

## BOTÂNICA E FISILOGIA/ BOTANY AND PHYSIOLOGY ANTIOXIDANT ACTIVITY AND PHYSICOCHEMICAL PARAMETERS IN ‘CUERNAVAQUEÑA’ MEXICAN PLUM (*Spondias purpurea* L.) AT DIFFERENT RIPENING STAGES<sup>1</sup>

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**ABSTRACT** - Mexican plum (*Spondias purpurea* L.) ‘Cuernavaqueña’ was harvested at four ripening stages, with the aim of evaluating the concentration of bioactive compounds and antioxidant capacity in the pulp and the epicarp. The highest ethylene production (9.43  $\mu\text{L kg h}^{-1}$ ) and total soluble solids concentration (23.9 °Brix) was observed in the fully ripe stage. Titratable acidity was higher in green stage compared to other analyzed ripening stages in both pulp (0.48 %) and epicarp (0.32 %). Fully ripe plum epicarp presented the highest content of total phenols (GAE 190  $\text{mg g}^{-1}$ ), flavonoids (QE 214  $\text{mg g}^{-1}$ ), and carotenoids (853  $\text{mg g}^{-1}$ ) compared to other ripening stages. The antioxidant capacity was higher in the epicarp of the fully ripe fruit compared to the other ripening stages: DPPH (1087  $\mu\text{M TE}/100\text{ g}$ ), ABTS (1534  $\mu\text{M TE}/100\text{ g}$ ), and FRAP (1764  $\mu\text{M TE}/100\text{ g}$ ). Significant correlations ( $r = 0.60$  \*\*\* to  $0.95$  \*\*) between bioactive compounds concentrations and antioxidant activity were obtained.

**Index terms:** *Spondias purpurea*; bioactive compounds; antioxidant activity.

## ATIVIDADE ANTIOXIDANTE E PARÂMETROS FISCOQUÍMICOS DA AMEIXA MEXICANA “CUERNAVAQUEÑA” (*Spondias purpurea* L.) EM DIFERENTES ESTÁGIOS DE AMADURECIMENTO

**RESUMO** - A ameixa mexicana (*Spondias purpurea* L.) ‘Cuernavaqueña’ foi colhida em quatro estádios de amadurecimento com o objetivo de avaliar a concentração de compostos bioativos e capacidade antioxidante da polpa e do epicarpo. A maior produção de etileno (9,43  $\text{mL kg h}^{-1}$ ) e concentração total de sólidos solúveis (23,9 ° Brix) foi observada no estágio totalmente maduro. A acidez titulável foi maior no estágio verde em comparação com outros estádios de amadurecimento analisados tanto na polpa (0,48%) como no epicarpo (0,32%). O epicarpo da ameixa totalmente madura apresentou o maior teor de fenóis totais (GAE 190  $\text{mg g}^{-1}$ ), flavonóides (QE 214  $\text{mg g}^{-1}$ ) e carotenóides (853  $\text{mg g}^{-1}$ ) em comparação com outros estádios de amadurecimento. A capacidade antioxidante foi maior no epicarpo do fruto totalmente maduro em comparação com os outros estádios de amadurecimento: DPPH (1087  $\mu\text{M TE}/100\text{ g}$ ), ABTS (1534  $\mu\text{M TE}/100\text{ g}$ ) e FRAP (1764  $\mu\text{M TE}/100\text{ g}$ ). Foram obtidas correlações significativas ( $r = 0,60$  \*\*\* a  $0,95$  \*\*) entre as concentrações de compostos bioativos e a atividade antioxidante.

**Termos para indexação:** *Spondias purpurea*; Compostos bioativos; atividade antioxidante.

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## INTRODUCTION

Mexican plum, *Spondias purpurea* L. (Anacardiaceae), is a tropical tree with round and ovoid fruits that vary on size (from 20-50 mm) and mass (from 4 to 43 g) which epicarp can be red, yellow, orange, or purple in the ripe fruit. It is considered a Mexican native species that can be found in low deciduous and semideciduous tropical forests which distribution comprises the Western coast and the Southeastern zone of the country. It is a fruit tree with a high commercial potential due to its low production cost and the fact that grows in the wild and adapts to poor and thin soils (AVITIA-GARCÍA et al., 2000). Its fruit is consumed fresh and due to its high acceptability, it has potential for commercial large-scale production (BAUTISTA-BAÑOS et al., 2003). Currently, the consumption of functional foods (fruits and vegetables) associated to prevention of chronic diseases has increased. These beneficial effects are due to the presence of bioactive compounds (vitamins, enzymes, carotenoids, flavonoid, and phenolic compounds) with antioxidant properties (HOOPER and CASSIDY, 2006; ISABELLE et al., 2010) that neutralize harmful molecules such as free radicals, that interact and destabilize important macromolecules like proteins, nucleic acids and lipids causing degenerative diseases in the organism (NIVA, 2007). There are different methods that use chromogenic compounds (DPPH, FRAP, ABTS and others) to determine the antioxidant capacity (BRAND-WILLIAMS et al., 1995; BENZIE and STRAIN, 1996; RE et al., 1999) of a product to neutralize reactive oxygen species (ROS) and free radicals (PRIOR et al., 2005). The studies that report the presence of bioactive compounds with antioxidant activity in Mexican native fruits are scarce. The objective of this research was to quantify the antioxidant capacity in pulp and epicarp of the Mexican plum 'Cuernavaqueña', a Mexican native fruit, at four different ripening stages and correlate it with the presence of bioactive compounds.

## MATERIALS AND METHODS

Mexican plum 'Cuernavaqueña' fruits were harvested in a commercial orchard located in Buenavista, Cuernavaca, Morelos, Mexico (18°56'55.71" N, 99°18'39.67" W, 1987 m.a.s.l.) in September 2014.

Fruits were sorted by color of the epicarp in four groups of 50 fruits each according to their ripening stage: green (100% green epicarp), ½ green

(75% green epicarp), ¼ ripe (25% green epicarp), and fully ripe (0% green epicarp). Fruits were disinfected with a 1% (v/v) hypochlorite solution and then let to stand at room temperature ( $22 \pm 2^\circ\text{C}$  and  $60 \pm 2\%$  RH).

Color parameters lightness ( $L^*$ ), chromaticity ( $C^*$ ), and angle hue ( $h^\circ$ ) were determined in the plum epicarp using a Spectrophotometer (X-Rite 3290®, USA). Measurements were taken from three different parts of each fruit (MCGUIRE, 1992). Twenty fruits from each ripening stage were evaluated.

Respiration rate ( $\text{CO}_2$  production) and ethylene production were quantified using a static system (MENDOZA-WILSON and BÁEZ-SAÑUDO, 2000) which consisted in placing two fruits of known mass (Average 70 g) in 145 mL glass jars hermetically sealed for 2 h. Afterwards, 1 mL of the headspace was taken from the jars and injected to a gas chromatograph (Agilent Technologies 7890A GC) with a porous layer open column simultaneously connected to a flame ionization detector (FID,  $170^\circ\text{C}$ ) and to a thermal conductivity detector (TCD,  $170^\circ\text{C}$ );  $\text{N}_2$  ( $2\text{ mL min}^{-1}$ ) was used as the carrier gas. The injector and the oven were maintained at 150 and  $80^\circ\text{C}$ , respectively, during measurements. Commercial standards of  $\text{CO}_2$  (460 ppm) and ethylene (100 ppm) (Quark INFRA®) were used for quantification. The experimental unit was of two fruits with six replicates for each ripening stage.

Soluble solids (SS) were determined from the juice of the fruit obtained with a Super Extractor (ATAGO®) using a refractometer (ATAGO PAL-1®, Japan). Results are reported in °Brix. Titratable acidity (TA) was determined by the AOAC method (1995) in the epicarp and the pulp; sample was titrated with 0.1 N NaOH using phenolphthalein as indicator. Results are expressed as percentage of citric acid. Flavor index (FI) was determined as the ratio of SS and TA (SS/TA) in the pulp and the epicarp. Ten fruits from each ripening stage were evaluated.

Total phenols concentration was determined by the Folin-Ciocalteu method (SINGLETON et al., 1999). One gram of tissue (pulp and epicarp) was homogenized with 20 mL of distilled water in a test tube using an Ultra Turrax (IKA®). The homogenate was filtered and 0.5 mL were mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10 v/v) and let to stand for 5 min, then 2 mL of sodium carbonate (7.5 % w/v) were added and let to stand for 2 h. Afterwards, absorbance at 760 nm was measured using a spectrophotometer (HACH DR 5000®). Results are expressed as mg gallic acid equivalents per 100 g ( $\text{mg GAE } 100\text{ g}^{-1}\text{ fw}$ ).

Total flavonoids were determined using the methodology reported by Arvouet-Grand et al. (1994). Pulp and epicarp samples (0.5 g) were homogenized with 10 mL of methanol using an Ultra Turrax (IKA®). The homogenate was filtered and 2 mL were mixed with 2 mL of 2% (w/v) aluminum chloride and let 15 min in the dark. Afterwards, absorbance at 415 nm was measured using a spectrophotometer (HACH DR 5000®). Results are expressed as mg quercetin equivalents (QE) per 100 g of fresh weight (mg QE 100 g<sup>-1</sup>).

Extraction and quantification of carotenoids was performed by the methodology described by Rodríguez-Amaya and Kimura (2004). One gram of sample was homogenized with 15 mL of cold acetone using an Ultra Turrax (IKA®) and the homogenate was filtered, then, 20 mL of hexane and 100 mL of distilled water were added and let to stand until two phases were separated. The aqueous phase (lower phase) was discharged and the upper phase was recovered, 100 µL 10 N NaOH were added and then, it was washed with 100 mL of distilled water four times. Afterwards, the extract was filtered using a filter paper coated with a layer of anhydrous sodium sulfate to remove water residues. Final volume was measured and absorbance at 452 nm recorded using a spectrophotometer (HACH DR 5000®). Samples that had an absorbance higher to 0.8 were diluted with hexane at a 1:1 v/v ratio. Total carotenoids content was calculated using the following formula:

$$\text{Total carotenoids content} = \frac{A \times \text{volume (mL)} \times 10^4}{A_{1\text{cm}}^{1\%} \times \text{sample weight (g)}}$$

Where A= absorbance, volume = total volume of the extract and A = absorption coefficient of β-carotene in hexane (2592). Results are expressed as µg 100 g<sup>-1</sup> fw.

Samples for measuring antioxidant capacity were prepared by homogenizing 1 g of tissue (pulp and epicarp) with 10 mL of distilled water. The DPPH method described by Brand-Williams et al. (1995) with slight modifications was used. In a 3 mL quartz cell, 6.1x10<sup>-5</sup> M methanolic DPPH solution (Sigma Aldrich, USA) was made to react with 100 µL of sample solution for 30 min at dark. Change in absorbance at 517 nm was determined. Results are expressed as mg ascorbic acid equivalents (AAE) and µM Trolox equivalents (TE) 100 g<sup>-1</sup> fresh weight.

A 1:1 v/v solution of 7 mM ABTS (Sigma-Aldrich) and 2.45 mM sodium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) was prepared and let to stand for 16 hours. This solution was diluted with 20 % ethanol until an

absorbance of 0.7 ± 0.02 at 734 nm was obtained; then 3 mL of the ABTS solution were made to react with 50 µL of sample solution for 15 min and absorbance was measured at 734 nm. Results are expressed as mg ascorbic acid equivalents (AAE) and µM Trolox equivalents (TE) 100 g<sup>-1</sup> fresh weight (RE et al., 1999).

The method developed by Benzie and Strain (1996) was followed. FRAP (ferric reduction) reagent was prepared (Tripyridil-s-triazina, FeCl<sub>3</sub> and acetate buffer) and 1.8 mL of the reagent were mixed with 140 µL distilled water and 60 µL of sample solution and incubated for 30 min at 37 °C. Afterwards, absorbance at 593 nm was measured. Results are expressed as mg ascorbic acid equivalents (AAE) and µM Trolox equivalents (TE) 100 g<sup>-1</sup> fresh weight.

Total phenols, flavonoids and carotenoids, as well as antioxidant capacity were determined in six replicates. Each replicate was obtained from a different experimental unit that consisted in two fruits.

Data were analyzed by analysis of variance (ANOVA) and Tukey mean comparisons (*P* < 0.05) using the software SAS version 9.2. Pearson correlation coefficient analysis was performed between the bioactive compounds and the antioxidant activity.

## RESULTS AND DISCUSSION

There were no significant differences in CO<sub>2</sub> production among the different ripening stages; the average value of this parameter was 1.11 mL kg h<sup>-1</sup> (Table 1). In a previous study, Pérez et al. (2004) did not observe significant differences in the respiration rate of Mexican plum fruit from Oaxaca at three different ripening stages: green, ½ yellow, and ¾ yellow. A similar response was reported by Kohatsu et al. (2011) in *Spondias purpurea* fruit grown in Brazil. In contrast, Dantas et al. (2016), Montalvo-González et al. (2011), Pareira et al. (2000) and Osuna et al. (2011) reported a climacteric behavior in Mexican plums grown in Mexico and Brazil. Recently, Maldonado-Astudillo et al. (2014) indicated that due to the variation in the behavior of CO<sub>2</sub> production, it is difficult to determine if Mexican plum is a climacteric fruit or not, even though physical and biochemical changes during ripening suggest it is a climacteric fruit.

Ethylene production increased with ripening stage from 3.92 to 9.43 µL kg h<sup>-1</sup>, the highest production was observed in the fully ripe stage (Table 1). Pérez et al. (2004) did not determine changes in ethylene production during ripening

of Mexican plum fruit from Oaxaca. On the other hand, Montalvo-González et al. (2011) detected a significant increase in ethylene production during ripening of yellow plum from Nayarit stored under different light conditions. In general, an increase in ethylene production acts as a trigger of fruit ripening, inducing the autocatalytic production that causes changes in color, texture, aroma, flavor, and other biochemical, physiological and physical attributes of the fruit (HIWASA-TANASE and EZURA, 2014) that the consumer consider important for its acquisition.

Color parameters in the epicarp of Mexican plum showed significant ( $P < 0.05$ ) changes among the four ripening stages (Table 1). Similar behavior to reported to Dantas et al. (2016). In the green stage, the epicarp was green ( $h^{\circ} = 108, 90$ ), dull ( $C^* = 26, 36$ ) and a little bright ( $L^* = 39, 87$ ); in the  $\frac{1}{2}$  green stage the color was close to yellow ( $h^{\circ} = 93, 7$ ), less dull ( $C^* = 34, 4$ ) and brighter; in the  $\frac{3}{4}$  ripe stage, fruits showed a angle hue tending towards orange ( $h^{\circ} = 75, 27$ ) with a chromaticity of  $C^* = 43, 39$  and more brightness ( $L^* = 50, 3$ ) compared to all the other ripening stages. In the fully ripe stage the color determined was tending toward red ( $h^{\circ} = 60, 39$ ), duller ( $C^* = 40, 48$ ) and less bright ( $L^* = 45, 91$ ) than the previous stage. Color change in Mexican plum might be due to a decrease in chlorophyll levels and an increase in carotenoids biosynthesis; however this hypothesis has not been confirmed.

Soluble solids (SS) significantly increased from the green stage (5.83 °Brix) to the ripe stage (23.9 °Brix) (Table 1). Alia et al. (2012) reported maximum values of 17.3 °Brix in 67 harvestings of Mexican plum from Morelos, Guerrero and Chiapas. Accordingly, Montalvo-González et al. (2011) reported maximum values of SS of 15.0 °Brix for yellow plum and Tiburski et al. (2011) obtained an average of 14.9 °Brix in yellow plum (*Spondias mombin* L.). An increase in the SS as fruit ripening process in Mexican plum and Brazilian has been reported by several authors (DANTAS et al., 2016; BAUTISTA-BAÑOS et al., 2003; PÉREZ et al., 2004; OSUNA et al., 2011).

Titrateable acidity, both in pulp and epicarp, was higher in the green stage and significantly decreased as the fruit ripened from 0.48% to 0.27% in pulp and from 0.42% to 0.23% in the epicarp. These results coincide with those reported by Dantas et al. (2016), Díaz-Pérez et al. (1999), Filgueiras et al. (2001) and Pérez et al. (2004) who mentioned a decrease of titrateable acidity as Mexican plum and Brazilian plums, fruit ripening process. Alia et al. (2012) obtained values between 0.2 and 2% of

citric acid, while Tiburski et al. (2011) reported and average value of 1.46 % of citric acid in yellow plum (*Spondias mombin* L.).

Mexican plum 'Cuernavaqueña' flavor index (FI) significantly increased with ripening stage both in pulp (from 12.35 to 87.62) and epicarp (from 18.47 to 105.16) (Table 1). The flavor index increase as a result of a decrease in TA and an increase in SS. Dantas et al. 2016, Filgueiras et al. (2001) and Pérez et al. (2004) reported a similar behavior. On another hand, Alia et al. (2012) reported a high variation (VC= 66%) on the FI going from 3.0 to 63.2; these results are attributed to the diversity of the evaluated genotypes.

The  $\frac{1}{2}$  green and the  $\frac{3}{4}$  ripe stages presented higher total phenols concentrations in the pulp, 89.21 and 77.7 mg GAE 100 g<sup>-1</sup> respectively, compared to the green and fully ripe stages (Table 2). On the other hand, the fully ripe stage showed the highest total phenols concentration in the epicarp (190.6 mg GAE 100 g<sup>-1</sup>). Filgueiras et al. (2001) quantified the highest concentration of total phenols in the ripe stage of *S. purpurea* grown in Brazil with values between 160 and 240 mg GAE 100 g<sup>-1</sup>. Beserra et al. (2011) and Vieira et al. (2011) reported total phenols in Brazilian plum of 55.0 ± 2.1, 70.92 ± 1.31 mg GAE 100 g<sup>-1</sup>, respectively. Tiburski et al. (2011) reported total phenols concentration in pulp of yellow plum (*Spondias mombin*) of 260 mg GAE 100 g<sup>-1</sup>. In the case of Mexican plum 'Cuernavaqueña', both the pulp and the epicarp are consumed, therefore the sum of the total phenol concentration of the pulp and the epicarp in ripe fruit make approximately 239 mg GAE 100 g<sup>-1</sup>. Phenols in *Spondias purpurea* have a natural antioxidant function and its consumption provides benefits against chronic and degenerative diseases (FILGUEIRAS et al., 2001; TIBURSKI et al., 2011). Mexican plum total phenols content is higher to those reported for papaya (54 mg GAE 100 g<sup>-1</sup>), banana (24-72 mg GAE 100 g<sup>-1</sup>) and pineapple (35-52 mg GAE 100 g<sup>-1</sup>) (THANARAJ and TERRY, 2011) and lower values (284.25 – 154.40 mg GAE 100 g<sup>-1</sup>) than Siqueira et al. (2015) in guanabana; therefore, it is considered a good source of metabolites with antioxidant capacity.

Flavonoids concentration in the pulp significantly increased from the green stage to the  $\frac{3}{4}$  ripen and the fully ripe stages, from 17 to 23-22 mg QE 100 g<sup>-1</sup> (Table 2). The highest concentration of total flavonoids was observed in the epicarp of ripe plums with 214 mg QE 100 g<sup>-1</sup> (Table 2). The total flavonoids content in ripe Mexican plum 'Cuernavaqueña' when the pulp and the epicarp flavonoids concentrations are added come to a total

of approximately 245 mg QE 100 g<sup>-1</sup> which is higher than the content reported for papaya (63.2 mg QE 100 g<sup>-1</sup>), grape (55.9 mg EQ 100 g<sup>-1</sup>), açaí (70.1 mg QE 100 g<sup>-1</sup>) and strawberry (21.8 mg QE 100 g<sup>-1</sup>) (ZIELINSKI et al., 2014). These results suggest that Mexican plum provides important quantities of flavonoids when it is consumed.

Total carotenoids content increased both in pulp and in epicarp as fruit ripening progressed in Mexican plum 'Cuernavaqueña' from initial values between 37.29 and 150.71 µg g<sup>-1</sup> to final values between 143.8 and 853.6 µg g<sup>-1</sup>, respectively (Table 2). A similar behavior was found by Solorzano et al. (2015). The sum of total carotenoids in pulp and epicarp of Mexican plum ripe fruit was of 1000 µg g<sup>-1</sup>. Tibursky et al. (2011) reported total carotenoids of 4869.5 µg g<sup>-1</sup> in yellow plum (*Spondias mombin*).

DPPH radical scavenging in pulp did not show significant differences among the four ripening stages with an average of 36.05 mg AAE 100 g<sup>-1</sup> and 396.62 µM TE 100 g<sup>-1</sup> (Table 3). In the ABTS assay, an increase on antioxidant activity in the pulp was observed in ½ green and ¾ ripe stages (Table 3), while in the FRAP assay, antioxidant activity decreased in the pulp as ripening progresses (Table 3). Spínola et al. (2015) reported antioxidant activity determined by the ABTS method in different tropical fruits, cherimoya, lime, papaya, passion fruit and strawberry with values between 121.2 and 316.15 AAE 100 g<sup>-1</sup> of juice and 675.14 and 1761.27 µmol TE 100 g<sup>-1</sup> of juice, respectively. The values obtained in Mexican plum 'Cuernavaqueña' compared to these data indicate that it has an acceptable antioxidant activity.

Antioxidant activity in epicarp of Mexican plum 'Cuernavaqueña' significantly increased as ripening progressed (Table 4). In the ripe stage, the epicarp showed the highest antioxidant activity against radicals DPPH, ABTS and FRAP. Kuskoski et al. (2005) reported antioxidant activity determined by DPPH in the pulp of tropical fruits (passion fruit, blackberry, pineapple, soursop, mango, grape) in the range of 174.3 to 41.1 mg AAE 100g<sup>-1</sup> and 12.9 to 0.5 µmol TE/g. For the ABTS assay, the same authors reported values between 37.0 and 224.7 mg AAE 100 g<sup>-1</sup> and 11.2 a 2.3 µ mol TE g<sup>-1</sup> which are similar to the values reported in the present work for Mexican plum. Tiburski et al. (2011) reported antioxidant activity of 17.47 mM TE g<sup>-1</sup> for yellow plum (*Spondias mombin*).

Mexican plum 'Cuernavaqueña' showed a good antioxidant activity with a higher free radical scavenging activity in the epicarp than in the pulp which can be attributed to the presence of higher

levels of bioactive compounds such as phenols and carotenoids. Among the methods used to determine antioxidant activity for free radicals scavenging, the DPPH radical, ABTS and FRAP methods are the fastest. In some works the antioxidant activity is related to the content of phenols, flavonoids and carotenoids; however, there might be other compounds with antioxidant properties (vitamins, enzymes, minerals) in the sample matrix (SERRANO et al., 2007).

The correlation between the evaluated bioactive compounds and the antioxidant activity (mg AAE g<sup>-1</sup> and µM TE100 g) determined by three different methods was performed; a high and significant correlation between phenols, carotenoids and flavonoids present in the epicarp and its activity for radical scavenging was found (Table 5). Bioactive compounds show different antioxidant activity depending on their structure (number of hydroxyl groups) as well as the matrix in which they are found (HEO et al., 2007). Besides, Palafox-Carlos et al. (2012) reported the interaction between the major phenolic compounds found in 'Ataulfo' mango pulp, which results in a significant synergism in the antioxidant capacity.

Several works have related the antioxidant capacity with the content of total phenols, flavonoids and carotenoids present in food (FRANKEL and MEYER, 2000; IMEH and KHOKHAR, 2002; PALAFOX-CARLOS et al., 2012). There are several methods for determining the antioxidant activity and it has been observed that each method show different tendencies and that is the reason for the need to use more than one method. The methods most commonly used are ABTS and FRAP, these have affinity for compounds of both hydrophilic and hydrophobic nature; on another hand, DPPH is more selective for antioxidants with hydrophilic nature (SÁNCHEZ-MORENO, 2002).

The results obtained in this research show that phenols, flavonoids and carotenoids with antioxidant capacity are naturally present in Mexican plum fruit and their concentration varies according to the ripening stage which agrees with previous reports indicating that bioactive compounds vary depending on ripening stage, cultural and processing practices (FALLER and FIALHO, 2009; VILLARODRIGUEZ et al., 2010; GAYOSSO-GARCÍA et al., 2011).

**TABLE 1-** Quality parameters in Mexican plum ‘Cuernavaqueña’ at four different ripening stages. Cuernavaca, 2014.

Ripening stage	Ethylene μL kg h <sup>-1</sup>	Respiration mL kg h <sup>-1</sup>	L*	C*	h	TSS °Brix	TA-P %	TA-E %	FI- P	FI- E
Green	3.92 c	1.01 a	39.87 c	26.3 c	108.9a	5.83 d	0.48 a	0.42 a	12.35c	18.47c
½ green	5.81 bc	1.20 a	45.33 b	34.4 b	93.7 b	10.1 c	0.4 a	0.32 b	24.67c	23.91c
¾ ripe	8.26 ab	1.03 a	50.3 a	43.3 a	75.2 c	18.7 b	0.33b	0.26bc	56.2 b	71.7 b
Fully ripe	9.43 a	1.23 a	45.91 b	40.4 a	60.3 d	23.9 a	0.27b	0.23 c	87.62a	105.1a
LSD	3.39	0.4651	2.18	3.68	6.52	1.72	0.067	0.082	13.28	19.22
VC (%)	23.7	22.96	2.98	6.30	4.52	7.29	9.89	14.45	16.23	19.37

L\*: lightness (0: white; 100: black); C\*: Chromaticity; h°: hue angle (0 = red; 90: yellow, 180 green); TSS: Total soluble solids; TA-P: Titratable acidity in pulp; TA-E: Titratable acidity in epicarp; FI-P: flavor index in pulp; FI-E: flavor index in epicarp. LSD: Least Significant Difference; VC: Variation Coefficient. Means followed by different letters in each column are significantly different according to the Tukey test  $P < 0.05$ .

**TABLE 2-** Bioactive compounds in Mexican plum ‘Cuernavaqueña’ at four different ripening stages. Cuernavaca, 2014.

Ripening stage	Pulp			Epicarp		
	Phenols (mg GAE 100 g <sup>-1</sup> )	Flavonoids (mg QE 100 g <sup>-1</sup> )	Carotenoids (μg g <sup>-1</sup> )	Phenols (mg GAE 100 g <sup>-1</sup> )	Flavonoids (mg QE 100 g <sup>-1</sup> )	Carotenoids (μg g <sup>-1</sup> )
Green	48.71 b	17.11 c	37.29 c	91.4 d	175.91 b	143.8 d
½ green	89.21 a	19.27 bc	43.64 c	117.17 c	169.62 b	390.9 c
¾ ripe	77.7 a	23.26 a	92.21 b	161.5 b	176.35 b	582.7 b
Fully ripe	48.1 b	22.07 ab	150.71 a	190.65 a	214.7 a	853.6 a
LSD	21.92	3.66	19.95	13.0	29.16	154.8
VC (%)	20.76	11.09	13.62	5.66	9.77	17.36

GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents. Means followed by different letters in each column are significantly different according to the Tukey test  $P < 0.05$ . LSD: Least Significant Difference, VC: Variation Coefficient.

**TABLE 3 - .** Antioxidant activity in the pulp of Mexican plum ‘Cuernavaqueña’ at four different ripening stages. Cuernavaca, 2014.

Ripening stage	Pulp					
	DPPH mg AAE 100 g <sup>-1</sup>	ABTS mg AAE 100 g <sup>-1</sup>	FRAP mg AAE 100 g <sup>-1</sup>	DPPH μM TE 100 g <sup>-1</sup>	ABTS μM TE 100 g <sup>-1</sup>	FRAP μM TE 100 g <sup>-1</sup>
Green	36.97 a	43.4 c	84.41 ab	398.63a	401.17 c	814.60 ab
½ green	36.92 a	61.5 ab	90.48 a	399.12a	563.3 ab	881.18 a
¾ ripe	37.64 a	73.7 a	73.69 b	405.75a	673.84 a	697.15 b
Fully ripe	32.53 a	57.37 bc	56.63 c	383.99a	524.58 bc	56.63 c
LSD	15.67	15.56	14.94	170.94	136.47	164.83
VC (%)	26.04	15.94	10.83	26.66	15.61	12.55

Ascorbic acid equivalent (AAE); Trolox equivalent (TE). LSD: Least Significant Difference, VC: Variation Coefficient.

**TABLE 4-** Antioxidant activity in the epicarp of Mexican plum ‘Cuernavaqueña’ at four different ripening stages. Cuernavaca, 2014.

Ripening stage	Epicarp					
	DPPH	ABTS	FRAP	DPPH	ABTS	FRAP
	Mg AAE 100 g <sup>-1</sup>	mg AAE 100 g <sup>-1</sup>	mg AAE 100 g <sup>-1</sup>	μM TE 100 g <sup>-1</sup>	μM TE 100 g <sup>-1</sup>	μM TE 100 g <sup>-1</sup>
Green	51.79 c	76.5 d	93.92 d	556.8 c	698.44 d	918.8 d
½ green	65.5 bc	101.6 c	111.7 c	702.99 bc	925.2 c	1114.79 c
¾ ripe	75.86 b	133.4 b	141.9 b	813.52 b	1210.9 b	1447.2 b
Fully ripe	98.87 a	167.4 a	170.7 a	1087.6 a	1534.7 a	1764.4 a
DMS	16.18	20.7	15.73	172.73	179.92	172.59
CV (%)	13.53	10.57	6.7	13.56	10.19	7.27

Ascorbic acid equivalent (AAE); Trolox equivalent (TE). LSD: Least Significant Difference, VC: Variation Coefficient.

**TABLE 5-** Positive Pearson correlation coefficients and level of significance between bioactive compounds and antioxidant activity. Cuernavaca, 2014.

	DPPH E	ABTS E	FRAP E	ABTS P	FRAP P
Phenols E	<sup>A</sup> 0.85*** <sup>B</sup> 0.871***	<sup>A</sup> 0.94 *** <sup>B</sup> 0.95 ***	<sup>A</sup> 0.941 *** <sup>B</sup> 0.943 ***		
Flavonoids E	<sup>A</sup> 0.68*** <sup>B</sup> 0.625 **	<sup>A</sup> 0.649*** <sup>B</sup> 0.601 **			
Carotenoids E	<sup>A</sup> 0.81*** <sup>B</sup> 0.76 ***	<sup>A</sup> 0.907*** <sup>B</sup> 0.884 ***	<sup>A</sup> 0.87 *** <sup>B</sup> 0.89***		
Phenols P					<sup>A</sup> 0.56** <sup>B</sup> 0.565 **
Flavonoids P				<sup>A</sup> 0.561** <sup>B</sup> 0.555 **	
Carotenoids P					<sup>A</sup> 0.83*** <sup>B</sup> 0.83 ***

E: Epicarp; P: Pulp. Level of significance (\* p<0.05, \*\* p<0.001, \*\*\* p<0.0001). A: results expressed as mg AAE g<sup>-1</sup>; B: results expressed as μM TE g<sup>-1</sup>.

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