PAPER

JUICES OF PRICKLY PEAR FRUITS (*OPUNTIA* SPP.) AS FUNCTIONAL FOODS

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ABSTRACT

The prickly pear (*Opuntia* spp.) usually is consumed as fresh fruit. In this study of prickly pear juice *in vitro* we characterized and quantified secondary metabolites including antioxidant capacity of ten *Opuntia* spp. variants. Gallic acid was abundant in most variants. Catechin and epicatechin isomers, and procyanidins B1 and B2 were present in most variants. Ascorbic acid content was higher than 84 mg. Betacyanins stand out in red-colored juices, betaxanthins in the yellow ones; this caused lack of relationship between antioxidant capacity and total phenolic content. The soluble fiber content, sugars, betalains and ascorbic acid position this juice as a functional food.

Keywords: ABTS, antioxidant, betalains, FRAP, juice, prickly pears

1. INTRODUCTION

Normal metabolic processes produce free radicals, which cause oxidative damage. The human body possesses endogenous antioxidant mechanisms using substances that significantly delay or prevent oxidation. These substances include the cellular enzymes superoxide dismutase, glutathione peroxidase and catalase (WANG and QUINN, 2000). There are also non-enzymatic defense substances against oxidation stress, including vitamin E, an effective antioxidant of polyunsaturated membrane lipids, and vitamin C which, as a reducing agent or electron donor, reacts rapidly with the $HO^{\cdot}$ and the superoxide anion and also prevents the oxidation of membrane lipids (WANG and QUINN, 2000).

When endogenous antioxidant mechanisms are insufficient to offset the imbalance resulting from oxidative stress, physiological and biochemical changes take place such as protein glycosylation, lipid peroxidation, and glucose auto-oxidation (OPERA, 2004). Diseases associated with oxidative stress include Type-2 diabetes mellitus (DM2), hypertension, renal and hepatic impairment, cancer, and neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's, Parkinson's and Huntington (JELLINGER, 2003; D'AMICO *et al*., 2013).

The intake of natural or synthetic antioxidants can reinforce the antioxidant capacity of the organism (HIDALGO *et al*., 2006). Recent research has shown that certain compounds present in plants, such as terpenes, flavonoids, betalains and anthocyanins, possess antioxidant properties that are more powerful than those of vitamins (HARASYM and OLEDZKI, 2014).

Global trends in food and nutrition indicate a growing interest in the consumption of fruits and vegetables, given their nutritional value and benefits for the functions of the human body. These trends in eating patterns have led to a new area of research and development in nutrition related to the so-called "functional foods", defined as any food, either natural or processed, which in addition to its nutritional components contains substances that boost a person's health, physical ability and mental state (KONIGSBERG-FAINSTEIN, 2008). Functional compounds include exogenous antioxidants, which safely interact with free radicals and disrupt their chain reaction before they damage vital molecules (OROIAN and ESCRICHE, 2015).

The prickly pear, the fruit of cacti of the genus *Opuntia* is widely available throughout Mexico's South Highland. More than 189 species of wild prickly pear cacti are known, 83 of which are Mexican; of these, 29 are distributed in the north-central region of Mexico, in an area of approximately 300 000 km^2 that stretches across part of the states of Aguascalientes, Guanajuato, Hidalgo, Jalisco, Queretaro, San Luis Potosí, Zacatecas, and around México City. In this area, a number of variants with different degrees of humanization can be found, from the wild *O. streptacantha* and the cultivated *O. hyptiacantha*, *O. megacantha* and *O. albicarpa*, to *O. ficus-indica*, the species considered as the one with the highest degree of domestication (REYES-AGÜERO *et al*., 2005). Prickly pears are consumed mainly as fresh fruit and display marked differences in size, shape, color and flavor, as well as in seed quantity, size and hardness; prickly pear is also processed to produce jelly, jam and paste. Chemical compounds found in prickly pear include polyphenols and betalains (FIGUEROA-CARES *et al*., 2010; YEDDES *et al*., 2013). These antioxidant metabolites either prevent or control the excessive production of highly unstable free radicals and reactive molecules that have the ability to disrupt the functions of various biomolecules, i.e., oxidative stress (RODRÍGUEZ *et al*., 2001; SOOBRATTEE *et al*., 2005).

Evaluations of the prickly pear fruit indicate its potential to be considered as a functional food due to its content of ascorbic acid, phenols, carotenoids and betalains at levels that

exceed those in plums, nectarines or peaches (FERNÁNDEZ-LÓPEZ *et al.*, 2010). These phytochemicals may contribute to mitigation of the effects of prolonged hyperglycemia and reinforcement of the antioxidant system in normal glycemic patients. Antioxidants have been shown to increase the sensitivity of insulin receptors or may moderate the rise in blood glucose concentration after the ingestion of carbohydrates by inhibiting the action of digestive enzymes and glucose transporters SGLT-1 (BRYANS *et al*., 2007). In addition, these phytochemicals have been associated with anti-inflammatory, antioxidant, immunomodulatory and apoptotic properties (KAULMANN and BOHN, 2016). Phytochemicals, which locally reduce oxidative stress, are widely studied as cancer-protective agents (MOORE *et al.*, 2016). Indeed, animal assays indicate that supplementation with green and black tea (rich in polyphenolic compounds) led to a decrease in postprandial blood glucose levels in Sprague-Dawley rats (ZEYUAN *et al*., 1998). Furthermore, *in vivo* studies showed a drop in glycosylated hemoglobin (FUKINO *et al*., 2008) and increased insulin activity after consumption of tea extracts (RICHARDA and DOLANSKY, 2002).
Based on the above, the objective of this study was to supplement existing assessments of prickly pear juice as a functional food by identifying and quantifying antioxidant compounds in the juice of fruits of *Opuntia* and to investigate their antioxidant capacity *in vitro*.

2. MATERIALS AND METHODS

2.1. Selection of variants and sample preparation

Ten prickly pear variants, six of them cultivated, were evaluated as ripe fruits: Rojo Pelón (*Opuntia ficus-indica*), Blanca (*O. albicarpa*), Amarilla Monteza, Pico Chulo, Torreoja and Sangre de Toro (*O. megacantha*), and four wild variants: Cardona (*O. streptacantha*), Charola (*O. streptacantha* ssp. *aguirrana*), Tapona and Tapón Rojo (*O. robusta*). Fruits were collected in the municipality of Villa de Arriaga, state of San Luis Potosí, México. *Opuntia* variants were selected based on: (a) degree of humanization, (b) abundance and economic potential in the state of San Luis Potosí, and (c) fruit color. The skin of prickly pears was removed, then the juice was extracted from the pulp with a stainless-steel blender (International LI-12-106), and seeds were separated with an 8 mesh filter; the juice was stored in sterile containers at -20°C until use.

2.2. Total phenolic compounds

Total phenolic compounds in prickly pear juice was quantified using the Folin-Ciocalteu method modified by YEDDES *et al*. (2013), and expressed as gallic acid equivalents (mg GAE g^{-1}). To extract phenols, cool absolute ethanol was added to 0.15 g of lyophilized juice stored at -50°C (Frezer dryers IIshin, Corea), the mixture was sonicated for 10 min and then maintained under constant stirring for 2 h at 4°C. The solution was filtered through Whatman Grade 42 filter paper. Extracts were brought to 15 mL with ethanol and stored protected from light at -20°C. Total phenols were measured in triplicate; to this end, 437.5 μL of 1N Folin-Ciocalteu reagent (Sigma) were added to 35 μL of the ethanol extract and were left to react at room temperature for 3 min. Afterwards, 2187.5 μL of a 20% Na_2CO_3 solution were added and the volume was brought to 3500 μL. The mixture was left to stand at room temperature in the dark for 2 h for the development of color. Absorbance was read at 760 nm in a spectrophotometer (Agilent Technologies, Germany), using blank samples made of distilled water and the reagents used. The amount of phenolic

compounds was estimated by comparing the absorbance values of samples with those of the gallic acid standards.

2.3. Phenolic acids and flavan-3-ols

Phenolic compounds were extracted with the method used by RODARTE *et al.* (2007). Two grams of lyophilized prickly pear juice were mixed with 3 mL of acidified methanol (0.1% hydrochloric acid), and sonicated in a water bath for 10 min; then, the supernatant was collected and the previous procedure was repeated five times with the precipitate to obtain six extractions. Supernatants were collected and centrifuged for 10 min at 5000 rpm; the centrifuged fraction was concentrated under vacuum on a rotary evaporator at 30°C (Heidolph, Alemania) followed by reconstitution with 2 mL of methanol. All samples were filtered through 0.45 μm nylon filters.
Phenolic acids were identified and quantified in a liquid chromatograph (Spectra-Physics UV6000LP), with a LiChrospher® 100 RP-18 column (250 mm x 4.6 mm, 5 μm particle size). Formic acid (10% in water) (A) and acetonitrile/water/formic acid (45:45:10) (B) were used as mobile phase, with a flow rate of 1 mL/min. The identification was made by comparing the retention times and UV-Vis spectra obtained using a diode array detection system (Thermo Scientific, USA), with reference standards. Hydroxybenzoic acids were quantified at 280 nm; hydroxycinnamic acid esters, at 315 nm.
Flavan-3-ols were identified and quantified in a liquid chromatograph (Thermo Spectra Physic Series P100, USA), coupled to a fluorescence detector (Perkin Elmer Series 200ª, USA), with a LiChrospher® 100 RP-18 column (250 mm x 4.6 mm and 5 μm particle size). Acetonitrile (A) and acetic acid (B) were used as mobile phase, with a flow rate of 1.4 mL/min. Flavanols were identified using the wavelengths λexc = 280 nm and λem = 320 nm. In this study, procyanidins were quantified as catechins.

2.4. Identification and quantification of betalains

Betalains were measured using the method of CASTELLANOS-SANTIAGO and YAHIA (2008). To do this, 100 mg of lyophilized prickly pear juice were weighed and 10 mL of 80% methanol acidified with 0.5% HCl were added; this mixture was sonicated for 15 min and filtered through a 0.45 μm nylon filter (Agilent Technologies, Alemania). An electronic scan was run between 400 and 700 nm in an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Alemania), which identified the absorption peaks of betalains at 547 nm and 490 nm. Absorbance units were converted to concentration units.

2.5. Ascorbic acid quantification

Ascorbic acid was quantified using the method of SDIRI *et al.* (2012). To this end, 2 mL of 4.5% meta-phosphoric acid were added to 0.1 g of lyophilized juice; this mixture was sonicated in a water bath for 2 min, then centrifuged for 10 min at 5000 rpm, and finally filtered through 0.45 μm nylon filters. Ascorbic acid was quantified in a HPLC chromatograph (Thermo Spectra Physic Series P100), coupled to a UV detector (Thermo Finnigan Spectra System UV2000), with a LiChrospher® 100 RP-18 column (250 mm x 4.6 mm and 5 μm particle size), and KH_2PO_4 (0.2 M at pH=2.3-2.4) was used as mobile phase, with a flow rate of 1.0 mL/min for 15 min at λ = 243 nm and an injection volume of 20 μL. This compound was estimated using the following calibration equation y = 76165x - 161251, r^2= 0.9993.

2.6. FRAP (ferric reducing antioxidant power) method

The FRAP assay assessed the capacity of juice samples to reduce the ferric ion (Fe^{+3}) in a complex with 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), to the ferrous ion (Fe^{+2}), in accordance with FIRUZI *et al.* (2005). The FRAP reagent was prepared daily by mixing 10 mL of 300 mM sodium acetate buffer solution (pH 3.6) with 1 mL of 20 mM ferric chloride hexahydrate and 1 mL of 10 mM TPTZ dissolved in 40 mM hydrochloric acid. Twenty five microliters of prickly pear juice diluted 1/20 with methanol were added to 96-well flat bottom microplates in triplicate, followed by the addition of 175 μL FRAP solution. A control treatment was prepared with 200 μL methanol; another control was prepared by mixing 25 μL methanol with 175 μL FRAP; for a third control, 25 μL ferric sulphate and 175 μL sodium acetate buffer were added; finally, 25 μL of juice sample were added to 175 μL sodium acetate buffer solution. Readings were recorded with a Multiskan Ascent reader (Thermo Electron Corporation 100-240 VAC Type: 354) at 595 nm. The first reading was taken at time 0, and the plate was incubated at 37°C immediately afterwards. The second reading was taken after 60 min.
The calibration curve was constructed with ferric sulphate heptahydrate (7.194 mM) dissolved in methanol at concentrations of 108 μM to 864 μM. The resulting calibration equation was y = 0.0011x - 0.069, r^2= 0.9971.
The FRAP value for the curve was calculated according to the following equation:

$$FRAP(M) = \left({}^{\Delta a_t FI}/_{\Delta a_t Fe^{2+}}\right) x10^{-5}$$

Where
$\Delta a_t FI$ = change in absorbance of the analyte after the time interval.
$\Delta a_t Fe^{2+}$= change in absorbance of iron sulfate at the same concentration and after the time interval.

The results of each sample are expressed as μM $FeSO_4$ eq.

2.7. Estimate of the trolox equivalent antioxidant capacity (TEAC) with the chemical mediator $ABTS^{\cdot+}$

This estimate was made in accordance with the methodology of NENADIS *et al.* (2004). The TEAC of samples was based on 2,2'-azino-bis(3-ethylenebenzothiazoline-6-sulfonic acid) (ABTS), which produces the radical $ABTS^{\cdot+}$ and is compared with an antioxidant (trolox). For each evaluation, the $ABTS^{\cdot+}$ solution was prepared by mixing 5 ml of 7 mM ABTS and 88 μL of 140 mM potassium persulphate; the mixture was stored in the dark covered with aluminum foil and left to stand for 12 h at room temperature to produce the radical; afterwards, 500 μL of the solution were mixed with 25 mL of ethanol and its absorbance was read in an Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, Alemania) to confirm that it was between 0.7 and 1 at 734 nm. Samples and controls were measured in triplicate (20 μL sample plus 230 μL $ABTS^{\bullet+}$) and placed in a 96-well flat bottom plate; after adding the radical ion, the plate was covered with aluminum foil and after 6 min the reading was recorded at 734 nm in a Multiskan Ascent Reader (Thermo Electron Corporation 100-240 VAC Tip: 354).
The percent inhibition of the standard was obtained with the following equation:

$$\% \text{ inhibition} = \left[\frac{Abs\ control - Abs\ sample}{\underset{control}{Abs}}\right] * 100$$

The absorbance of the sample was subtracted from the absorbance of the control to obtain the true absorbance.
The calibration curve was constructed with 50 μM, 100 μM, 200 μM, 300 μM, 400 μM and 500 μM trolox standards, adding 20 μL of the standard solution and 230 μL $ABTS^{\cdot+}$; the resulting regression equation was y = 0.2263x + 7.5033; r^2= 0.9979. The results were expressed as μg·mol trolox equivalent (TE) per gram of juice (dry weight).

2.8. Experimental design and statistical analysis

The experiment was performed according to a completely randomized experimental design. Treatments were the juices from the 10 prickly pear variants, which were tested for content of phenolic compounds, betalains and ascorbic acid, as well as their antioxidant capacity through FRAP and ABTS. Three replicates were used for each of these measurements. The data were subjected to an analysis of variance and Tukey's multiple comparison test. A Pearson correlation was carried out between FRAP and ABTS variables (SAS, version 8.0; SAS Institute, Cary, North Carolina).

3. RESULTS AND DISCUSSION

The potential of these prickly pear juices as functional foods is high due to outstanding content of soluble fiber and the adequate content and proportion of glucose to fructose (ZENTENO-RAMÍREZ *et al*., 2015). In order to determine whether consumption of prickly pear juices can help prevent or cure diseases associated with the excess of free radicals it is essential to identify and quantify the compounds with antioxidant capacity contained in prickly pear juice. Phenols in fruits, flowers and vegetables have attracted the attention due to their antioxidant potential. It has been shown that various parts of *Opuntia* (pulp, fruit skin, seeds and cladodes) are rich in polyphenols (GALATI *et al*., 2003; VALENTE *et al*., 2010; TOUNSI-SAIDANI *et al*., 2011). In addition, various studies have demonstrated their antioxidant effects (DOK-GO *et al*., 2003; TESORIERE *et al*., 2004; SIRIWARDHANA and JEON, 2004; OSORIO-ESQUIVEL *et al*., 2011).

3.1. Total phenol content

Due to their ubiquitous presence in plant foods, phenolic compounds are normally included in the daily human diet. The daily intake ranges between 25 mg and 1 g, depending on the amount of fruits, vegetables, pulses, tea and spices consumed (HAGERMAN *et al*., 1998). Raw extracts of phenol-rich plant products are attracting interest in the food industry, since these slow down the oxidative degradation of lipids, and hence improve the quality and nutritional value of food; their antioxidant power protects against heart disease and cancer, in addition to other chronic degenerative diseases (KÄKHÖNEN *et al*., 1999). The protection against LDL oxidation is not due to a single compound, but results from the effect of several phenolic compounds (RICCHELLE *et al*., 2001).
The total content of polyphenols was estimated in the ethanol extracts of lyophilized juice samples (Table 1). The statistical differences between prickly pear variants seem to be unrelated to fruit color and degree of humanization, and it should be noted that the four

O. megacantha variants evaluated showed the highest total content of phenolic compounds. Among the variants evaluated by MABROUKI *et al.* (2015), the highest concentration was observed in the pulp of *O. streptacantha*, followed by *O. ficus-indica*, with 104.6 GAE per 100 g of juice. In general, it has been pointed out that the concentration of phenolic compounds in prickly pears range from 54 mg/100 g to 104 mg/100 g fresh weight (KATABI *el al*., 2013; FIGUEROA-CARES, *et al.*, 2010). Thus, the concentration of phenolic compounds in prickly pear juice is similar or higher than in pineapple, tomato, banana, mango and cucumber (1.7, 2.0, 2.3, 2.6, and 3.8, all in mg/g dry weight, respectively) (MUÑÓZ-JÁUREGUI and RAMOS-ESCUDERO, 2007).

3.2. Quantification of phenols by high performance liquid chromatography (HPLC)

Table 1 shows the concentration of phenolic acids in the studied prickly pear variants, which show significant differences ($P < 0.0001$). Gallic acid was recorded in all variants except Tapona, and was the main phenolic compound in most of them, with varying concentrations between 32.6 μg/g and 81.2 μg/g. Syringic acid was absent only in Torreoja and Cardona, and ellagic acid in Blanca, Sangre de Toro and Tapón Rojo. Protocatechic acid was recorded only in Pico Chulo (41.6 μg/g). Pico Chulo showed the four phenolic acids and recorded the highest total phenolic acid concentration (176 μg/g). By contrast, Blanca showed the lowest total phenolic acid content (79.4 μg/g).

Table 1. Average concentration (μg/g) of total phenols and phenolic acids in lyophilized juices of 10 prickly pear variants.

Variant*	Total phenols	Gallic acid	Syringic acid	Ellagic acid	Total phenolics acids
Rojo Pelón	1.92±0.11^{de}	32.6±0.6^{g}	29.2±0.9^{d}	25.0±0.9^{e}	86.9±2.3^{e}
Blanca	1.93±0.25^{de}	53.7±0.6^{e}	25.6±0.4^{e}	n.d.	79.4±0.8^{e}
Amarilla Monteza	3.81±0.75^{bc}	74.8±3.6^{bc}	13.6±0.3^{h}	33.5±0.1^{d}	122.0±3.8^{b}
Pico Chulo	2.81±0.39^{bcd}	63.6±0.6^{d}	20.0±2.7^{f}	50.5±2.3^{b}	176±7.9^{a}
Torreoja	3.90±0.06^{ab}	49.7±1.8^{e}	n.d.	41.9±1.9^{c}	91.6±3.6^{d}
Sangre de Toro	5.21±0.83^{a}	42.4±0.5^{f}	66.5±0.1^{a}	n.d.	109±1.2^{c}
Cardona	1.67±0.36^{de}	81.2±0.7^{a}	n.d.	26.7±0.4^{e}	108.0±1.0^{c}
Charola	1.69±0.48^{de}	78.3±1.0^{ab}	16.9±0.3^{g}	73.2±1.5^{a}	168±2.2^{a}
Tapona	2.52±0.42^{cde}	n.d.	45.3±1.1^{b}	68.3±4.1^{a}	114.0±5.2^{bc}
Tapón Rojo	1.45±0.14^{e}	71.5±0.1^{c}	38.4±0.3^{c}	n.d.	110.0±1.2^{c}
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Variants are sorted from highest to lowest degree of humanization.
n = 3. Treatments with different letters in the same column are statistically different (<0.05).
n.d.= not detected

Flavonoids are the dominant class of phenols in food, accounting for approximately two thirds of the phenols consumed in the human diet (LOTITO and FREI, 2006). Table 2 shows the concentrations of the flavan-3-ol derivatives found in the juice of all prickly pear variants studied, with significant differences ($P < 0.0001$) between them. These four derivatives were recorded in the juice of all variants; however, catechin was not found in Blanca, being the derivative found at the lowest concentration in all variants except Charola, where epicatechin attained the lowest concentration. The derivative registered at the highest concentration in these prickly pear juices was either epicatechin or procyanidin

B2, according to the variant. The highest epicatechin concentrations were found in Tapona juice, and the lowest in Cardona, with 90.8 μg/g and 17.2 μg/g, respectively. With regard to the total content of flavan-3-ol derivatives, the Tapona juice showed the highest concentration (223±6.09 μg/g), and also the highest levels of each individual derivative; in contrast, the Cardona juice showed the lowest concentration of these derivatives (73.7 μg/g).

Table 2. Average concentration (μg/g) of flavan-3-oles in lyophilized juices of 10 prickly pear variants.

Species	Variant*	Catechin	Epicatechin	Procyanidin B1	Procyanidin B2	Total flavan-3-oles
O. ficus-indica	Rojo Pelón	13.30±0.63^{de}	19.26±1.68^{f}	16.71±0.13^{f}	28.36±0.38^{e}	77.6±1.30^{f}
O. albicarpa	Blanca	n.d.	60.94±1.26^{b}	32.50±1.49^{d}	38.76±0.43^{c}	132±3.18^{c}
O. megacantha	Amarilla Monteza	14.23±0.69^{cd}	37.4±0.16^{c}	21.03±0.74^{ef}	33.93±0.55^{d}	107±4.82^{d}
	Pico Chulo	14.87±0.6^{cd}	24.58±0.02^{e}	23.59±1.25^{e}	29.51±1.19^{de}	92.5±1.86^{e}
	Torreoja	19.61±0.94^{b}	32.10±0.39^{d}	25.40±0.87^{e}	20.37±0.40^{f}	97.5±1.80^{de}
	Sangre de Toro	19.61±1.60^{b}	61.93±0.77^{b}	40.67±1.40^{c}	51.62±1.31^{a}	174±1.88^{b}
O. streptacantha	Cardona	10.44±0.18^{e}	17.15±0.45^{f}	22.41±0.51^{e}	23.68±1.08^{f}	73.7±2.21^{f}
O. streptacantha ssp. *aguirrana*	Charola	27.25±0.95^{a}	17.97±0.98^{f}	47.06±2.19^{b}	44.45±0.28^{b}	137±4.40^{c}
O. robusta	Tapona	27.89±0.97^{a}	90.81±2.18^{a}	59.47±0.90^{a}	45.20±2.05^{b}	223±6.09^{a}
	Tapón Rojo	17.56±1.14^{bc}	19.16±0.30^{f}	24.67±1.56^{e}	23.63±1.81^{f}	85.1±4.21^{ef}
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*Variants are sorted from highest to lowest degree of humanization.
n = 3. Treatments with different letters in the same column are statistically different (<0.05).
n.d.= not detected

3.3. Concentration and identification of betalains

According to STINTZING *et al*. (2005), prickly pear color is due to betalains, since these authors recorded indicaxanthin and betaxanthins (84 mg/kg and 100 mg/kg, respectively) in yellow-orange prickly pears, while red prickly pears contained betacyanins at concentrations of 400 mg/kg, as the chemicals responsible for this color. As shown in Table 3, the juice of red-colored prickly pears have a higher betacyanin content, while betaxanthins predominate in the yellow variants (Amarilla Monteza and Pico Chulo), a finding that is consistent with other studies (STINTZING *et al*., 2005; CHÁVEZ *et al*., 2009; YAHIA and MONDRAGÓN, 2011). The Tapona juice showed the highest content of betacyanins and betaxanthins, but its purple-red color derives from the prevalence of betacyanins. The intermediate values of both compounds in the juice of the red *O. megacantha* variants is worth noting, as well as the minimum content of them in Blanca juice; this finding coincides with the results of CASTELLANOS-SANTIAGO and YAHIA (2008) for the same species.

Table 3. Average content of betaxanthins and betacyanins (mg/g dry weight) in lyophilized juices of 10 prickly pear variants.

Species	Variant*	Betaxanthins	Betacyanins	Total betalalains
O. ficus-indica	Rojo Pelón	0.148±0.005^{f}	0.149±0.010^{g}	0.298^{g}
O. albicarpa	Blanca	0.018±0.003 ^{g}	0.021±0.004^{h}	0.044^{h}
O. megacantha	Amarilla Monteza	0.120±0.007 ^{f}	0.011±0.001^{h}	0.130^{h}
	Pico Chulo	0.085±0.017^{f}	0.019±0.004^{h}	0.105^{f}
	Torreoja	0.313±0.029^{e}	0.358±0.030^{f}	0.671^{ef}
	Sangre de Toro	0.810±0.007^{c}	1.580±0.030^{c}	2.390^{c}
O. streptacantha	Cardona	0.423±0.030^{d}	0.800±0.008^{d}	1.213^{d}
O. streptacantha ssp. *aguirrana*	Charola	0.290±0.007^{e}	0.660±0.020^{e}	0.945^{e}
O. robusta	Tapona	1.450±0.017^{a}	2.610±0.030^{a}	4.074^{a}
	Tapón Rojo	1.230±0.04^{b}	2.380±0.060^{b}	3.610^{b}
P value		<0.0001	<0.0001	<0.0001

*Variants are sorted from highest to lowest degree of humanization.
n = 3. Treatments with different letters in the same column are statistically different (<0.05).

3.4. Quantification of ascorbic acid by HPLC

Ascorbic acid is one of the most effective and abundant antioxidants in fruits and vegetables (LOGANAKI and MANIAN 2010), participating in various biological functions that include the synthesis of collagen, hormones and neurotransmitters. The increase in the consumption of ascorbic acid is associated with a lower risk of chronic diseases such as cancer, cardiovascular disease and cataracts. This may be due to its ability to eliminate free radicals in biological systems. This study only measured ascorbic acid content (Table 4) without performing the reduction of dehydroascorbic acid (DHAA), necessary to obtain the total vitamin C content (SDIRI *et al*., 2012).

Table 4. Average concentration of ascorbic acid (mg/g dry weight) in lyophilized juices of 10 prickly pear variants.

Species	Variant*	Ascorbic acid
O. ficus-indica	Rojo Pelón	1.328±0.003^{a}
O. albicarpa	Blanca	0.316±0.003^{d}
O. megacantha	Amarilla Monteza	0.327±0.016^{d}
	Pico Chulo	0.542±0.004^{c}
	Torreoja	0.327±0.004^{d}
	Sangre de Toro	0.652±0.041^{b}
O. streptacanta	Cardona	0.325±0.006^{d}
O. streptacanta ssp. *aguirrana*	Charola	0.191±0.000^{e}
O. robusta	Tapona	0.527±0.029^{c}
	Tapón Rojo	0.691±0.006^{b}
P value		<0.0001

n=3. Treatments with different letters in the same column are statistically different (<0.05).
*Variants are sorted from highest to lowest degree of humanization.

Ascorbic acid concentration showed significant differences between the prickly pear juices evaluated ($P < 0.0001$).
The highest ascorbic acid content was recorded in Rojo Pelón, followed by Sangre de Toro and Tapón Rojo; Charola was the variant with the lowest ascorbic acid concentration in juice. However, all concentrations measured were sufficient to meet easily the minimum daily intake (84 mg) of ascorbic acid in the human diet (SÁENZ *et al*., 2007).
Among the variants evaluated by YAHIA and MONDRAGÓN (2011), the highest ascorbic acid concentration was recorded in the juice of the Camuesa prickly pear (*O. robusta*), followed by Cardona (*O. streptacantha*), with 4.0 mg/100 g and 2.1 mg/100 g fresh weight, respectively, while the lowest concentration was observed in the juice of Naranjona (*O. megacantha*), ranging between 1.2 mg/100 g and 1.4 mg/100 g fresh weight; according to these authors, DHAA showed a pattern similar to that of ascorbic acid. In general, it has been pointed out that the concentration of ascorbic acid in prickly pear (*Opuntia* spp.) ranges from 12 mg/100 g to 81 mg/100 g fresh weight (FEUGANG *et al*., 2006). Thus, the concentration of ascorbic acid in prickly pear juice is similar to or higher than in grapes, apple and pear (0.5 mg/g, 0.3 mg/g and 0.2 mg/g edible dry weight, respectively), but lower than in guava and kiwi fruit (9.4 mg/g and 4.9 mg/g edible dry weight, respectively) (LOTITO and FREI, 2006). To note, the variants with the highest and lowest degree of humanization showed the highest concentrations of this antioxidant, suggesting that this process is unrelated to the concentration of this antioxidant.

3.5. Antioxidant capacity of prickly pear juice *in vitro*

The antioxidant capacity of prickly pear juice was estimated through ABTS and FRAP, since both are the assays most frequently used and they measure most antioxidants present. ABTS is typically used for mixtures or complex beverages, and measures mainly SET (single electron transfer) antioxidants, without excluding HAT (hydrogen atom transfer) antioxidants, in both water-soluble and fat-soluble media. In contrast, FRAP is applicable mostly to vegetables with SET and HAT antioxidants, mainly phenols and ascorbic acid (SURVESWARAN *et al.*, 2007; GÜLÇIN, 2012). These methods were considered as mutually complementary and were contrasted through the correlation between their respective results.
SURVESWARAN *et al*. (2007) point out that various herbs, fruits and vegetables show a direct relationship between antioxidant capacity and total phenolic content. In the prickly pear juices evaluated, this trend was not observed due to their contrasting differences in color, related to the presence of antioxidants such as ascorbic acid and betalains (Table 5). The data obtained with ABTS were normally distributed, but those with FRAP had to be log-transformed before being analyzed. The estimates of antioxidant capacity obtained with both methods (FRAP and ABTS) for total phenols, betalains and ascorbic acid in the juice of the 10 prickly pear variants were compared through a simple linear correlation analysis (Table 6).
All correlation values for each comparison had the same sign, which evidences a consistent general trend in the estimates obtained with both methods. The results show that the estimates of the reduction ability of betalains with FRAP and ABTS were positively and significantly correlated ($P < 0.0001$). On the other hand, the estimates for ascorbic acid and phenolic compounds were not significantly correlated. The significant correlation of the total antioxidant capacity between both methods is explained by the abundance of betalains and because both methods produced similar estimates of the antioxidant capacity for all other compounds tested.

Table 5. Antioxidant total capacity of juices of 10 prickly pear variants.

Species	Variant*	ABTS$^{\cdot+}$ TEAC (μM/g dry weight)	FRAP (μM eq. FeSO4/g dry weight)
O. ficus-indica	Rojo Pelón	43837±2601^{abc}	49102±4280^{cd}
O. albicarpa	Blanca	39570±8473 ^{c}	45202±4098^{cd}
O. megacantha	Amarilla Monteza	37504±6726^{c}	38490±2591^{d}
	Pico Chulo	38307±6833^{c}	39157±2583^{d}
	Torreoja	46264±8198^{abc}	42378±9272^{cd}
	Sangre de Toro	51422±400^{abc}	79066±7562^{b}
O. streptacantha	Cardona	45501±3565^{abc}	60141±4645^{bc}
O. streptacantha spp. aguirrana	Charola	40778±3741^{bc}	57543±4843^{cd}
O. robusta	Tapona	62117±10439^{a}	112651±15066^{a}
	Tapón Rojo	59968±12243^{ab}	118790±16262^{a}
P value		<0.0016	<0.0001

*Variants are sorted from highest to lowest degree of humanization.
n=3. Treatments with different letters in the same column are statistically different (<0.05).

Table 6. Correlation (r) between estimates of antioxidant capacity generated by ABTS and FRAP methods in the juices of 10 prickly pear variants.

Antioxidant	ABTS	FRAP
Total	0.7845 ***	0.9436 ***
Total phenolic compounds	0.0848	-0.11811
Phenolic acids	-0.2033	-0.0943
Flavan-3 ols	0.3943	0.4781
Betalains	0.7828***	0.9511***
Ascorbic acid	0.1829	0.1635

Significance *** $P < 0.0001$.

4. CONCLUSIONS

The higher content of betalains in the red prickly pear variants Tapona, Tapón Rojo and Sangre de Toro, and of ascorbic acid in Rojo Pelón, Tapón Rojo and Sangre de Toro, resulted in their total antioxidant capacity being higher than in the other color variants, and explains the lack of a significant correlation between the estimates of the antioxidant capacity of phenols. Of the variants evaluated, Pico Chulo was the richest in phenolic acids, and Tapona in flavan-3-ols. Unlike other table fruits, prickly pear is an important source of phenols, in addition to having the most common antioxidant phytochemicals, such as betalains and ascorbic acid.
Therefore, this study of prickly pear juice *in vitro* provides further support for recommending consumption of prickly pear juice as a functional food due to its antioxidant properties similar or superior to the juice of various marketed fruits. The study confirms the antioxidant capacity of the analyzed fruit, however before prickly pear fruit can be considered a potential functional food it is important to highlight the importance of running *in vivo* studies (animals and humans) in order to confirm

bioavailability of the compounds analyzed in the study and to find whether consumption of prickly pear fruit actually induces positive effect in promoting a healthy status.

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