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Storage of ‘Cuernavaqueña’ Mexican plum (*Spondias purpurea* L.) fruits in a passive modified atmosphere

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Abstract

Mexican plum (*Spondias purpurea* L.) fruits are highly perishable, so passive modification of the product's surrounding atmosphere and a low storage temperature can help increase its shelf life. The objective was to evaluate the effect of storage temperature on the postharvest behavior of Mexican plums packaged in a passive modified atmosphere. Physiologically mature ‘Cuernavaqueña’ Mexican plum fruits were placed in low-density polyethylene bags (0.062 μm thick) and stored at 14 and 25 °C and 93 ± 2 % relative humidity for eight days. The results in CO₂ and ethylene production, weight loss and firmness indicate that the use of low-density packaging, a polyethylene bag, and storage at a temperature of 14 °C delays the ripening process of the fruits. Regarding the bioactive compounds evaluated in pulp and peel, in both treatments, their content is attractive for the purpose of enhancing fresh consumption of these fruits that are beneficial to human health.

► **Keywords:** *Spondias purpurea* L., antioxidant capacity, bioactive compounds, ethylene, firmness, respiration.

Introduction

The Mexican plum (*Spondias purpurea* L.) is a native species of Mexico. Some authors indicate that it can be classified into three types: 1) dry-season plums with fruiting from February to May; 2) wet-season plums with fruiting from September to December and, 3) mid-season plums with fruiting in June and July (Álvarez-Vargas et al., 2019). The ‘Cuernavaqueña’ Mexican plum is a wet-season plum that enjoys great regional acceptance due to its flavor and epicarp color. In addition, its consumption provides polyphenols, vitamins A, B, C, and E, and carotenoids, which can provide protection against chronic-degenerative diseases, allowing high marketing potential in the export market (Maldonado-Astudillo et al., 2014; Shahidi & Ambigaipalan, 2015).

However, the concentration of these bioactive compounds in fruits is highly associated with the species, cultivar, degree of ripeness and pre- and postharvest handling conditions, leading to important changes in the nutraceutical quality of the product (Tomás-Barberán & Espín, 2001; Belitz et al., 2008; Li et al., 2014). Álvarez-Vargas et al. (2019) reported in the ‘Cuernavaqueña’ Mexican plum at harvest (October) a total carotenoid content of 50.8 $\mu\text{g} \cdot \text{g}^{-1}$ in fresh weight in the epicarp and 27 $\mu\text{g} \cdot \text{g}^{-1}$ in fresh weight in the mesocarp, which coincided with the yellow coloration in the exocarp. Likewise, the authors indicated mean values for total phenols of 266.7 $\mu\text{g} \cdot \text{g}^{-1}$ in fresh weight of pulp.

The Mexican plum is a highly perishable climacteric fruit that is sensitive to low temperatures due to its tropical origin. Therefore,

it is susceptible to cold damage and, consequently, during postharvest handling, the quality of the fruit decreases rapidly, making it necessary to use technologies for its preservation, such as refrigeration and storage in modified atmospheres (Tomás-Barberán & Espín, 2001; Maldonado-Astudillo et al., 2014; Vargas-Simón, 2018). As a result, the combination of different preservation techniques, such as barrier technologies, is an alternative to avoid microbial growth and fruit quality losses. One of these techniques is the passive modification of the atmosphere surrounding the product, by the effect of its respiration and permeation of gases through the plastic container, in combination with a low storage temperature to increase shelf life (Ospina-Meneses & Cartagena-Valenzuela, 2008; Barrios et al., 2014; Oliveira et al., 2015).

In passive modified atmosphere packaging (MAP), low-barrier (low density) material, such as polyethylene, is used, which once the package is sealed allows a balance of gases to be established within the container during storage (Priyadarshi et al., 2020). In fruits such as the Mexican plum, whose behavior is climacteric, shelf life depends on the storage temperature, since one of the objectives of using MAP is to minimize the respiration rate and ethylene production and thereby delay the ripening and senescence process and prevent product deterioration (García-González et al., 2016; Priyadarshi et al., 2020).

Considering the above, this research aimed to evaluate the effect of storage temperature (14 and 25 °C) and passive modified atmosphere packaging on some physiological, physical and biochemical parameters during postharvest of 'Cuernavaqueña' Mexican plum fruits.

Materials and methods

Plant material and treatments

'Cuernavaqueña' Mexican plum (*Spondias purpurea* L.) fruits were harvested in the municipality of Atlatahuacan, Morelos, Mexico. The color of the fruit epidermis was used as a harvest index, with 75 % green coloration, homogeneous fruit size (30 g), and free of physical damage and pathogens. A total of 180 fruits were harvested and transported in plastic boxes by land to the multipurpose Plant Science Department laboratory of the *Universidad Autónoma Chapingo*. The fruits were washed with a chlorinated solution (1 %) to disinfect them and were left exposed to ambient conditions for 4 h to dry.

Two storage temperatures were evaluated, 14 and 25 °C, as well as a storage condition in a low-density polyethylene bag (Ziploc®) (16.5 x 14.9 cm) with a thickness of 0.062 µm. A completely randomized experimental design was used, with an experimental unit of six Mexican plum fruits and three replicates. After the treatments began, evaluations were made at 1, 2, 4, 6 and 8 d of some physiological, physical and biochemical variables.

Variables evaluated

Respiration and ethylene production were determined using a static system. Six fruits were placed in an airtight container of known volume and, after one hour, a 1 mL sample was taken from the headspace to be injected into a Varian gas chromatograph (3400CX®, USA) equipped with a Chrompack® poraPLOT Q-type capillary column, a thermal conductivity detector (TCD) and a flame ionization detector (FID) (Tovar et al., 2011). Temperatures of 80, 150 and 170 °C were used in the column, injector and detectors, respectively, with N₂ as carrier gas and column pressure of 158.5 KPa. Respiration data were reported as mL·kg⁻¹·h⁻¹ CO₂ and ethylene concentration µL·kg⁻¹·h⁻¹, using a calibration curve.

Fruit weight was recorded using a digital balance (Ohaus, USA) and results were expressed as percentage of weight loss (%). Firmness was measured at the equatorial part of each fruit using an FDV-3 texture analyzer (Greenwich, CT 06836, USA) with a conical strut (8 mm diameter) and results were expressed in newtons (N).

For the quantification of total soluble solids, two fruits were used, from which juice was extracted from the pulp and 1 mL was placed in the reader of a digital refractometer (ATAGO PAL-1, Japan) with results expressed in degrees Brix (°Brix). Titratable acidity was determined using the methodology of Marsh et al. (2011), for which 5 g of pulp was homogenized with 10 mL of distilled water, filtered and a 5 mL aliquot was taken from the recovered liquid phase, to which two drops of phenolphthalein were added as an indicator and titrated with 0.1 N sodium hydroxide (NaOH). Results were expressed as % citric acid.

Total carotenoids were quantified in pulp and epicarp using the method proposed by Speek et al. (1988), in which 10 mL of a n-hexane-acetone-ethanol solution (50:25:25 v/v) was added to 2 g of sample, keeping the solution in darkness at room temperature (20 °C) for one hour. Subsequently, centrifugation (Thermo Scientific Sorvall RC 6, centrifuge) was applied at 1000 x g for 5 min and the organic phase was made up to 25 mL with hexane, to which the absorbance was measured at 450 nm in a 10s-vis spectrophotometer (Thermo Scientific, Florida, USA). The concentration was calculated using the extinction coefficient of β-carotene, ε=2505 as standard and results were reported in µg β-carotene·g⁻¹ of fresh weight (µg β-carotene·g⁻¹ fw).

Total chlorophyll content was determined in the fruit epidermis according to the method proposed by Pompelli et al. (2013). For this purpose, 1 g of the epidermis was weighed and homogenized with 10 mL of acetone-water (8:2 v/v) for 15 s and filtered. Subsequently, absorbance was measured at three wavelengths (445, 663 and 645 nm) and results were expressed as µg of total chlorophyll·g⁻¹ fresh weight (µg·g⁻¹ fw).

The quantification of the content of phenolic compounds, flavonoids and antioxidant activity was carried out from a

methanolic extract of the pulp for each treatment, according to the method described by Chang et al. (2002). To 1 g of pulp, 10 mL of a methanol-water solution (80:20 v/v) was added. The mixture was homogenized with a vortex mixer (Vortex Synergy, WVR International) for 15 s, placed in sonication (Branson 1510 Ultrasonic Cleaner) for 15 min and left to stand for 24 h under refrigeration (8 °C); finally, it was centrifuged in a Thermo Scientific Sorvall device (RC 6, USA) at 600 x g for 10 min.

The concentration of total phenolic compounds was determined by the Folin-Ciocalteu method (Jiménez-Arellanes et al., 2011), where 0.5 mL of Folin-Ciocalteu reagent (0.2 N) and 4 mL of a 0.7 M Na₂CO₃ solution were added to 0.5 mL of the methanolic extract; the mixture was then left to rest for 2 h in darkness at room temperature. Finally, absorbance was measured at 760 nm in a Genesys 10s-vis spectrophotometer (Thermoscientific, Florida, USA). The concentration was calculated from a standard curve ($y = 0.0074x + 0.0118$; $R^2 = 0.999$) prepared on the basis of gallic acid (20-200 mg) and the content of phenolic compounds was expressed in mg gallic acid equivalents per 100 g fresh weight (mg GAE 100·g⁻¹ fw).

Total flavonoids were quantified according to the method proposed by Chang et al. (2002) where to 0.5 mL of the methanolic extract prepared above, 1.5 mL of ethanol:water (90:10 v/v), 0.1 mL of AlCl₃-water solution (1:9 w/v), 0.1 mL of 1 M potassium acetate solution (CH₃COOK) and 2.8 mL of distilled water were added. The mixture was incubated for 30 min at room temperature (25 ± 1 °C). The absorbance was quantified at 415 nm. Results were reported in mg quercetin equivalents per 100 g fresh weight (mg QE 100·g⁻¹ fresh weight). To determine flavonoid concentration, a standard curve ($y = 0.0365x - 0.0047$; $R^2 = 0.999$) of quercetin (3-30 mg) was constructed.

Antioxidant capacity was determined according to the method described by (Re et al., 1999). The free radical ABTS^{•+} (2,2'-azino-bis(3-ethylbenzothiazolin)-6-sulfonic acid) was generated by mixing 10 mL of a 7 mM solution of ABTS^{•+} with 6.61 mg of K₂S₂O₈. The mixture was incubated for 12-16 h in the dark to generate the ABTS^{•+} radical. The ABTS^{•+} radical solution was diluted with anhydrous ethanol until obtaining an absorbance of 0.7 ± 0.1 (maximum concentration of ABTS^{•+} radical formed) measured in a Genesys 10s-vis spectrophotometer (Thermoscientific, Florida, USA) at a wavelength of 734 nm. To determine antioxidant activity, 10 µL of the methanolic extract of the sample was added to 1 mL of the ABTS^{•+} free radical solution and the mixture was incubated in a water bath at 30 °C in darkness for 7 min. Subsequently, the absorbance was measured at a wavelength of 734 nm. To quantify antioxidant activity, a standard curve ($y = -0.0393x + 0.6931$; $R^2 = 0.999$) based on trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was made, for which a solution of trolox was prepared at a concentration of 17.5 mg and, from this solution, different concentrations were obtained. Results were expressed in mg trolox equivalents per 100 g fresh weight (mg TE 100·g⁻¹ fw).

Statistical analysis

Data are presented as mean ± standard deviation (SD). A comparison of means was performed using the statistical t-test at $P=0.05$. The mean was obtained over time for each treatment and for each variable. Statistical analysis was carried out using the SAS statistical package (SAS Institute Inc., 1999).

Results and discussion

Maximum CO₂ and ethylene production in fruits stored at 25 °C was obtained on the fourth day, with 78.77 mL CO₂·kg⁻¹·h⁻¹ and 78.15 µL·kg⁻¹·h⁻¹, respectively (Figures 1 A and B). In fruits stored at 14 °C, respiration and ethylene production were significantly lower, between 40 and 60 % compared to fruits stored at 25 °C (Figure 1 A and B). Maldonado-Astudillo et al. (2014) reported that the 'Cuernavaqueña' Mexican plum is climacteric, presenting an increase in respiration and ethylene production seven days after harvest. Fruits stored at low temperature and with a low-density barrier (polyethylene) significantly slowed down fruit respiration. The decrease in the respiration rate and ethylene production is due to the fact that modified atmosphere packaging slows down the physiological and biochemical processes and delays senescence (Caleb et al., 2012).

In the 'Cuernavaqueña' Mexican plum, after eight days of evaluation, the weight loss of fruits stored at 14 °C was less than 1.0 %, while in those exposed to 25 °C the weight loss was greater than 2.5 % (Figure 1 C). Maldonado-Astudillo et al. (2014) noted that 'Cuernavaqueña' *Spondias purpurea* fruits stored at 25 °C lose 11.46, 11.09 and 12.38 % of the weight of fruits harvested at the unripe, half-ripe and ripe stages after six days of storage, respectively. Pérez-López et al. (2004) reported weight losses of up to 17.6 % in 'Amarilla' Mexican plum fruits stored at 20 °C for four days. The use of a low-density barrier material (polyethylene) prevented excessive water loss through transpiration, minimizing weight loss. Weight loss was influenced by storage temperature and relative humidity. In addition, it is associated with fruit transpiration and deterioration (Maldonado-Astudillo et al., 2014).

Firmness in fruits stored at 25 °C decreased by 57.37 % after eight days, while in those stored at 14 °C it decreased by 23.94 % with respect to initial firmness (6.0 N; Figure 1 D). The results suggest that the lower temperature allowed preserving fruit quality and delaying softening. During the ripening process, protopectin, a cell adhering substance, is degraded along with pectic substances, which modifies the texture and consistency of the fruit. The use of temperatures that favor ripening increases the loss of firmness associated with water loss, synthesis of free carbohydrates from reserve carbohydrates and increased activity of enzymes that act on the cell wall, causing softening (Arenas-Ocampo et al., 2007; Prasanna, et al., 2007).

Total soluble solids increased in both treatments, ranging from 11 to 14 °Brix at the beginning of the experiment, while at the end

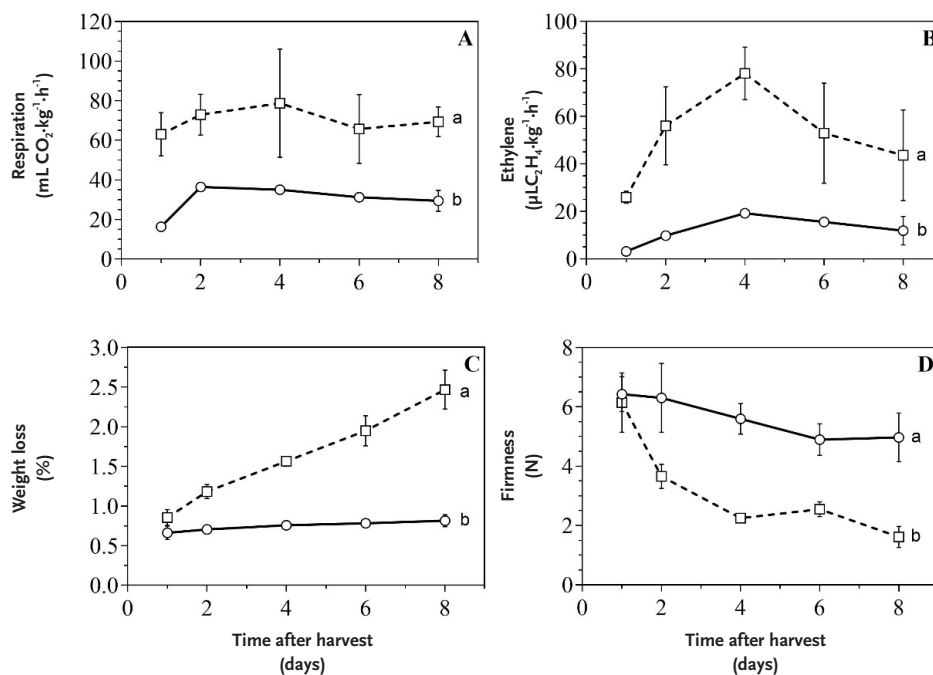


Figure 1. Respiration rate (A), ethylene production (B), weight loss (C) and firmness (D) of 'Cuernavaqueña' Mexican plum fruits stored at 14 °C (—○—) and 25 °C (---□---) in polyethylene bags. Means \pm SD, n=3. Means with different letters indicate significant differences for the t-student test ($P < 0.05$).

of storage they reached between 14.15 and 17.90 °Brix (Figure 2 A). Statistical analysis determined significant differences between the two treatments, where fruits stored at 25 °C obtained a greater accumulation of total soluble solids. Pérez-Arias et al. (2008) reported between 12.47 and 17.43 °Brix in several Mexican plum ecotypes at the fully ripe stage. García-González et al. (2016) evaluated 'Cuernavaqueña' Mexican plum fruits at three ripeness stages, green, changing and ripe, reporting that, after nine days stored at 20 °C, TSS increased between 23.4 and 25.8 °Brix. TSS are frequently used to evaluate fruit quality. Furthermore, this variable is influenced by ripeness stage, storage conditions and postharvest treatments (Maldonado-Astudillo et al., 2014), so the differences found for this variable between treatments can be explained by the effect of using low-density polyethylene bags and storage temperature, suggesting a more accelerated ripening process in fruits stored at 25 °C.

Titrate acidity decreased during the evaluation period in both treatments. Initially, it was 1.2%, and after eight days the value was around 0.09 % (Figure 2 B). Although on days 2 and 4 the fruits stored at 14 °C maintained higher values, there was no effect of storage temperature on the ripening process (Figure 2 B). As part of the ripening process of a climacteric fruit, the content of organic acids, in this case citric acid, is reduced (Parra & Fischer, 2013), as occurred in Mexican plum fruits where citric acid content decreased significantly throughout the ripening process (Figure 2 B). According to the results obtained, the use of plastic bags allowed modifying the fruit atmosphere, which contributed to reducing the incidence and maintaining the quality of Mexican plum fruits during storage time.

In the epicarp, total chlorophyll content in fruits stored at 25 °C decreased from 16.0 to 5.0 $\mu\text{g}\cdot\text{g}^{-1}$ fw in eight days and total carotenoids increased from 9.9 to 19.7 $\mu\text{g}\beta\text{-carotene}\cdot\text{g}^{-1}$ fw in the same period (Figure 3 A and B). In contrast, in plum fruits stored at 14 °C, chlorophyll content remained at 16.0 $\mu\text{g}\cdot\text{g}^{-1}$ fw and total carotenoids remained at approximately 9.5 $\mu\text{g}\beta\text{-carotene}\cdot\text{g}^{-1}$ fw, suggesting that fruits from the latter treatment did not change in epidermis color (Figure 3). In 'Cuernavaqueña' Mexican plum fruits, a decrease in chlorophylls present in the peel begins during the pre-climacteric stage and the total carotenoid content increases resulting in yellow and reddish colorations once the climacteric stage is reached (Maldonado-Astudillo et al., 2014; Álvarez-Vargas et al., 2019). Álvarez-Vargas et al. (2017) reported for *Spondias purpurea* L. collected in the states of Guerrero, Morelos and Chiapas, Mexico and evaluated at the fully ripe stage a concentration from 148.21 to 1993.12 $\mu\text{g}\beta\text{-carotene}\cdot\text{g}^{-1}$ fw, values higher than those found in this research. The results are consistent with those reported by Zuo et al. (2024) who stored fruits of the Rhamnaceae family in low-density polyethylene bags, where chlorophyll content gradually decreased during storage time, while carotenoid content showed an increasing trend.

Spondias purpurea L. fruits have high commercial potential due to their sensory and nutritional qualities. In addition, they are a source of bioactive compounds such as phenolics, vitamins, carotenoids and minerals, which make them functional and nutraceutical foods, due to their antioxidant properties (Beserra-Almeida et al., 2011; Villa-Hernández et al., 2017). During storage, the concentration of phenolic compounds in fruits stored at 14 and 25 °C did not show significant differences

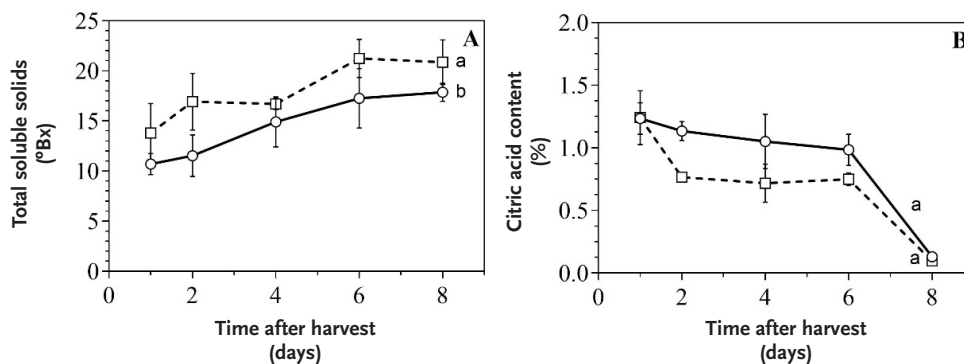


Figure 2. Total soluble solids (A) and titratable acidity in 'Cuernavaqueña' Mexican plum fruits stored at 14 °C (—○—) and 25 °C (---□---) in polyethylene bags. Means \pm SD, n=3. Means with different letters indicate significant differences for the t-student test ($P < 0.05$).

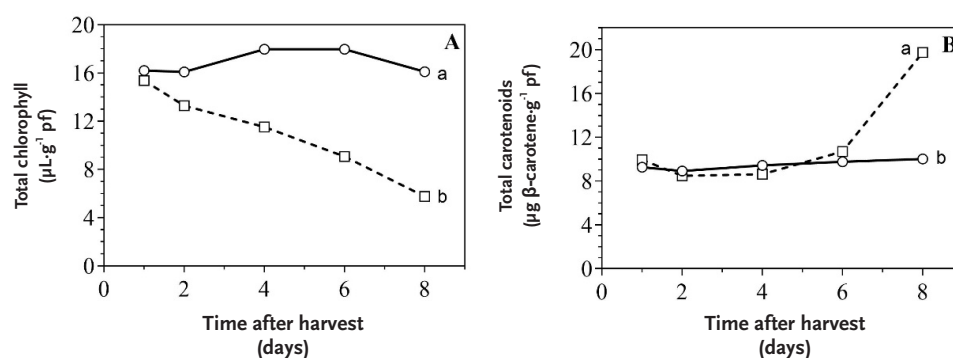


Figure 3. Behavior of total chlorophyll (A) and total carotenoids (B) in the epicarp of Mexican plum stored at 14 °C (—○—) and 25 °C (---□---) in polyethylene bags. Means \pm SD, n=3. Means with different letters indicate significant differences for the t-student test ($P < 0.05$).

(Figure 4 A) on the eighth day, with both treatments presenting a concentration of $73.53 \text{ mg GAE } 100\cdot\text{g}^{-1} \text{ fw}$. The values of phenolic compounds in this work are lower than those reported by Suárez-Vargas et al. (2017), who obtained values of $89.21 \text{ mg GAE } 100\cdot\text{g}^{-1} \text{ fw}$ in 'Cuernavaqueña' Mexican plum pulp at four ripening stages. These results may be due to the different extraction methods used in both investigations.

Regarding total flavonoid content, in both treatments it increased during the first four days, reaching $8.0 \text{ mg QE } 100\cdot\text{g}^{-1} \text{ fw}$; however, although its content subsequently decreased, statistically the highest concentration occurred in fruits stored at 14 °C ($5.49 \text{ mg QE } 100\cdot\text{g}^{-1}$) with respect to those exposed to 25 °C ($5.22 \text{ mg QE } 100\cdot\text{g}^{-1}$) (Figure 4 B). Suárez-Vargas et al. (2017) reported total flavonoid content values between 17.11 and $23.26 \text{ mg QE } 100\cdot\text{g}^{-1}$ in pulp for 'Cuernavaqueña' Mexican plum at different ripening stages. These values are higher than those reported in the present investigation, possibly as a result of the different quantification methods.

Total carotenoid content in *S. purpurea* ecotype 'Cuernavaqueña' fruits increases during the ripening process, with a higher concentration in the epicarp and a lower concentration in the

pulp (Maldonado-Astudillo et al., 2014), which coincides with what was found in this research, where the total carotenoid content present in the pulp, in both treatments, was lower compared to that found in the epicarp (Figure 3 B and 4 C). In the pulp, $5.2 \mu\text{g } \beta\text{-carotene}\cdot\text{g}^{-1} \text{ fw}$ was quantified on day one and $6.4 \mu\text{g } \beta\text{-carotene}\cdot\text{g}^{-1} \text{ fw}$ on day eight for both treatments (Figure 4 C), while in the epicarp a significant increase in total carotenoid content was observed in fruits stored at 25 °C (Figure 4C). Solorzano-Morán et al. (2015) determined that the total carotenoid content in the pulp of 11 evaluated ecotypes of Mexican plum ranged from 0.5 to $3.4 \text{ mg } 100\cdot\text{g}^{-1} \text{ fw}$. On the other hand, Suárez-Vargas et al. (2017) reported values between 37 and $150.2 \mu\text{g } \beta\text{-carotene}\cdot\text{g}^{-1}$, which are higher than those found in the fruits evaluated in this work.

The nutraceutical quality of a food and its antioxidant content are estimated through total antioxidant capacity, which is given by a mixture of various antioxidants that can prevent or delay oxidation processes caused by free radicals and reactive oxygen species (Heo et al., 2007; Beserra-Almeida et al., 2011; Solorzano-Morán et al., 2015). Various studies have been carried out on Mexican plums with the aim of obtaining genetic materials with outstanding characteristics in terms of quality and contribution

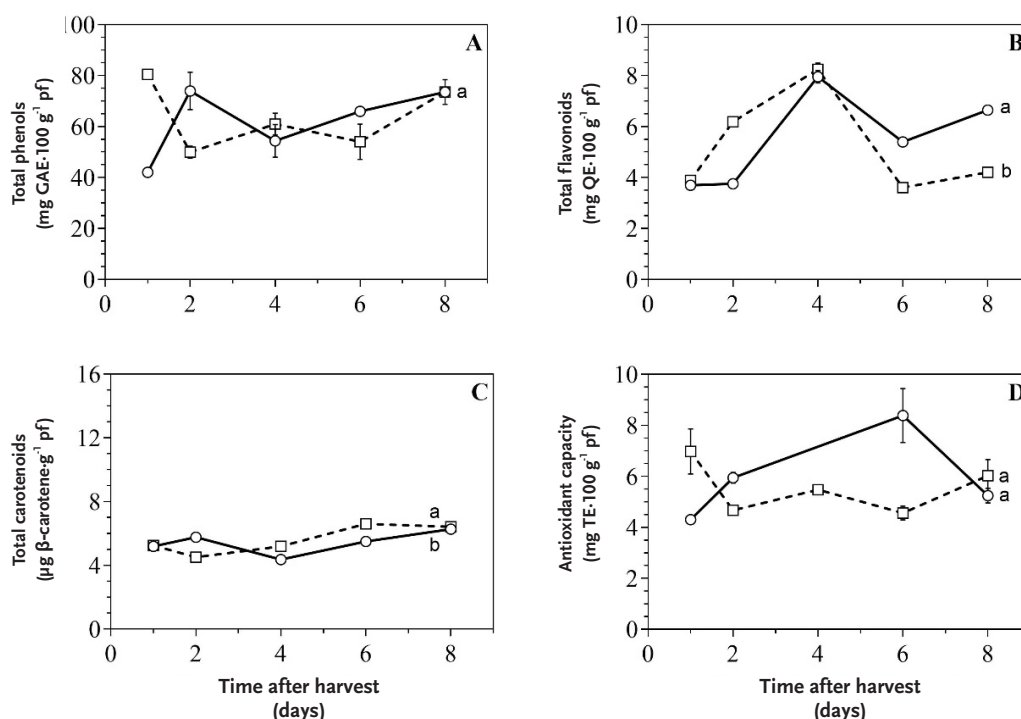


Figure 4. Antioxidant compounds and their activity in Mexican plum pulp stored at 14 °C (—○—) and 25°C (---□---) in polyethylene bags. Means ± SD, n=3. Means with different letters indicate significant differences for the t-student test ($P < 0.05$).

of bioactive compounds, thus enhancing their marketing (Álvarez-Vargas et al. 2017).

Regarding the antioxidant capacity determined by the ABTS method in the study fruits, an increase from 4.3 to 8.3 mg TE $100 \cdot g^{-1}$ fw was observed during the first six days of evaluation in fruits stored at 14 °C, but on the eighth day it decreased to 5.2 mg TE $100 \cdot g^{-1}$ fw. On the other hand, in fruits stored at 25 °C the antioxidant capacity decreased from 6.9 to 6.0 mg TE $100 \cdot g^{-1}$ fw (Figure 4 D). In this regard, Suárez-Vargas et al. (2017) reported similar behavior in antioxidant capacity measured by the ABTS method, where it increased from 43.4 to 73.7 mg ascorbic acid equivalent (AAE) $100 \cdot g^{-1}$ fw, at the physiological maturity stage. However, once consumption maturity was reached, it decreased to 57.3 mg AAE $100 \cdot g^{-1}$ fw. Villarreal-Fuentes et al. (2019) evaluated the antioxidant capacity in fruits of various Mexican plum genotypes at the fully ripe fruit stage, where they found a minimum of 21.4 mg EAA $100 \cdot g^{-1}$ fw and a maximum of 377.8 mg AAE $100 \cdot g^{-1}$ fw for the antioxidant capacity evaluated with the ABTS method.

Conclusions

The use of low-density packaging, combined with storage at 14 °C, delayed the ripening process of *Spondias purpurea* L. ecotype 'Cuernavaqueña' fruits, due to a decrease in respiration intensity, ethylene production and weight loss, as well as lower flavor development, indicated by a higher TSS content

and a lower citric acid content. In the epicarp, minimal color development was found due to a higher chlorophyll content and a lower carotenoid concentration, compared to fruits stored in polyethylene bags at 25 °C, after eight days of storage. Regarding the bioactive compounds present in the pulp, their content is attractive for the purpose of enhancing the fresh consumption of these exotic tropical fruits. Finally, by modifying the atmosphere, the fruits extended their shelf life and maintained their quality.

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