had very little impact on the availability of carotenoids and antioxidant capacity of either carrots or broccoli (McINERNEY et al., 2007). Using HHP improved the extraction of phenolic compounds of *Rubus coreanus* 'Miguel' fruits. An increase in the extraction was associated with alteration of the membrane cell permeability during pressurization, allowing the solvents to easily penetrate into the cytoplasm (YONG et al., 2011). HHP improved the extraction of bioactive compounds from 'Rojo Brillante' persimmon fruit at two stages of maturation. Samples treated at 400 MPa showed higher extractability of compounds than the samples treated at 200 MPa at these two stages of maturity. The improvement in extractability was associated with the cell wall rupture and dispersion of intracellular components through the tissue causing an overall decrease in firmness at both ripening stages (VÁZQUEZ-GUTIÉRREZ et al., 2011a).

Recently, HHP has been proposed as a quarantine treatment to destroy eggs and larvae of several genera and species of insects infesting raw fruits. However, preliminary reports have indicated that pressure levels required to destroy eggs and larvae of targeted pests (above 100 MPa) can induce detrimental changes in hosted fruits and modify their postharvest physiology (NEVEN; FOLLETT; RAGHUBEER, 2007; CANDELARIO et al., 2009; VELÁZQUEZ et al., 2010a, b). Consumers concerns regarding the nutritional quality and presence of bioactive compounds in fruits and vegetables has increased recently; therefore, the use of any postharvest technology in fruits and vegetables requires preserving their nutritional quality (WOLBANG; FITOS; TREEBY, 2008). On this regard, the effect of high pressure processing on changes in post-harvest physiology, nutritional composition, and overall quality of fruits requires further research.

Candelario et al. (2009) reported that HHP at 100 MPa for 5 min in whole mango cv Tommy Atkins, showed minimal visible changes in fruit quality. On the other hand, 'Ataulfo' mangoes pressurized at 15 to 60 MPa showed changes in their postharvest physiology, but their ripening process was not inhibited; the effect was dependent on the level of pressure,

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pressurization time, and interaction of both parameters (ÁLVAREZ-VIRRUETA et al., 2012). On the other hand, heat shock and UV-C irradiation have been reported as effective methods to induce abiotic stress in carrots, altering the respiratory metabolism and improving the synthesis of bioactive compounds (ALEGRIA et al., 2012). Nevertheless, information of using HHP to induce abiotic stress in fruits and vegetables to improve the synthesis of bioactive compounds is scarce.

The production of 'Ataulfo' mango in Mexico is important because it is one of the main varieties for export (MÉXICO, 2011). Currently, there have been scarce reports on the effect of HHP on the nutritional components of whole Ataulfo mango postharvest; therefore, the aim of this study was to evaluate the effect of HHP on changes in the concentration of different antioxidant compounds during postharvest maturation of 'Ataulfo' mango.

2 Materials and methods

2.1 Plant material

'Ataulfo' mangoes (*Mangifera indica* L) at physiological maturity were harvested in the 5 de Mayo Village in the municipality of Tepic Nayarit, Mexico. The fruits with the following characteristics were selected: light green color and sunk peduncle. The fruits were transported to the laboratory, washed, and immersed in a solution of 2-(4-thiazolyl)-1H-benzimidazole (20 mg/L) for 5 minutes to prevent fungal growth. Then they were dried and immediately used for this study. The fruits were pressurized on the harvest day (day 1) and stored at 25 °C along with the control fruits. The response variables were measured starting on the second day of storage.

2.2 High hydrostatic pressure treatment

The mangoes were distributed into seven groups of 50 fruits per group. Each group was subjected to different pressure treatments: 15, 30, or 60 MPa for 10 or 20 minutes at 25 °C. A control group remained untreated. Hydrostatic pressure treatments were applied using an isostatic press; model CIP42260 (Avure Autoclave Systems, Erie PA USA) with a pressure chamber of 101.6 mm internal diameter and 584.2 mm in length, which operates at a maximum capacity of 400 MPa. A mixture of water and anticorrosive lubricant (diethyl-amino-ethanol; Hydrolubric 120-B, EF Houghton and Co., Valley Forge, PA) in 5:1 (v/v) ratio was used as a pressurizing fluid. The mixture temperature was adjusted to 25 °C before pressurizing. The time required to reach the hydrostatic pressures of 15, 30, and 60 MPa was 3, 10, and 28 s, respectively. The time to release the pressure was always less than 20 s. The pressurized fluid temperature was not higher than 26 °C at the end of each treatment. The fruits were covered with high-density polyethylene and vacuum-sealed (Multivac model C100) at room temperature before introduced into the treatment chamber to avoid direct contact with the pressurizing liquid. Pressurized mangoes were stored at 25 ± 1 °C and 85-90% relative humidity (RH) until senescence.

2.3 Ascorbic acid (AA)

Ascorbic acid (AA) was analyzed using the technique reported by Suntornsuk et al. (2002); 10 g of mango pulp were homogenized with 25 mL of sulfuric acid (2 N), 25 mL of distilled water, and 3 mL of starch used as indicator. The mixture was titrated with iodine (0.05 N), and the results were reported in mg ascorbic acid/100 g fresh weight (FW).

2.4 Total carotenoids (TC)

Two g of mango pulp were mixed with 7 mL of acetone:petroleum ether 80:20 (v/v), and 0.5 g of $MgCO_3$ were homogenized for 1 min before centrifuged at 15000 rpm for 30 minutes at 4 °C. The supernatant was placed in a separator funnel adding 15 mL of NaCl (20%). The aqueous phase was drained, and the organic layer was diluted to 10 mL with petroleum ether. Absorbance was measured at 448 nm using a UV/Vis spectrophotometer (Jenway model 6705). The quantification was performed using a calibration curve of β-carotene (PHILIP; CHEN, 1988). To express β-carotene concentration on dry weight, the moisture content was measured in the mango pulp using the AOAC method (ASSOCIATION..., 1984).

2.5 Extractable phenols (EP)

10 g of pulp were homogenized with 96% ethanol and sonicated (sonicator Cole-Parmer model 8891) for 30 minutes. Subsequently, the sample was centrifuged (centrifuge Hermle Labortechnick model Z36HK) at 11500 rpm and 10 °C for 10 minutes. The supernatant was concentrated to a final volume of 4 mL; then 5 mL Folin Ciocalteu's reagent and 20 mL of sodium bicarbonate 20% were added. The reaction was allowed to stand for 30 minutes, and absorbance was measured at 760 nm using a spectrophotometer. A calibration curve with standard chlorogenic acid was constructed to quantify the EP. The results were expressed as mg of chlorogenic acid/100 g dw (VIÑA; CHAVES, 2006).

2.6 Total flavonoids (TF)

The flavonoid extraction was performed as EP; 0.5 mL of extract was mixed with 3.1 mL of methanol and 0.15 mL of $NaNO_2$:water (1:20 w/w); the reaction was carried out in complete darkness. After 5 and 6 minues, 0.15 mL of $AlCl_3$:water (1:10 w/w) and 0.01 mL of NaOH 11.25 M were added, respectively. The reaction was allowed to stand for 15 min. Absorbance was measured at 415 nm using a spectrophotometer. The quantification was performed using a calibration curve with quercetin. The results were expressed as mg of quercetin/100 g dw (ZHISHEN; MENGCHENG; JIANMING, 1999).

2.7 Antiradical efficiency (AE)

Antiradical efficiency was performed following the method of Sánchez-Moreno, Larrauri and Saura-Calixto (1998). To obtain the antioxidant extract, 5 g of pulp were stirred with 10 mL of absolute methanol for 2 h before centrifuged for

30 minutes at 4 °C and 15000 rpm. A solution containing 25 mg L^{-1} of the radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) was prepared. Portions of 5, 10, 20, 30, 40, and 50 µl of antioxidant extract were reacted separately with 3.5 mL of the DPPH·solution. Absorbance was measured at 515 nm every minute for 5 minutes using a spectrophotometer. Reduced DPPH· was calculated over time with a calibration curve of this compound. The percentage of remaining DPPH per minutes was calculated using the following Equation 1:

$$\% \text{ DPPH}\cdot_{\text{remaining}} = \text{DPPH}\cdot_{T} / \text{DPPH}\cdot_{T=0} \quad (1)$$

where: $\text{DPPH}\cdot_{T}$ = Final concentration of DPPH· 1 to 5 minutes; $\text{DPPH}\cdot_{T=0}$ = Initial concentration of DPPH·

The amount of antioxidant extract necessary to decrease the initial concentration of DPPH· (EC_{50}, mL extract/kg de DPPH) to 50% was calculated plotting the % DPPH remaining against microliters of extract, using a model fit to the data and calculating the amount of extract antioxidant that reduces by half DPPH. Time was plotted against microliters of antioxidant extract to get the time in min (T_{EC50}) needed to achieve the EC_{50}. Finally, the antiradical efficiency (AE) was calculated using the following Equation 2

$$EA = 1/ EC_{50} * T_{EC50} \quad (2)$$

2.8 Statistical analysis

Data were analyzed with a randomized complete block design with factorial 3 × 2 (pressure of 15, 30, and 60 MPa × time of 10 and 20 minutes), in which the blocks were the days of storage. Analysis of variance was carried out using the general linear model (GLM) of SAS (The SAS System for Windows, Version 6.11). Two replicates were performed, and each analysis was conducted in triplicate.

3 Results and discussion

3.1 Ascorbic acid (AA)

Figure 1 shows the AA content of control and pressurized fruits. The control mangoes showed an initial AA content of 134.98 mg/100 g FW reaching a maximum of 149.14 mg/100 g FW on day 11, and then it decreased when the fruits were senescent. The content of ascorbic acid was similar to the previously reported values for 'Kent' mango (ROBLES-SÁNCHEZ et al., 2009).

The concentration of ascorbic acid in pressurized fruits was significantly affected ($P \leq 0.05$) by both factors: the level of pressure and the treatment time (Figure 1). Álvarez-Virrueta et al. (2012) reported that 'Ataulfo' mango ripened after pressurization at 15 MPa (10 and 20 minutes). Therefore, the ascorbic acid content in 'Ataulfo' mango after the 15 MPa treatment was not affected. An increase in ascorbic acid during the first 8 days of storage was observed in all samples although it was evident that the highest pressure level and treatment time increased the ascorbic acid content more than the other treatments.

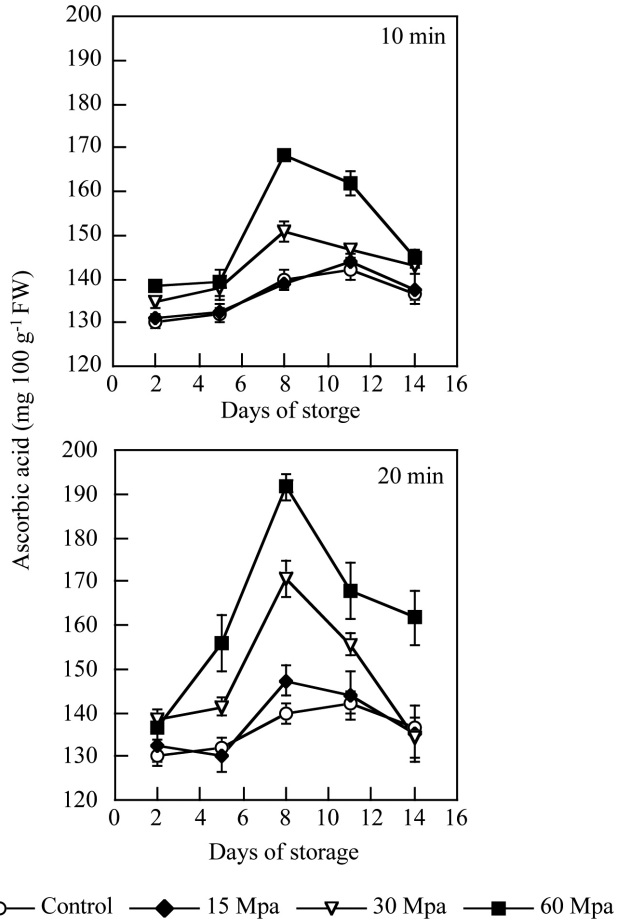

Figure 1. Effect of pressure treatments on the concentration of ascorbic acid during ripening of 'Ataulfo' mango stored at 25 °C.

In previous studies, 'Ataulfo' mango pressurized at 60 MPa (10 or 20 minutes) showed lower respiration rate and ethylene production rate (ÁLVAREZ-VIRRUETA et al., 2012). Thus, an increase in ascorbic acid concentration in fruits pressurized at 30 and 60 MPa might be associated with the structural alteration of the cells yielding a higher amount of extracted metabolite. Other possible explanation is that the higher concentration of ascorbic acid is a physiological response of the fruit to the stress conditions of higher pressurization levels. Eltelib et al. (2011) reported that the activity of monodehydroascorbate reductase and dehydroascorbate reductase, enzymes of the ascorbate-glutathione cycle, was significantly up-regulated under stress conditions in Acerola (*Malpighia glabra*). On the other hand, it has been reported that pressurized mango and melon pulps showed changes in metabolites concentration associated with cell rupture (GUERRERO; SWASON; BARBOSA, 2005; WOLBANG; FITOS; TREEBY, 2008).

3.2 Total carotenoids (TC)

Changes in the concentration of total carotenoids during the ripening of control and pressurized fruits are shown in Figure 2. Control fruits showed a gradual increase in the content of total carotenoids during storage related to changes in the internal color, which is common during the ripening of the fruit.

Total carotenoids in pressurized fruits were affected by the both factors: the level of pressure and the treatment time. Fruits pressurized for 10 minutes showed a higher level of total carotenoids with lower pressure levels ($P \leq 0.05$).

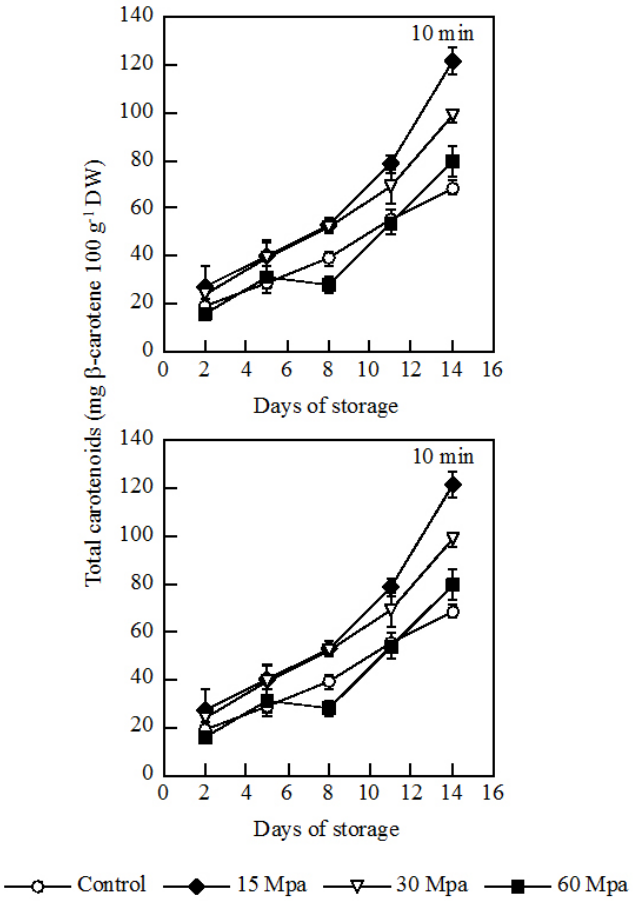

Figure 2. Effect of pressure treatments on the concentration of total carotenoids during ripening of 'Ataulfo' mango stored at 25 °C.

Samples pressurized at 15 y 30 MPa showed higher content of total carotenoids (121.22 and 98.67 mg β-carotene/100 g DW, respectively) than that of control samples (68.65 mg β-carotene/100 g DW) after 14 days of storage. Fruits pressurized for 20 minutes at 15 and 30 MPa showed lower values of total carotenoids than that of fruits pressurized for 10 minutes. Mango pressurized at 60 MPa for 10 or 20 minutes showed lower value of total carotenoids than that of control fruits (see Figure 3).

Similar results have been reported in persimmon fruit pressurized at 50 MPa, showing higher carotenoid content than that of fruits processed at higher level (> 50 MPa) (DE ANCOS; GONZÁLEZ; CANO, 2000). The increased level of carotenes extracted from fruits as a result of high pressure has been reported in cultivars of melon (ROBLES-SÁNCHEZ et al., 2009) and persimmon fruits at maturity stages III and IV (PLAZA et al., 2012). The increasing in the extraction of carotenoids and other bioactive compounds due to high pressure level has been associated with cell wall disruption, inducing the intracellular component dispersion throughout the tissue (VÁZQUEZ-GUTIÉRREZ et al., 2011b). In 'Ataulfo' mango, the results indicate that the increase in total carotenoids at low levels of pressure (lower than 50 MPa) seems to be associated with an improvement in the synthesis of this metabolite and not with an improvement in the extraction of the pulp, usually caused by alterations in the cellular structure.

3.3 Extractable phenols (EP)

The effect of HHP on the extractable phenols is shown in Figure 4. Control fruits (non-pressurized) showed an initial content (day 2) of extractable phenols of 105.83 mg of chlorogenic acid/100 g DW, which decreased after day 5 of storage, reaching a lower concentration of 63.53 mg of chlorogenic acid/100 g dw on day 14, which means a decrease of 38% in the initial concentration of extractable phenols. This

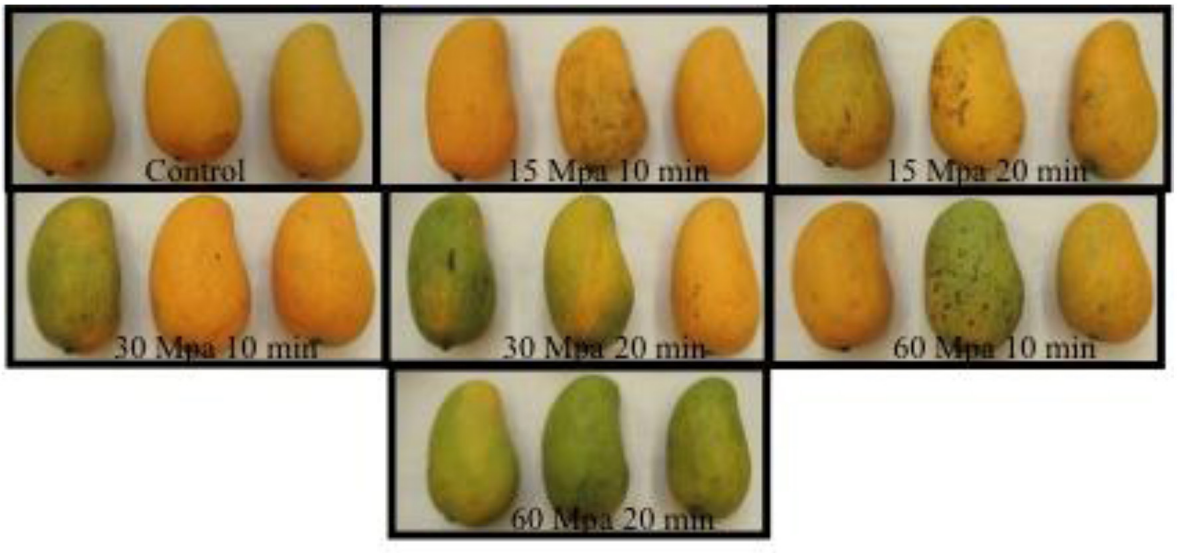

Figure 3. Visual effect of pressure treatments on 'Ataulfo' mango storage at 25 °C for 14 days.

behavior is characteristic of mango fruits since they contain a higher concentration of phenolic compounds at the unripe stage, decreasing their concentration during maturation (MASIBO; HE, 2008).

Mangoes pressurized at 15 or 30 MPa showed a higher level of EP than that of control fruits after day 8 of storage. The level of EP was higher in mangoes pressurized at 15 MPa than mango pressurized at 30 MPa; this behavior was more evident in mangoes pressurized for 10 minutes than fruits pressurized for 20 minutes, suggesting an improved response in the production of this antioxidant metabolites induced by pressurization. Phenolic compounds are antioxidant compounds synthesized to target the oxidative damage caused by the ripening of fruit or mechanical damage (GRASSMANN; HIPPELI; ELSTNER, 2002). The use of HHP in whole fruits is a physical phenomenon that can cause stress to the fruits and induce the synthesis of secondary metabolites.

Mangoes pressurized at 60 MPa (10 or 20 minutes) showed a different behavior, with an initial increase in the level of EP on day 5 and a subsequent and gradual decrease during storage. The initial improvement in the content of EP might be associated to an increase in the synthesis of this metabolite as a response to the oxidative stress generated immediately after the high pressure treatments. However, the reduction in the content of EP in these fruits might be associated with damage in the cellular structures involved in the synthesis of this metabolite.

HHP has been reported as an effective method to improve the extraction of phenolic content in fruit products (juices, mousses) increasing the antioxidant activity (FERRARI; MARESCA; CICCARONE, 2011). The effect of HHP treatments has been studied on fresh-cut fruits, but not on whole fruit. In an experiment using high pressure combined with argon in fresh-cut apples stored at 4 °C, it was found significantly reduction in the total phenolics content (WU; ZHANG; WANG, 2012). In the present study, the content of EP was higher at lower values of pressure, suggesting that 15 Mpa had a positive effect on the synthesis of these bioactive compounds during the storage of whole fruits.

3.4 Total flavonoids (TF)

The effect of HHP on the content of TF is shown in Figure 5. The concentration of flavonoids in the control fruits reached the maximum level on day 5 and decreased gradually to reach the lowest level on day 14. This behavior has been previously reported in the literature. The flavonoid content of fruits increased or kept unchanged during ripening or storage, but during senescence, most flavonoids decreased (KEVERS et al., 2007).

Pressure levels affected the synthesis of flavonoids. On the first eight days, the synthesis of flavonoids was low and depended on the level of pressure, this means that at the lowest pressure,

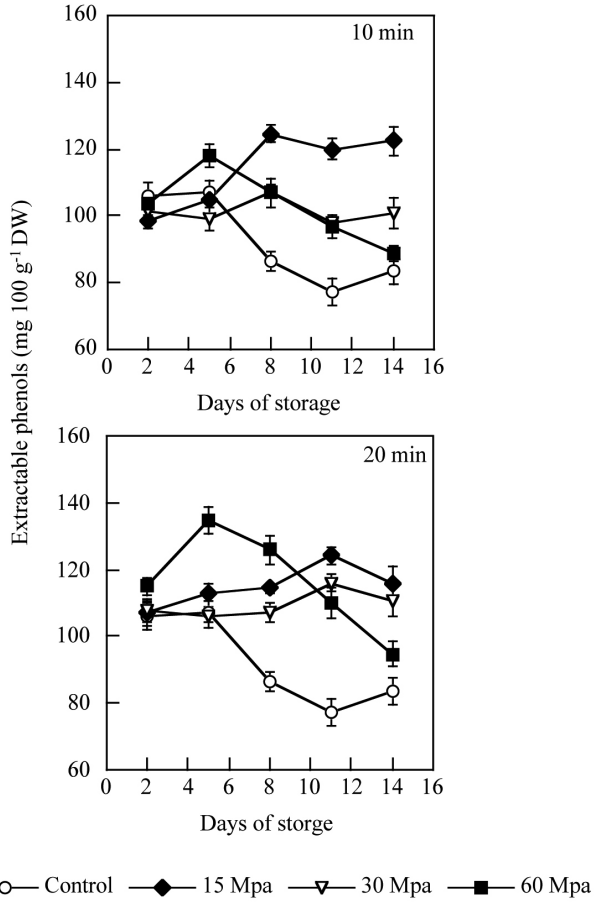

Figure 4. Effect of pressure treatments on the concentration of extractable polyphenols during ripening of 'Ataulfo' mango stored at 25 °C.

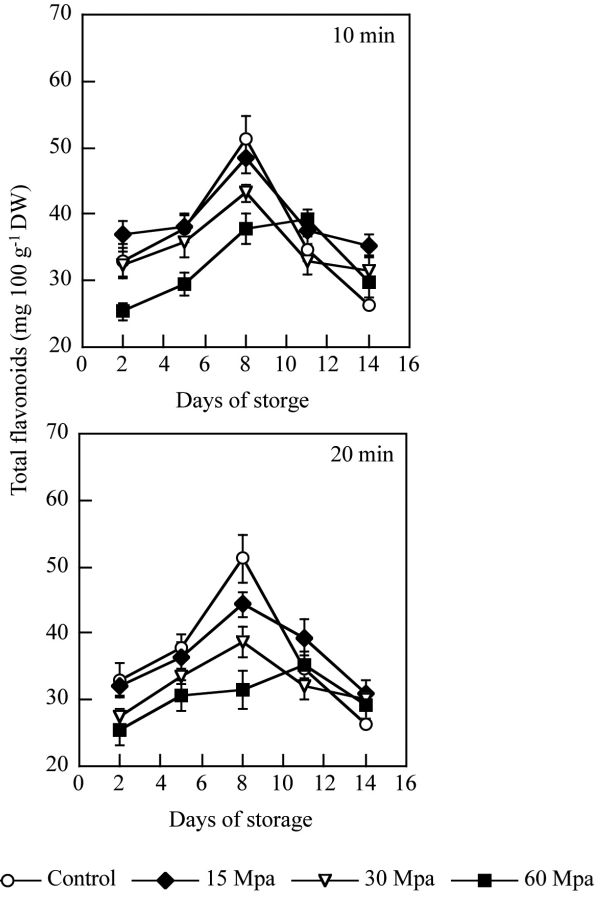

Figure 5. Effect of pressure treatments on the concentration of total flavonoids during ripening of 'Ataulfo' mango stored at 25 °C.

the highest content of flavonoids synthesized and vice versa. On day 8, the mangoes pressurized at 15 MPa (10 or 20 minutes) showed the highest level of total flavonoids ($P \leq 0.05$). Different factors, including ultraviolet rays and physical alteration for HHP, can change the metabolism of polyphenols, including flavonoids inducing their synthesis or degradation (ABOUZID; ELSHERBEINY, 2008; SHIRLEY, 2002). In the present study, HHP affected the content of flavonoids during ripening, and this phenomenon could be associated with changes in the activity of enzymes involved in their synthesis although more research is needed to support this hypothesis. Menezes et al. (2008) reported that polyphenoloxidase activity was stimulated when acai pulp was subjected to 500 MPa at 25 °C (5 to 15 minutes) in comparison with the pulp pressurized to 300 MPa under the same conditions. Additionally, HHP can damage the vegetable tissue inducing changes in the membrane permeability of cells, facilitating the contact of substrate and enzymes (TANGWONGCHAI; LEDWARD; AMES, 2000). Maybe the TF decrease is due to increased activity of polyphenoloxidase by the interaction of the enzyme with polyphenols.

3.5 Antiradical efficiency (AE)

The AE of control fruits decreased slightly after day 2 and increased gradually to reach its maximum level on day 14 (Figure 6). This behavior is consistent with the antioxidant capacity reported for 'Keitt' mango, which increased during ripening (TALCOTT et al., 2005). The antioxidant capacity reported for 'Ataulfo' mango in this study could be considered in the middle level of activity as compared with the capacity reported for other fruits (ZHISHEN; MENGCHENG; JIANMING, 1999).

Pressure treatments increased significantly ($P \leq 0.05$) the AE of mango fruits compared to that of control fruits during ripening, depending on the level of pressure. Fruits pressurized at 15 MPa showed higher levels of AE than fruits pressurized at 30 or 60 MPa (Figure 6). This behavior was more evident when fruits were pressurized for 10 minutes than when they were pressurized for 20 minutes. Mangoes pressurized at 15 MPa for 10 minutes reached its maximum level of AE on day 11, while mangoes pressurized at 30 and 60 MPa reached their maximum AE on day 8 and remained lower than the value of fruits pressurized at 15 MPa.

The increase in antiradical efficiency of fruits could be associated to an increase in the synthesis of bioactive compounds and/or an increase in the extraction of bioactive compounds from the fruit pulp. As previously discussed, HPP can induce changes in the structure of fruit tissue and in the cell membrane permeability, resulting in the release of antioxidant compounds into the extracellular medium. However, an increase in the extractability of bioactive compounds is always dependent on a higher level of pressure (McINERNEY et al., 2007; GRASSMANN; HIPPELI; ELSTNER, 2002; VÁZQUEZ-GUTIÉRREZ et al., 2011a). In the present study, the increase in the concentration of bioactive compounds and antiradical efficiency seemed to depend on a lower level of pressure. This behavior indicates a physiological response associated to the oxidative stress induced by external factors, which in this case were the high pressure treatments.

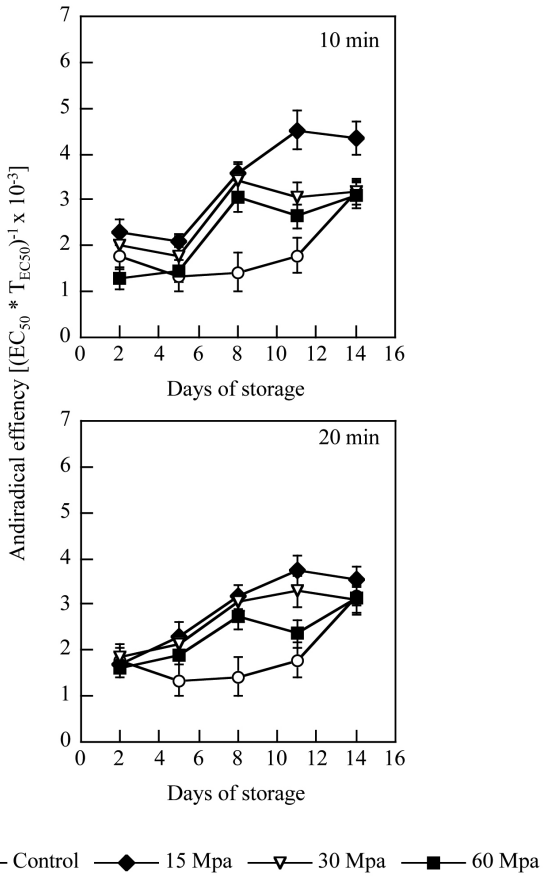

Figure 6. Effect of pressure treatments on the concentration of antiradical efficiency during ripening of 'Ataulfo' mango stored at 25 °C.

The increase in the antiradical efficiency of pressurized mango fruits (Figure 6) seemed to be highly dependent on the total carotenoids concentration (Figure 2). However, it is important to consider that AE depends not only on carotenoids, but also on other bioactive compounds present in the fruit. Kevers et al. (2007) reported a relationship between the antiradical efficiency and the phenolic content in different fruits, so it is possible that these compounds and carotenoids have a significant influence on the AE in fruits treated at 15 MPa.

4 Conclusions

High hydrostatic pressure caused changes in the content of antioxidants in 'Ataulfo' mango during postharvest ripening at 25 °C. The effect of HHP on mango fruits depended on the level of pressure and time of processing. The fruits treated with less pressure and time (15 MPa for 10 minutes) showed higher values of extractable phenols content, total carotenoids, total flavonoids, and antiradical efficiency than those of the control fruits during storage.

Acknowledgments

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