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Post-harvest quality and quantification of betalains, phenolic compounds and antioxidant activity in fruits of three cultivars of prickly pear (*Opuntia ficus-indica* L. Mill)

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ABSTRACT

Postharvest quality, quantification of betalains, phenolic compounds and antioxidant activity of peel, pulp and juice of fruits of three prickly pears (*Opuntia ficus-indica* L. Mill.) cultivars of Colegio de Postgraduados in México, were measured. The red and orange cultivars showed outstanding features of postharvest quality (size, texture, TSS and pulp and juice content), highest content of betalains and phenolic compounds. Therefore, highest antioxidant activity. In general, highest content of bioactive compounds was detected in peel, besides the content in pulp and juice did not show statistically significant differences. Phenolic content is very high in comparison with other fruits. Antioxidant activity was measured by three assays: FRAP, ABTS and DPPH. Three cultivars showed high correlation between antioxidant activity and phenolic compounds. The methodologies used in this work are a very useful tool for the quantification of bioactive compounds in *O. ficus-indica* fruit tissues.

Keywords **:** Betalains, Flavonoids, *Opuntia ficus-indica*, Phenolic compounds and Prickly pear

INTRODUCTION

Prickly pear (*Opuntia ficus-indica* L. Mill.) is the species of cacti with the greatest economic importance in the world (Bravo, 1978); (Kiesling, 1999); (Griffith, 2004); (Feugang *et al*., 2006). It is cultivated in several continents, but is native to America, where, there are more than 93 species of *Opuntia* (Hunt, 1999). In the southern highlands of Mexico, there are more than 243 varieties, used as fodder, vegetables and fruit. Most of the prickly pear cactus is collected from the wild, since there are only approximately 20,000 commercial plantations of prickly pear cactus. The semiarid regions of central Mexico hosted the greatest genetic diversity, as well as the largest cultivated area of prickly pear cactus in the world. Variability is found in both cultivated and wild populations. Prickly pear has become an important fruit crop in the semi-arid lands of Mexico, where it plays a strategic role in subsistence agriculture (Pimienta, 1994). The prickly pear has been recognized for its numerous nutritional virtues, nutritional and functional properties. Recent data have revealed the high content of some chemical components, which can give added value to this fruit. High levels of betalains, taurine, calcium, magnesium and antioxidants stand out. In addition, some of the components show promising characteristics in terms of functionality (Piga, 2004).

The diversity of betalains found in these prickly pear cultivars, indicate the potential value of Opuntia cactus pear fruit, as a good source of pigments, and their potential industrial exploitation for drinks and food products. Therefore, consumption of cactus pear fruit may provide nutritional and health benefits (Castellanos & Yahia, 2008). Flavonoids have been reported by several authors (Feugang *et al*., 2006); (García *et al*., 2019). Also, Kuti (2000) reported about the presence of phenolic compounds in fresh prickly pear fruits. (Lee *et al*., 2002) also reported the antioxidant effects of *Opuntia* extracts. There is few information on the quantification of betalains and

phenolic compounds in different fruit tissues, and juice of *Opuntia ficus-indica* cultivars. The purpose of the following work was to evaluate the postharvest quality, quantification of betalains, phenolic compounds and antioxidant activity of fruit tissues of three prickly pears (*Opuntia ficus-indica* L. Mill.) cultivars grown at Colegio de Postgraduados.

MATERIALS AND METHODS

Plant material

Three *O. ficus-indica* cultivars colors red, white and orange developed in the fruticulture experimental field of Colegio de Postgraduados, located in Montecillo, State of Mexico (coordinates 19°272 513 N 98°542 153 O, altitude 2250 msnm), high altitude, temperate climate, the driest of the sub-humid, with rainfall in summer, precipitation 572. 25 mm and mean annual temperature of 15.3 ºC (García, 1988) were selected for the study according to flesh color, identified as CP1 (red), CP3 (white) and CP4 (orange). For fruit harvesting, the criteria established were the flattening of the floral cavity and the moment when the glochids or thorns fell (Cantwell, 1995).

Color characteristics

The fruit color was measured by CIELAB system. The epicarp color was measured on two opposite sides of the equatorial zone of the fruit, with a Hunter-Lab model D-25 reflection colorimeter (Reston, Virginia, USA); CIELAB parameters L*, a*, b* were recorded and the hue angle (°h=tan-1(b^*/a^*) and saturation index (Chroma (C) (a^2+b^2) $^1/_2$) were calculated (McGuire, 1992).

Postharvest quality

A total of 50 fruits per cultivar were harvested and measured for size, structural components (peel, pulp and seeds), epicarp (peel) color, texture, juice content, total soluble solids, juice pH, betalains, flavonoids, phenols contents and antioxidant capacity. Size was determined based on longitudinal and equatorial diameter, measured with a trupper-14388 digital vernier on a total of 15 fruits; data were reported in millimeters (mm). The structural components evaluated were the proportion of peel, pulp and seeds, determined on a weight basis with an Ohaus Scout-Pro electronic balance with a sensitivity of 0.1 mg and the percentage of peel, pulp and seeds was calculated; in addition, the number and area (mm^2) of seeds was determined using an Epson Scan scanner with WinSeedle TM 2013 software. Firmness was measured based on the deformation of the fruit when a force of 1 kg was applied with a Chantillon texturometer (Wagner Force Five model FDV-30) with a flat strut; the results were expressed in Newtons/cm^2 (N/cm^2).

Juice extraction

To determine the juice content, the juice was extracted from a total of 15 fruits separately with an Oster ® FPSTJE317 centrifugal extractor; for the calculations, the equation % juice= (juice weight/pulp weight) x100 was applied. Total soluble solids (%) and pH were measured according to the methods of the (AOAC, 1990) using a portable refractometer Palette Atago, PR-320 (0-.32%) and a Corning Model 12 potentiometer, NY, USA, respectively.

Obtaining prickly pear tissues

Samples of the epicarp (30g), mesocarp and endocarp (30 g), as well as juice from the pulp (15 mL) were obtained separately by hand using an Oster ® FPSTJE317 centrifugal extractor. All samples were kept in Ultrafreeze at -65°C and subsequently freeze-dried for 3 days at -45°C and 1.3 × 10^{-3} MPa in a Labconco Freezone 2.5 L equipment. The freeze-dried samples were homogenized using a Nutribullet Nb-101b to obtain a fine particle. Finally, they were preserved in airtight aluminum bags for storage at -18°C until analysis.

Extraction procedure of freeze-dried prickly pear tissues

Extraction was performed by placing 1 g of freeze-dried prickly pear sample in 50 mL of methanol: water (80/20, v/v) and mixed by vortex for 3 min, subsequently pH was adjusted to 3 with hydrochloric acid, and put in an ultrasonic bath (Bransonic™ CPXH series) for 15 min. After that, the samples were rotated for 30 min at 150 rpm and 27°C. Finally, they were centrifuged for 15 min (3500 rpm) and the supernatant was separated. The extracts were stored at -18°C in dark for further analysis.

Spectrophotometric quantification of total betalains and phenolic compounds

For the determination of total betalains and phenolic compounds, the prickly pear extracts mentioned above were used. Betalain content was measured

according to the method of (Castellanos & Yahia, 2008) using a Sinergy 2 microplate multidetector equipped with Gen 5 Data Analysis Software (Biotek Instruments Inc., Winoosky, VT USA). The absorption spectrum was obtained from 200 to 700 nm to obtain the absorption maximum and an OD <1. Readings were obtained for each extract in triplicate. The betalain content was expressed as: µg betanin equivalents for betacyanin content (BC) and µg indicaxanthin equivalents for betaxanthin content (BX). The calculation was made using the following formula: BC or BX (mg/g) = [A(Df)(Mw)(Vd)/ ε(L)(Wd)] where A is the absorption value at the absorption maximum of 535 and 483 nm for betacyanins and betaxanthins, respectively, DF is the dilution factor, Vd is the dried pulp solution volume (mL), Wd is the dried pulp weight (g), and L is the path-length (0.38 cm) of the cuvette. The molecular weight (Mw) and molar extinction coefficient (ε) of betanin [Mw) 550 g/mol; ε) 60,000 L/(mol cm) in H2O] were applied in order to quantify the betacyanins. Quantitative equivalents of the major betaxanthins (Bx) were determined by applying the mean molar extinction coefficient [ε) 48,000 L/(mol cm) in H^2O]. In all cases, water extracted the highest level of pigments.

The total flavonoid determination was conducted according to the colorimetric method defined by Chang *et al*. (2002) with modifications. The prickly pear extract was mixed with 100 µL of potassium acetate, 100 µL of 10% aluminum chloride and 4.7 mL of distilled water. After incubation at room temperature for 30 min in darkness, the absorbance of the reaction mixture was measured at 415 nm in a microplate multidetector mentioned in section 2.7 placing 200 µL of sample and reagent blank in respective microwells. The amount of 10% aluminum chloride was substituted by the same amount of methanol: water (80/20, v/v) in blank. Quercetin (0.4 – 1.6 µg/mL) was used to make the calibration curve and the results were expressed as mg quercetin equivalents per g dry weight (mg EQ/ g dry weight).

The total phenolic determination was expressed as µg gallic acid equivalents per g of dry weight (mg GAE g dry weight), according to the Folin-Ciocalteau assay which detects electron transfer by measuring the reducing capacity of the sample and can therefore also be considered as antioxidant activity assay (Cano *et al*. 2017).

Antioxidant activity

The antioxidant activity of each cultivar of prickly pear was determined using three assays: FRAP, ABTS and DPPH which have been widely applied in the analysis of food samples (Re *et al*., 1999). The FRAP assay was performed according to the methodology (Benzie & Strain, 1996) with some modifications. The FRAP solution includes 10 mL of 300 mM acetate buffer at pH 3.6, 1 mL of 10 mM TPTZ and 1 mL of 20 mM $FeCl_3 6H^2O$. The prickly pear extracts (20 µL) were allowed to react with 180 µL of FRAP solution and 60 µL of distilled water for 10 minutes in dark conditions. Readings were taken at 595 nm. The calibration curve was linear between 50 and 600 µM Trolox. Results were expressed in µM Trolox equivalents (µM TE)/g dry weight. For ABTS assay, the procedure of (Re, 1999) was followed with some modifications. The $ABTS^{-+}$ radical solution included 7.4 mM $ABTS^{-+}$ and 2.6 mM sodium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react in the dark for 16 hours. The solution was then diluted by mixing 600 µL of $ABTS^{-+}$ solution in 9.4 mL of methanol. The prickly pear extracts (20 µL) were allowed to react with 180 µL of ABTS solution for 10 minutes in dark conditions. Readings were taken at 734 nm. The calibration curve was linear from 50 to 500 µM Trolox. Results are expressed in µM Trolox equivalents (µM TE/g dry weight).

DPPH assay was done according to the method of Williams *et al*. (1995) with some modifications. The DPPH stock solution was prepared by dissolving 19.7 mg of DPPH in 100 mL of 80% methanol. Prickly pear extracts (200 µL) were allowed to react with 50 µL of DPPH solution for 30 min in dark conditions. Readings were taken at 515 nm. The calibration curve was linear from 50 to 500 µL of Trolox. The results were expressed in µM Trolox equivalents (µM TE/g dry weight). Additional dilutions were made when the values obtained from the samples were outside the linear range of the calibration curve.

Statistical analysis

The compositional data were expressed as mean ± standard deviation of at least five independent determinations. Significant differences between results were calculated by one-way analysis of variance

(ANOVA), followed by a post hoc Tukey's test. A level of $p < 0.05$ was considered a significant difference**.** To investigate the relationship between main phytochemicals, a bilateral Pearson correlation analysis was performed with a significance of $p < 0.01$ and $p < 0.05$. All statistical analyses were executed with SAS Institute, Inc 9.4.

RESULTS AND DISCUSSION

Morphological characterization

The morphological and physical characteristics of three prickly pear cultivars are directly influenced by selection (Table 1). Fruit length averages (mm) were significantly different among them, with CP4 and CP3 obtaining the highest and lowest values (97.15 and 73.2 mm, respectively). Regarding diameter, no significant differences were found between selections with averages of 52 and 55 mm respectively. The values of both lower and upper limits are very similar to those reported by Parish and Felker (1997) with average ranges of 73 to 88 mm for length and 56 to 57 mm for diameter. Cerezal & Duarte (2005) evaluated prickly pears harvested in the Andean highlands of the 2nd Region of Chile, reporting average length values of 62 to 78 mm and 46 to 52 mm in diameter. Karababa *et al.* (2004) reported fruit length values ranging from 66 to 71 mm and diameter values from 45 to 52 mm for a variety harvested in five locations in Turkey. On the other hand, Singh (2003) reported length and diameter values lower than those found in this study for prickly pear clones from the USA and introduced to India with average ranges of 55 to 76 mm in length and 33 to 46 mm in diameter.

CP1 and CP4 had values of epicarp firmness of 32 and 36.5 N/cm^2 respectively, higher than CP3 (26.6 N/cm^2). Weight of fruit of CP3 was significantly lower (124 g) compared to CP1 and CP4 (160 and 164 g respectively). There are other published works about the size of fruit, weight, TSS, pH and number of seeds (Cerezal & Duarte, 2005); (Karababa *et al*., 2004); (Parish & Felker, 1997); (Singh, 2003).

Table 1: Morphological, physical and physico-chemical characteristics of fresh fruits of three prickly pear cultivars (*Opuntia ûcus-indica* L. Mill.)

	CP1 (red)	CP3 (white)	CP4 (orange)
Size of fruit (mm)	87.18±6.64^{b}	73.19±8.72^{c}	97.15±6.62^{a}
Diameter (mm)	55.39±2.95^{a}	54.02±6.7^{a}	52.22±4.24^{a}
Firmness (N/cm^2)	31.99±9.54^{a}	26.63±6.94^{b}	36.51±6.98^{a}
Total weight of whole fruit (g)	159.91±23.81^{a}	123.95±33.55^{b}	154.26±17.67^{a}
Peel content (%)	37.19±4.15^{b}	40.9±3.33^{a}	39.67±3.51^{ab}
Pulp content (%)	62.3±4.18^{a}	57.57±5.2^{b}	60.54±2.93^{ab}
TSS of pulp (%)	15.53±1.22^{a}	12.59±1.73^{b}	11.4±0.78^{ab}
Juice content (%)	74.99±5.36^{a}	66.57±7.11^{b}	67.72±5.64^{b}
pH	7.31±0.16^{a}	6.55±0.15^{c}	7.03±0.13^{b}
TSS of juice (%)	13.52±0.86^{a}	12.86±2.33^{a}	11.91±0.54^{a}
Seed content (%)	2.74±0.17^{ab}	2.08±0.61^{b}	3.09±0.60^{a}
Weight of seeds (g)	4.18±0.86^{a}	3.11±0.39^{b}	4.12±0.69^{a}
Number of seeds	329.67±61.8^{a}	188.11±32.65^{bc}	231.56±50.7^{c}
Average area of seeds (mm^2)	15.75±0.7^{c}	18.41±0.68^{b}	19.592±1^{a}

*Values are the mean of 15 independent determinations ± standard deviation.

*Different letters indicate statistically significant differences (pd" 0.05) between columns.

In contrast with studies of Barbera *et al*. (1994) the biggest fruit (CP4) don´t have the high quantity of seeds, in this case the fruit of CP1 had high quantity of seeds. The cultivar with less number of seeds was CP3 (white), it has been cultivated to produce prickly pear for many years. So, it has had a selection process. El Behi *et al.* (2015); Barbera *et al.* (1994); Mejía & Cantwell (2003) mention in their studies that the relationship between fruit size and seed content is highly variable and influenced by factors such as genotype, crop load and fruit position within the canopy.

Firmness is a mechanical property gives post-harvest quality in fresh fruits. A loss of firmness is caused by loss of cell turgor due to aging or dehydration. Both thinning and softening of the peel contribute to increased susceptibility to physical damage and deterioration of prickly pears during handling (Cantwell, 1995). However, this characteristic is also due to genetic and nutritional issues of the crop. Guerrero (2018) reports firmness values for white prickly pear *Opuntia amyclaea* green mature (36.28 N/cm^2) and mature (26.48 N/cm^2). In this study we obtained values between 23.63 N/cm^2 for CP3 and 36.51 N/cm^2 for CP4.

Red cultivar (CP1) was characterized by the significantly higher content of pulp (62.3%), TSS of pulp (15.53 Brix) and juice (13.52%), juice content (74.99%), and lower content of peel (37.19%). Significant differences in the pH of the three cultivars were observed with values between 6.55 (CP3) and 7.31 (CP1).

This values were higher than reported by Andreu *et al*. (2018) in six cultivars of prickly pears grown in Spain, who showed values of pH between 5.2 and 6.06. Regarding seed content, cultivars CP1 (red) and CP4 (orange) showed higher seed weight (4.18 and 4.12 g respectively), and higher seed quantity (329 and 231 seeds respectively) than CP3 (white). However, CP1 (red) has significantly smaller seeds (15.75 mm) than CP3 and CP4.

Color

Table 2 shows that the three cultivars had L* values less than 50, the CP3 (white) was the closest with (L= 47.5), so it is the one with the least dark color. Between CP1 (red) and CP4 (orange) cultivars, no significant differences were observed for lightness. Hue values suggest that there are three types of shades; white with high hue values (112.27), red with intermediate value (25.72) and orange with low hue values (7.49). The highest chroma values were also presented by CP3 (white) (21.88), CP1 (red) and CP4 (orange) obtained very close values (16.17 and 15.32) respectively.

Table 2: Color of fruit or three prickly pear cultivars (*Opuntia ficus-indica* L. Mill.)

	CP1 (red)	CP3 (white)	CP4 (orange)
L*	35.19±2.76^b	47.5±3.96^a	34.33±1.86^b
a	6.2±2.5^b	-8.3±2.3^c	9.6±2.1^a
b	9.3±3.0^b	19.8±1.5^a	11.4±1.2^b
Hue	25.72±9.59^b	112.27±5.52^a	7.49±7.49^c
Chroma	16.17±3.97^b	21.88±2.4286^a	15.32±1.7^b

* Values are the mean of 15 independent determinations ± standard deviation.

* Different letters indicate statistically significant differences ($p \leq 0.05$) between columns.

Quantification of betalains

Betalains are water soluble compounds present in a restricted number of families of plants from the *Caryophyllale* family. They are classified in two chemical families: betacyanins and betaxanthins with 540 and 480 nm absorption maxima. Betalains are powerful radical eliminators in chemical system and act as an efficient antioxidant in biological models (Cano *et al*., 2017).

Betalain content was measured in CP1 (red) and CP4 (orange) cultivars, in the peel, pulp and juice of prickly pear. The CP1 cultivar showed higher betacyanins (BC) and betaxanthins (BX) content than CP4 (orange) with values of 1181 and 1137 µg/g d.w in peel, respectively for CP1 (red) and values of 161 and 408 µg/g d.w in peel for CP4 (orange), respectively. These compounds are responsible for the red and orange shades respectively.

Betacyanins appear to be in higher concentration in the peels of both prickly pear cultivars (red and orange), however, betaxanthins are observed evenly distributed in both peel, pulp and juice in the CP4 (orange) cultivar. This is consistent with the findings of (Cano *et al.*, 2017).

On the other hand, no significant differences are shown between BC and BX content in pulp and juice

for both selections (Table 3). In this sense, we could assume that no significant betalain content is lost during the juice extraction process.

Table 3: Betalain content in two prickly pear cultivars (*Opuntia ficus-indica* L. Mill.)

		CP1 (red)	CP4 (orange)
BC[1]	Peel	1181.67±151.3^{aA}	161±6.08^{bA}
	Pulp	496±30.51^{aB}	69.67±0.58^{bB}
	Juice	472.33±12.74^{aB}	65.67±5.69^{bB}
BX[2]	Peel	1137.67±169.82^{aA}	408±2.65^{bA}
	Pulp	552.67±26.65^{aB}	435.33±58.77^{aA}
	Juice	398±19^{aB}	457±21.07^{aA}

* Values are the mean of 3 independent determinations ± standard deviation.

* Lowercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

* Uppercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

BC[1]: Betacyanins expressed as µg of betanin equivalents per gram of dry weight.

BX : Betaxanthins expressed as µg of indicaxanthin equivalents per gram of dry weight.

Castellanos & Yahia (2008) reported values of betacyanins of 5290 µg/g dw in Camuesa cultivar, followed by 2060 µg/g in Roja Pelota, 2040 µg/g dw in Cardona and much lower contents in the Reyna variety (50 µg/g dw). Betaxanthins were found in the yellow prickly pear varieties Naranjona, 2651 and 21441 with values of 160, 140 and 120 µg/g dry weigt, respectively. These values differ greatly from those found in this work.

García *et al.* (2019) reported betacyanin values of 1670 µg/g d.w and 450 µg/g and betaxanthin values of 730 and 370 µg/g in the pulp of Mexican varieties of purple and red prickly pear, respectively. The values reported for red tuna are more consistent with what was found in this study.

Quantification of total phenols (TP) and total flavonoids (TF)

Some of the published works on the chemical composition of prickly pear showed information about the main compounds with antioxidant activity (Fernández *et al.*, 2010). Phenolic compounds are known as bioactive or functional compounds that serve as protectors against certain diseases (Butera *et al.*, 2002), which are mainly characterized by their antioxidant activity (Andreu *et al.*, 2018).

Table 4 shows the content of total phenols in the peel, pulp and juice of the three cultivars evaluated. CP1 (red) and CP3 (white) presented the highest Total phenols content (TP) in peel (7225.67 and 7486.67 µg GAE. g^{-1} dw, respectively), which was significantly different for CP4 (orange), which obtained 59.39% with respect to the CP3 (white) cultivar. No significant differences were found in the total flavonoid content in the peel of the three selections studied (2505, 2114 and 2239 µg QE g^{-1} d.w.) respectively.

The Total Flavonoids content (TF) in pulp and juice of the three cultivars did not show significant differences with average values of 2121, 1422.5 and 1911 µg GAE. g^{-1} dw for CP1, CP3 and CP4, respectively). García *et al*. (2019) found values of 2067 µg GAE. g^{-1} dw for red prickly pear fruit pulp and 3501 µg GAE. g^{-1} dw in peel. This value is close to that we found in this study for the orange selection (4446 µg GAE. g^{-1} p.s.).

TP and TF were found in 70 and 83% higher concentrations in peel than in pulp and juice in CP1 (red). In 82 and 83% in CP3 (white) and 62 and 93% in CP4 (orange). This corresponds with the findings of several authors, giving clear evidence that the highest antioxidant contents are present in the peel of the fruit (Andreu *et al.,* 2018); (García *et al*., 2019); (Morales, 2009).

CP1 presented the highest content of TP and TF in pulp (2149 µg GAE. g^{-1} dw and 558 µg QE g^{-1} dw respectively). In addition, cultivars CP1 and CP4 had the highest TP in juice (2092 and 2138 µg GAE. g^{-1} dw and CP3 had the highest TF in juice (555.33 µg QE g^{-1} dw).

The content of total phenols in prickly pear is very high compared to other fruits. The TP ranges (µg GAE. g^{-1} dw) are 140 to 1020 in nectarines, 210 to 110 in peaches and 420 to 1090 in plums (Gil *et al.*, 2002). On the other hand, the results are close to other fruits with high antioxidant capacity such as guava, which obtained values of 1700 to 3000 µg GAE. g^{-1} dw in a study carried out on pink-fleshed clones (Thaipong *et al.*, 2006).

Table 4: Content of total phenols, total flavonoids and antioxidant activity (FRAP, ABTS y DPPH) in three prickly pear cultivars (*Opuntia ficus-indica* L. Mill.)

		CP1 (red)	CP3 (white)	CP4 (orange)
Total Phenols[1]	Peel	7225.67±198.07^{aA}	7486.67±461.24^{aA}	4446.67±295.5^{bA}
	Pulp	2149.33±211.05^{aB}	1529.67±163.09^{bB}	1683.33±54.37^{bB}
	Juice	2092.67±132.08^{aB}	1315.33±155.58^{bB}	2138.33±127.45^{aB}
Total Flavonoids[2]	Peel	2505.33±194.54^{aA}	2114.67±78.56^{aA}	2239.67±176.52^{aA}
	Pulp	558±55.51^{aB}	249±21.52^{bC}	168±5.57^{bB}
	Juice	425.67±68.38^{bB}	555.33±25.66^{aB}	148.67±2.08^{cB}
FRAP[3]	Peel	17.68±0.74^{aA}	14.94±0.48^{bA}	19.13±0.35^{aA}
	Pulp	8.63±0.75^{aB}	6.83±0.84^{aB}	7.71±0.32^{aB}
	Juice	7.48±0.49^{aB}	5.23±0.16^{bB}	8.14±0.17^{aB}
ABTS[4]	Peel	20.61±0.74^{aA}	20.49±0.32^{aA}	19.08±0.35^{aA}
	Pulp	18.34±1.34^{aA}	7.39±0.45^{bB}	7.65±0.32^{bB}
	Juice	14.38±1.21^{aB}	6.09±0.19^{cC}	8.09±0.17^{bB}
DPPH[5]	Peel	16.03±4.23^{bA}	32.38±1.61^{aA}	19.82±5.65^{aA}
	Pulp	8.96±0.74^{aAB}	6.56±0.89^{bB}	2.41±0.24^{cB}
	Juice	5.05±0.37^{aB}	2.89±0.15^{bC}	2.38±0.22^{bB}

* Values are the mean of 3 independent determinations ± standard deviation.

* Lowercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

* Uppercase letters indicate statistically significant differences ($p \leq 0.05$) between cultivars of the same tissue for each given compound.

[1] expressed as µg of gallic acid equivalents per gram of dry weight.

[2] expresado as µg of quercetin equivalents per gram of dry weight.

[3,4,5] expressed as µmol de trolox equivalents per gram of dry weight.

*DPPH (2,2-difenil-1-picrilhidrazilo), ABTS (ácido -3 etilbenzotiazolino-6-sulfónico) y FRAP (Ferric Reducing Antioxidant Power).

On the other hand, other species such as xoconostle (*Opuntia matudae*) have shown higher values of these compounds (TP) with values of up to 8590 and 9180 µg GAE. g^{-1} dw in pulp and peel (Morales, 2009). Similarly, values from 4950 to 9800 µg GAE. g^{-1} dw have been reported in blueberry (Wada, 2002) and from 526 to 6819 µg GAE. g^{-1} dw at different maturity stages in garambullo (Felix, 2018).

Antioxidant activity (AOA)

Antioxidant activity, is one of the main mechanisms in which vegetables and fruits provide health benefits to humans (Andreu *et al.,* 2018). Several studies have established inverse correlations in the consumption of fruits and vegetables and cardiovascular, inflammatory, cancer and age-dependent diseases (Willet, 2001).

The use of a single technique to determine antioxidant activity may prove to be unrealistic and not as useful, however there are a large number of published techniques that purport to measure antioxidant activity *in vivo* (Wuang *et al.,* 2005). The measurement of antioxidant activity in prickly pear fruits was evaluated based on three spectrophotometric assays; DPPH, ABTS and FRAP. The results are shown in Table 4. As with total phenols and flavonoids, the highest antioxidant activity was clearly observed for the three assays and three cultivars (CP1, CP3 and CP4) in the fruit peel, except in the peel and pulp of CP1 (red) by ABTS.

For the FRAP assay, CP1 (red) and CP4 (orange) show higher antioxidant activity (17.6 and 19.13 µmol ET g^{-1} dw) than CP3 (white) in peel. CP3

shows the lowest antioxidant activity in this assay in the three tissues (14.9, 6.8 and 5.2 µmol ET g^{-1} dw) in peel, pulp and juice, respectively. In the ABTS assay, CP1 (red) showed higher antioxidant activity in pulp and juice with values of 18.34 and 14.38 µmol ET g^{-1}dw, respectively. There are no significant differences in the antioxidant activity of the three cultivars in peel. In DPPH, CP3 (white) and CP4 (orange) showed higher antioxidant activity in peel 32.3 and 19.8 µmol ET g^{-1}dw, respectively. On the contrary, CP1 (red) showed higher antioxidant activity in juice and pulp than the other cultivars with values of (8.96 and 5.05 µmol ET g^{-1}dw), respectively.

FRAP technique estimates the reducing activity of Fe(III), which is not necessarily relevant for calculating its antioxidant capacity (Ou, *et al*., 2002). Taking into account that not all antioxidants reduce Fe(III) as fast as required (Pulido *et al.,* 2000), their antioxidant capacity could be underestimated. The ABTS technique is considered to be highly sensitive (Kuskoski *et al.,* 2005), however, the working solution for this technique needs to be kept in the dark for 12 hours to generate free radicals. As the reacting solution is not always of the same age, this can lead to differences in values depending on the determination times (Thaipong *et al.*, 2006).

The DPPH assay has been a widely used method to detect the ability of compounds to scavenge free radicals or the antioxidant activity of extracts (Hou, *et al*., 2003). Sanchez suggested that 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is an easy and accurate method to measure the antioxidant capacity in fruit and vegetable extracts (Sánchez, 2002).

As concluded by Frankel and Meyer, these assays differ from each other in terms of substrates, probes, reaction conditions and quantification methods, making it very difficult to compare the results obtained between them (Frankel & Meyer, 2000).

A single method is not sufficient to determine the antioxidant capacity of plant extracts; more than one type of AOA determination is required to represent the different modes of action of antioxidants. The methods used are basically classified into two types: assays based on hydrogen atom transfer (HAT) and assays based on electron transfer (ET) (Dudonné *et al.*, 2009). In this study, AOA was determined by two HAT-type assays: ABTS and DPPH, as well as Fe reduction capacity, using the FRAP assay.

The presence of phenolic compounds in plant extracts contributes significantly to their antioxidant potential (Dudonné *et al.*, 2009). Part of this AOA comes from flavonoids, low molecular weight polyphenolic compounds distributed in fruits and vegetables (Hertog *et al*., 1992). For their part, betalains are powerful free radical scavengers that act as efficient antioxidants in biological models (Cano *et al*., 2017).

Antioxidant capacity varies considerably from one type of fruit to another. (Wuang *et al*., 2005) and coworkers conducted a study in which the antioxidant capacity of 12 fruits and 5 commercial juices was measured by ORAC assay, resulting in strawberry having the highest AOA (15.36), followed by plum (9.49), orange (7.50), grape (7.39), kiwi (6.02) and melon (0.97 µmol ET g^{-1} fresh fruit).

(Andreu *et al*., 2018) and coworkers reported high levels of antioxidant capacity in prickly pear fruits of different cultivars for peel and pulp showing higher values than those found in this study in the three methods. By the ABTS technique, they reported the lowest AOA value in peel for cultivar NA (14.7) and the highest value for cultivar NA (14.7 µmol ET g^{-1} dw). In pulp, the lowest value was obtained by cultivar NJ (6.4) and the highest value by NT (30 µmol ET g^{-1} dw). By the DPPH technique, the lowest AOA value in peel was obtained by cultivar NE (54.8) and the highest value in cultivar FR (60 µmol ET g^{-1} dw). In pulp, the lowest value was obtained by cultivar NO (57.4) and the highest value by NT (60 µmol ET g-1 dw). Finally, measured by FRAP, the lowest value of AOA in peel was obtained by cultivar NE (40.2) and the highest value by cultivar NA (116 µmol ET g^{-1} dw). In pulp, the lowest value was obtained by cultivar NA (15) and the highest value by FR (32 µmol ET g-1 dw).

This exceeds the results found for antioxidant capacity in this study for the three selections, with

the highest values found by the DPPH technique for CP3 peel (32.3) and for CP1 pulp by the ABTS technique (18.3 µmol ET g^{-1} dw).

Some authors have reported results consistent with this study, finding a higher antioxidant capacity in fruit peel than in the pulp of pomegranate (Calín *et al.*, 2013), guava (Marquina *et al.*, 2008) and berries (Oszmiański *et al*., 2016).

Correlation between tests

To determine the linear relationship between the antioxidant capacity methods performed and the phenolic compounds and betalains, Pearson's correlation coefficient was calculated. Table 5 shows high correlations between the three methods and phenolic compounds (Phenols and Flavonoids). The correlation coefficient between total phenols and flavonoids and the AOA measured by the FRAP assay was 0.85 and 0.93, respectively. The correlation between total phenols and flavonoids and AOA measured by ABTS was 0.79 and 0.81 and by DPPH was 0.85 and 0.77, respectively.

The correlation coefficients of betalains (betacyanins and betaxanthins) and AOA by FRAP were lower, with values of 0.41 and 0.48, respectively, 0.67 and 0.51 by ABTS and 0.50 and 0.466 by DPPH.

All techniques used for the determination of antioxidant capacity (AOA) showed a high correlation with TP and TF for three evaluated cultivars (CP1, CP3 and CP4). This may be because phenolic compounds, known as hydrophilic antioxidant compounds, are the most abundant secondary metabolites in plants (Gil *et al*., 2002). This corresponds with what has been found by other authors such as (Thaipong *et al.,* 2006) in guava extracts (r=0.97) using the FRAP technique and by (Dudonné *et al.,* 2009) in Pinus bark (r=0.96) using the ABTS technique. In addition, high correlations have been reported between total phenols and antioxidant activity by FRAP in fruit juices (Gardner *et al.*, 2000). Kuti also reports similar correlations to those found in this work between total flavonoids and the antioxidant capacity of four varieties of prickly pear with values ranging from 0.76 to 0.88 using the ORAC technique (Kuti, 2000).

Table 5: Pearson correlation matrix

	FRAP	ABTS	DPPH
BC	0.415*	0.670**	0.504*
BX	0.489*	0.516*	0.466*
FT	0.854**	0.798**	0.853**
FL	0.938**	0.811**	0.775**

*,**= significant ($p \leq$ 0.05 y 0.01 respectively).

BC: Betacyanins, BX: Betaxanthins. TP: Total phenols, TF: Total Flavonoids.

FRAP= total antioxidant capacity determined using Cu (III) complex as oxidant. ABTS= total antioxidant capacity determined with the 2, 2'-azino-bis-3-ethylbenzothiazoline6-sulfonic radical (ABTS•+); DPPH= total antioxidant capacity determined with the radical 2,2-diphenyl-1-picrylhydracil (DPPH •).

The high correlation shown by both TP and TF, as determined by the three techniques, indicates that both compounds are important contributors to the antioxidant activity of prickly pear fruit.

In the case of betalains, low correlations were found with the three techniques, ranging from 0.41 to 0.67: the lowest correlation was by the FRAP technique and the highest by ABTS. This may be attributed to the assays used, considering the fact that individual antioxidants may, in some cases, act by multiple mechanisms depending on the reaction system (Fernández *et al.,* 2010). Cano and collaborators reported a negative correlation of total betalain content and antioxidant capacity determined by the DPPH technique (-0.08) (Cano *et al.,* 2017).

The body's defense system is composed of several antioxidant components. Supplementation with one or few antioxidants may not be as effective. Fruits contain a group of natural antioxidants that could have not only high antioxidant activity, but also a good combination or mixture of antioxidants (Wuang *et al*., 2005).

CONCLUSIONS

The present study provides information on physicochemical characterization and antioxidant properties of three selections of prickly pear (*Opuntia ficus-indica* Mill) grown at the Colegio de Postgraduados, Mexico. The results show that prickly pear has considerable levels of phenolic compounds that play an important role against oxidation. The highest content of these compounds is found in the peel of the fruit and there are no significant differences between the content in pulp

and juice. Therefore, prickly pear peel has a great potential for obtaining bioactive compounds, antioxidants. These natural antioxidants can be formulated to give nutraceuticals, which can help prevent oxidative damage from occurring in the body.

In relation to quality and physicochemical characteristics, CP1 (red) and CP4 (orange) were outstanding in aspects of size, weight, greater resistance to deformation, higher total soluble solids content, greater quantity of pulp and juice, and smaller seed.

All these aspects make the CP1(red) and CP4(orange) selections interesting materials for both fresh and processed products. Further research is needed to find alternatives to take full advantage of the compounds found in all parts of the fruit, as well as to understand the role played by betalains in the antioxidant activity of the fruit.

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