Journal of the Professional Association for Cactus Development

Chemical and antioxidant analysis of peels from two red-purple cactus pears (*Opuntia streptacantha*) from Altos Norte of Jalisco, Mexico

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Abstract. The cactus pears, fruits of the *Opuntia* genus, exhibit a wide variety of colors and feature a juicy, fleshy pulp with numerous seeds enclosed within a thick peel. The peel, which accounts for approximately 50% of the fruit's weight, contains valuable components, including pigments, dietary fiber, and antioxidants. However, it is currently regarded as a waste product. The objective of the present research was to evaluate the chemical composition and antioxidant capacity of the peel from "Redondilla" (*Opuntia streptacantha* cv. Dojä) and "Cardona" (*O. streptacantha* cv. Jocoquillo), which are abundant in the Altos Norte of Jalisco, Mexico. The fruit cultivars were collected and assessed for their physical characteristics, including size, weight, and peel content. The peels were then analyzed for their chemical composition (proximate composition, qualitative phytochemical profile, total phenolic and betalain contents), as well as their antioxidant properties (DPPH, iron-reducing power), using spectrophotometric techniques. The fruits of the "Cardona" cultivar were larger and heavier (83 g) than those of the "Redondilla" (40 g). In both cultivars, 54% of the fruit weight is derived from the peel. The moisture (88%) and protein (1.0%) content of the peel of both *Opuntia* cultivars was found to be similar. However, the total dietary fiber (41.4% DW) and ash (10.9% DW) contents were higher in "Redondilla". The phytochemical profile of by-products of both cultivars was found to be similar, with the presence of alkaloids, sterols, tannins, flavonoids, and saponins. The aqueous extracts of the peels were used for antioxidant properties determination. The pigments content, comprising betacyanins and betaxanthins (131.86 and 102.81 mg/100 g DW, respectively), and the iron-reducing power (1,116.67 mmol TE/kg DW) were found to be greater in "Cardona". In contrast, the total phenolic content (1,197.04 mg gallic acid eq./100 g DW) and DPPH antiradical capacity (39.19 mmol Trolox eq./kg DW) were higher in "Redondilla". The results demonstrate that peels from *Opuntia* cultivars native to Altos Norte of Jalisco, Mexico, represent a source of biologically active molecules, including dietary fiber, betalains, and phenolic compounds with high antioxidant value and potential for use as antioxidant fiber and for the extraction and application of natural pigments and antioxidants in pharmaceutical, food and manufacturing industries with benefits in terms of health and sustainability.

Keywords: *Cactus pear peal, betalains, polyphenols, phytochemicals*

Cite: Ortega-Cardona, C. E., Loza-Cornejo, S., Gómez-Velazquez, H. D. de J., Ochoa-Velasco, C. E. and Aparicio-Fernández, X. 2025. Chemical and antioxidant analysis of peels from two red-purple cactus pears (*Opuntia streptacantha*) from Altos Norte of Jalisco, Mexico. *Journal of the Professional Association for Cactus Development. 27*: 81-101. https://doi.org/10.56890/jpacd.v27i.568

Associate Editor: Pablo Misael Arce-Amezquita.

Technical Editor: Tomás Rivas-García.

Received date: 24 September 2024

Accepted date: 23 April 2025

Published date: 15 May 2025

Introduction

The genus *Opuntia*, which belongs to the Cactaceae family, comprises over 130 genera and 1500 species (Guzmán *et al*., 2003; Tahir *et al*., 2019). It is primarily cultivated in arid and semi-arid zones due to its distinctive water retention capacity and its ability to adapt to extreme climatic conditions (Bensadón *et al*., 2010; Reyes-Agüero and Rivera, 2011; Astello-García *et al*., 2015). *Opuntia* spp. is native to the American continent and has subsequently been dispersed worldwide due to human colonization. Mexico is considered the origin center of the *Opuntia* genus, with over 100 documented species, including both cultivated and wild varieties (Guzmán *et al*., 2003; Scheinvar *et al*., 2015). In the Altos Norte region of Jalisco state, western of Mexico, Aparicio-Fernández *et al*. (2017) reported the presence of 15 varieties of *Opuntia* species, including both wild and cultivated, specifically in the municipalities of Lagos de Moreno and Ojuelos. The authors described the wide variability of morphometric and chemical characteristics of fruits among the different species. The cultivated *O. streptacantha* cv. Jocoquillo, also known as "Cardona", showed larger fruits (in terms of size and weight) with a high pulp percentage and high total soluble solids content. That is why fruits from this species are widely commercialized in the region and used to prepare candies (such as “melcocha”, cactus pear cheese, and jelly) and fermented beverages (such as “colonche” and wine). In contrast, the fruits from wild species *O. streptacantha* cv. Dojä, also known as "Redondilla", does not meet the quality requirements for marketing due to its small size, low sweetness level, and intense red-purple color; therefore, its use is limited to local consumption. Regardless of their use, the fruits of both species have a thick peel that is not consumed, representing between 40% and 45% of their weight (Aparicio-Fernández *et al*., 2017).

The different species of the *Opuntia* genus have been extensively studied for the beneficial effects of their chemical components. The polysaccharides, organic acids, amino acids, vitamins, minerals, carotenoids, components of essential oils, polyphenolic compounds, phytosterols, and betalain-type pigments are the groups of compounds most studied and reported in the tissues of *Opuntia* species (Mazari *et al*., 2018; Amaya-Cruz *et al*., 2019; Madrigal-Santillan *et al*., 2022; Chahdoura *et al*., 2024). More than 40 phenolic compounds, including hydroxybenzoic acids, hydroxycinnamic acids, flavonols, flavonoids, and flavanones have been reported in the edible portions of *Opuntia* species (Zeghbib *et al*., 2022). According to the results of *in vitro*, *in vivo*, and clinical trials, the dietary fiber, polyphenolic compounds, and betalains are some of the phytochemicals that have been attributed to the biological effects of *Opuntia* species, such as chemo preventive, antigenotoxic, antiatherogenic, hypolipidemic, hypocholesterolemic, hepatoprotective; as well as the beneficial effects on type 2 diabetes and obesity (Abdel-Hameed *et al*., 2014; Daniloski *et al*., 2022; Madrigal-Santillan *et al*., 2022). Specifically, cactus pears are considered a potential nutraceutical agent against metabolic syndromes, primarily due to their high content of fiber, vitamins, protective peptides; and antioxidants with lipid-lowering, antidiabetic and antiatherogenic effects. Other components with antioxidant properties, such as ascorbic acid, phenolic compounds, and betalain pigments, have also been associated with their biological effects (Jiménez-Aguilar *et al*., 2015; Daniloski *et al*., 2022).

The presence of all these chemical components, and their biological effects, has been evidenced not only in the edible portions of the plant but also in by-products such as seed and fruit peels (Jiménez-Aguilar *et al*., 2015; Amaya-Cruz *et al*., 2019; García-Cayuela *et al*., 2019; Manzur-Valdespino *et al*., 2022). Issami *et al*. (2024) performed the phytochemical characterization of the ethanolic extract of the pulp, peel, and seeds of Tunisian barbary fig (*Opuntia ficus-indica*) of three different colors (purple, orange and green). Using reverse-phase HPLC, the authors identified 36 polyphenolic compounds, including flavonoids, phenolic acids, one stilbene (resveratrol), and three simple phenolics. Interestingly, only 7 of these compounds were detected in the three parts of the analyzed fruit varieties; and the rest, including all the identified flavonoids, were only present in the by-products. In addition to polyphenols, other phytochemicals such as polysaccharides and betalains have been identified in

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cactus pear by-products, and their use has been proposed for food additives, food supplements, and wastewater treatment (Amaya-Cruz *et al*., 2019; Manzur-Valdespino *et al*., 2022).

The chemical composition of *Opuntia* varies considerably between species, cultivars, the type of tissue analyzed, and due to the environmental conditions during their cultivation (Jiménez-Aguilar *et al*., 2015; Zeghbib *et al*., 2022; Giraldo-Silva *et al*., 2023). The annual production of prickly pear in Mexico exceeds 400 thousand tons (Servicio de Información y Estadística Agroalimentaria y Pesquera, 2023); and peels represent between 33 to 55% of the total weight, which reaches this by-product an alternative source of valuable phytochemicals that must be exploited (Bensadón *et al*., 2010; Chougui *et al*., 2015; Melgar *et al*., 2017; Aparicio-Fernández *et al*., 2018; Amaya-Cruz *et al*., 2019). *Opuntia ficus-indica* (OFI) is the most widely distributed species in Mexico and globally (Madrigal-Santillán *et al*., 2022; Giraldo-Silva *et al*., 2023); therefore, it is also the species that has been most studied. Given the variety of *Opuntia* cultivars found in Jalisco (Aparicio-Fernández *et al*., 2017), some of which are underutilized, and the knowledge of the presence of valuable phytochemicals in the peels; the study of phytochemicals present in peels of native and under-exploited species is relevant for their use. Therefore, the present study aimed to evaluate the proximate chemical composition, qualitative phytochemical profile, betalains, and total phenolic compounds content; as well as the antioxidant capacities of the peel of two red-purple cactus pears (*Opuntia streptacantha*) from Altos Norte of Jalisco, Mexico; for their revalorization as a promising source of phytochemicals for their use in pharmaceutical and food industries.

Material and Methods

Plant material

Ripe and healthy fruits from *O. streptacantha* cv. Jocoquillo, "Cardona" (Figure 1), and *O. streptacantha* cv. Dojä, "Redondilla" (Figure 2) were collected in Ojuelos and Lagos de Moreno, Jalisco (Table 1), respectively. The sampling period was established between August and September 2018. The fruits were transported to the laboratory, where they were thoroughly cleaned from glochids, rinsed, and disinfected (NaClO, 100 ppm, 5 min). The physical parameters were determined on whole fruits. Then, the cactus pear peels (CPP) were manually separated from the pulp, cut into 1 cm^2 pieces, and subjected to a blanching process (boiling water, 3 min). The drying process was carried out in an oven with natural convection (BINDER® GmbH, ED, Germany) at 60 °C until constant weight. The dried peels were subjected to a grinding process using an electric food grinder (Moulinex®, DPA 141, France) and sieved (Retsch® GmbH, AS 200 DIGIT, Germany) to obtain 63 µm particles. The obtained powders were stored in glass containers, protected from light, and refrigerated until analysis within the same week.

Reagents

The chemicals used were of analytical grade and were obtained from Sigma-Aldrich®. These included: ethyl alcohol (C_2H_6O), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), Folin-Ciocalteu reagent, gallic acid, sodium carbonate (Na_2CO_3), potassium hexacyanoferrate ($K_3Fe(CN)_6$), trichloroacetic acid ($C_2HO_2Cl_3$), ferric chloride ($FeCl_3$).

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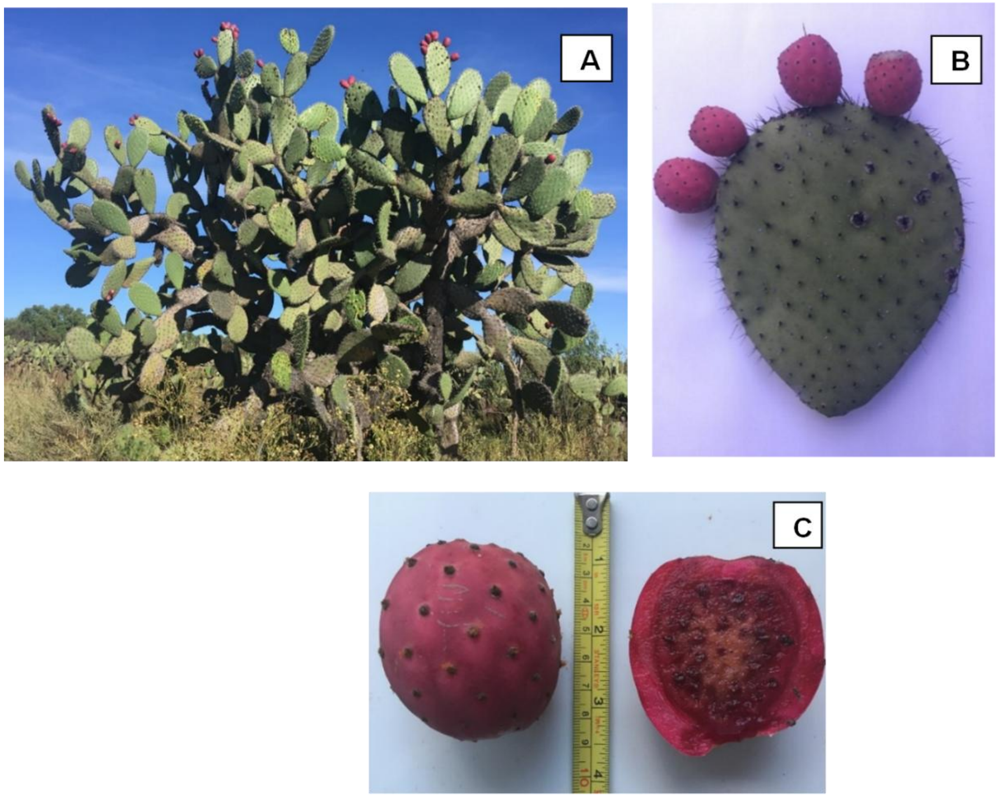

Figure 1. *Opuntia streptacantha* cv. Jocoquillo, "Cardona", plant (A), cladode with fruits (B), whole fruit and longitudinal cross-section (C).

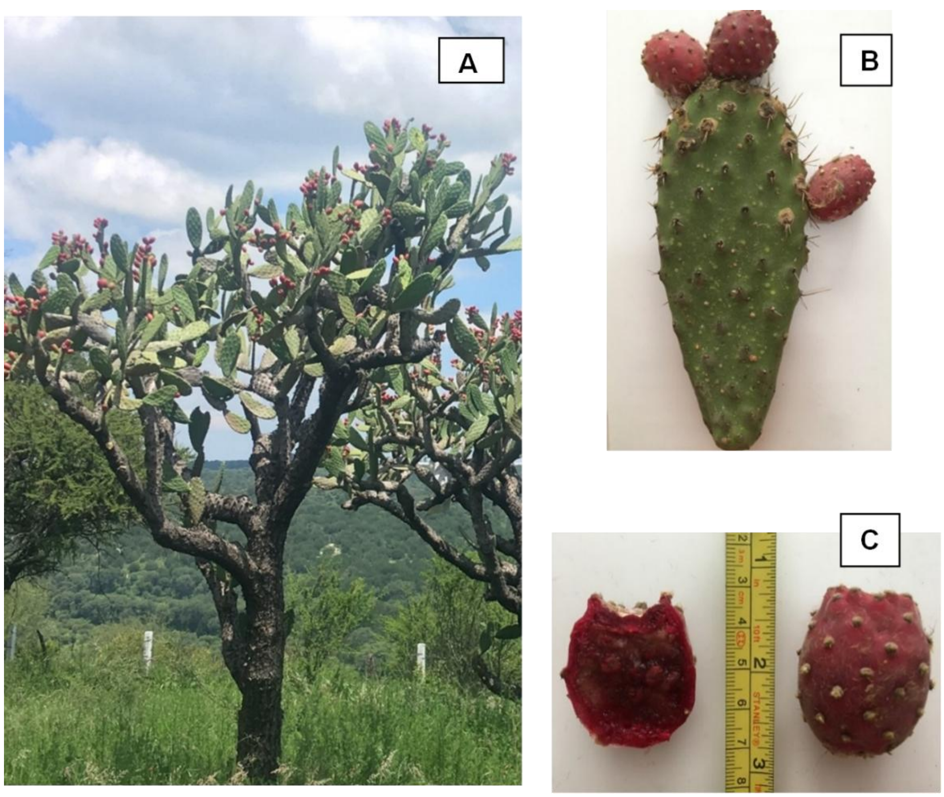

Figure 2. *Opuntia streptacantha* cv. Dojä, "Redondilla", plant (A), cladode with fruits (B), whole and longitudinal cross-section of fruit (C).

Table 1. Location and climate conditions of sampling areas.

	Ojuelos de Jalisco	**Lagos de Moreno**
Geographic location	21°33'N - 103°09'W	19°50'N - 103°59'W
Altitude (m.a.s.l.)	2219	1877
Climate	Semi-arid temperate with warm summer (BS_1kw)	Semi-arid temperate with warm summer (BS_1kw)
Average annual temperature (°C)	19	19
Average annual precipitation (mm)	594	600
Average evaporation (mm.)	500	1900

Source: Comisión Estatal del Agua Jalisco, n.d.; Ruiz-Corral *et al*., 2021

Physical parameters

Fifteen fruit units per cultivar randomly selected, were used to determine the physical parameters of the entire fruit and its peel. The determinations included the weight, length, and diameter of the whole fruit, as well as the weight and thickness of the CPPs and the weight of the pulp. The calibrated instruments were employed, a digital micrometer (Mitutoyo®, 500-473 CAL, Mexico) and a laboratory precision scale (Precisa Gravimetrics®, AG, T220A, Switzerland).

Chemical parameters

Proximate composition

The CPPs were analyzed for proximate chemical composition. The contents of moisture, ash, lipid, protein, and total dietary fiber (soluble and insoluble fiber) were determined using the AOAC (2000) official methods (923.03; 920.39C; 960.52; and 991.43). The total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber. The nitrogen-free extract was calculated as the difference between 100% of the sample and the sum of the determined parameters. This provides an approximation of the digestible carbohydrate content. The analyses were performed in triplicate, and the results were expressed as g/100 g of dry matter (DW).

Qualitative phytochemical profile

The presence of secondary metabolites, alkaloids, sterols, tannins, flavonoids, quinones, and saponins, in the CPP of both cultivars was determined through qualitative chemical reactions (Domínguez, 1973). In order to carry out an exhaustive extraction of the phytochemicals present in the by-products under study, sequential extractions were carried out under reflux (30 min; boiling point), with solvents of increasing polarity (*n*−hexane, ethyl acetate, 80% ethanol, water) (Sánchez-Herrera *et al*., 2011). The extracts were stored in darkness at 4 °C until further analysis. Dragendorff's, Mayer's, Warner's, and Hager's reagents were used for alkaloid presence visualization. The turbidity or precipitation in three of the four reagents evidenced the presence of alkaloids. Libermann−Burchard's and Salkowski's reagents were used to determine the presence of sterols (triterpenes). For tannins presumptive detection, $FeCl_3$ solution was used, and their presence was confirmed by the formation of precipitate in 1% gelatin solution and 1% gelatin solution in 1% NaCl but not in 1% NaCl solution. The flavonoid's presence was demonstrated using Shinoda's test. The quinone presence was detected using toluene and alkalis after the extracts were treated with H_2O_2 and H_2SO_4. The saponins were

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detected by the foam test and Rosenthaler's reaction. All the techniques employed are in accordance with the description by Sánchez-Herrera *et al*. (2011).

Aqueous peel extract preparation

The quantification of betalains and total phenolics, as well as the antioxidant capacity assays was determined using a 2% aqueous extract from each CPP cultivar, based on the abundance of polyphenolics observed in the phytochemical profile and on some preliminary experiments in the laboratory. Briefly, 2 g of dry peel powder was mixed with 100 mL of distilled water and magnetically stirred (Thermo Scientific®, Cimarec, USA) at 700 rpm. The extraction process was conducted for two hours at room temperature (25 °C), with the samples protected from light exposure. The extracts were vacuum filtered, and their volume was determined for calculation purposes. Quantifications were performed immediately after extraction by spectrophotometric methods in a calibrated instrument (Jenway Instruments®, 6305 Spectrophotometer, United Kingdom).

Betalain contents

The betacyanin (Bc) and betaxanthin (Bx) concentrations were determined at 535 and 483 nm, respectively, using aqueous extracts diluted in Mc-Ilvaine buffer (Stintzing *et al*., 2005). For quantification, the molecular weights (MW) and molar extinction coefficients (Ɛ) of betanin (MW = 550 g/mol; Ɛ = 60,000 L mol^{-1} cm; λ = 538 nm) and indicaxanthin (MW=308 g/mol; Ɛ = 48,000 L mol^{-1} cm; λ = 480 nm) were used.

Total phenolic contents

Total phenolic contents (TPC) were quantified as described by Hernández-Carranza *et al*. (2016) with modifications. Aliquots (one mL) of aqueous extracts were mixed with one mL of 0.1 M Folin-Ciocalteau solution and allowed to react for 3 minutes. Then, one mL of 0.5% sodium carbonate was added and mixed. After 30 min of reacting at room temperature (25 °C), the absorbance was measured at 765 nm. TPC were calculated using a standard curve of gallic acid (0-70 ppm) and expressed as mg of gallic acid equivalent (GAE) /100 g DW.

Antioxidant capacities

The antioxidant capacities were analyzed by the radical scavenging capacity (DPPH) and the iron-reducing power assays.

Radical scavenging activity (DPPH assay)

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging capacity was determined as described by Hernández-Carranza *et al*. (2016) with modifications. The aliquots of aqueous extracts were diluted in methanol (25 – 300 µL mL^{-1}) and mixed with the same volume of DPPH solution (40 ppm). The reaction was carried out at room temperature for 30 min, protected from light; and finally, the absorbance was read at 517 nm.

The DPPH radical inhibition percentage was calculated as follows [1]:

$$\text{Radical scavenging (\%)} = [1- (A_1 / A_0)] \times 100 \quad [1]$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample. The radical scavenging capacity was evaluated with a Trolox regression line (0-20 ppm of Trolox); results were expressed as mmol of Trolox equivalents (TE) kg^{-1} DW.

Iron-reducing power

The iron-reducing power of CPPs was evaluated as described by Oyaizu (1986) with modifications. One milliliter-aliquots of properly diluted peel's aqueous extracts (25-300 µL mL^{-1}) were mixed with 2.5 mL of a sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The samples were incubated at 50°C for 20 minutes. After that, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with the same volume of distilled water, and 0.5 mL of ferric chloride solution (1%) was added. The absorbance of the resulting solutions was measured at 700 nm. The iron-reducing power was evaluated with a Trolox regression line (0-700 ppm of Trolox); results were expressed as mmol TE kg^{-1} DW.

Statistical analysis

All analytical measurements were performed in triplicate. The results were expressed as the mean ± standard deviation. The numerical data were subjected to one-way ANOVA, and comparative mean significant differences were analyzed by Tukey's test ($p < 0.05$) using Minitab Software (Minitab Inc., Pennsylvania, USA). A Pearson correlation analysis was carried out between antioxidant parameters and was conducted using SAS software v. 9.4 (SAS, 2002).

Results and Discussion

Physical parameters

The fruit sizes of both cultivars, *O. streptacantha* cv. Jocoquillo, "Cardona", and cv. Dojä, "Redondilla" were similar (Table 2); fruits' length and diameter ranged from 3.95 to 4.75 cm, and from 3.70 to 4.36 cm, respectively. However, significant differences in weight were observed. The "Cardona" fruits showed greater size and weight, and their peels were significantly thicker and heavier than those from the "Redondilla"; these differences are attributable to the morphometric characteristics of each cultivar, as described previously (Aparicio-Fernández *et al*., 2017). The fruits studied in the present study are smaller and lighter than those previously analyzed by the research team for the same cactus pear cultivars collected in 2010 in the same geographic regions (Aparicio-Fernández *et al*., 2017). The fifferences in fruit characteristics from one season to another are caused by variations in environmental conditions that occur during their development and harvest, even in highly domesticated varieties such as *Opuntia ficus-indica* (OFI). For example, Jiménez-Aguilar *et al.* (2015) reported weight differences between 198.3 g and 54.5 g in fruits of the "Verde Villanueva" (OFI) variety collected in Mexico, in 2010 and 2011, respectively. In both cultivars, peel accounted for 54% of the total fruit weight, which is consistent with the information reported by Jiménez-Aguilar *et al*. (2015), who found that OFI fruits have a peel composition ranging from 24 to 58%.

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Table 2. Physical parameters of fruits from two *Opuntia streptacantha* cultivars.

	Opuntia streptacantha	
Parameter	**“Cardona”**	**“Redondilla”**
Length (cm)	4.75 ± 0.47^a	3.95 ± 0.49^a
Equatorial diameter (cm)	4.36 ± 0.31^a	3.70 ± 0.34^a
Fruit weight (g)	82.96 ± 12.37^a	40.05 ± 7.71^b
Peel thickness (cm)	1.00 ± 0.0^a	0.50 ± 0.0^b
Peel weight (g)	44.95 ± 6.48^a	21.69 ± 4.98^b
Peel percentage (%)	54.18 ± 7.81^a	54.16 ± 12.43^a
Pulp percentage (%)	45.96 ± 10.64^a	37.80 ± 8.86^a

Data represents the mean ± standard deviation (n=15); statistical significance ($p<0.05$) represented by different superscripts.

Chemical parameters

Proximate composition

The moisture content in the CPP of both cultivars was very similar (88.08 ± 0.45% and 88.05 ± 0.70 %, for “Cardona” and “Redondilla”, respectively). The results of the proximate chemical composition of CPPs from both cultivars showed no significant difference in lipids and protein contents (Figure 3A). The peel of the “Redondilla” cultivar had significantly higher ash and total dietary fiber contents as well as lower nitrogen-free extract (NFE) content (Figure 3B). The total dietary fiber (TDF) content determined in "Redondilla" (41.4%) was 1.8 times greater than in "Cardona" (22.4%). Proportions of insoluble (IF) and soluble fiber (SF) also varied, containing 52.2 and 37.4% of IF and 47.8 and 62.6% of SF for "Redondilla" and "Cardona," respectively.

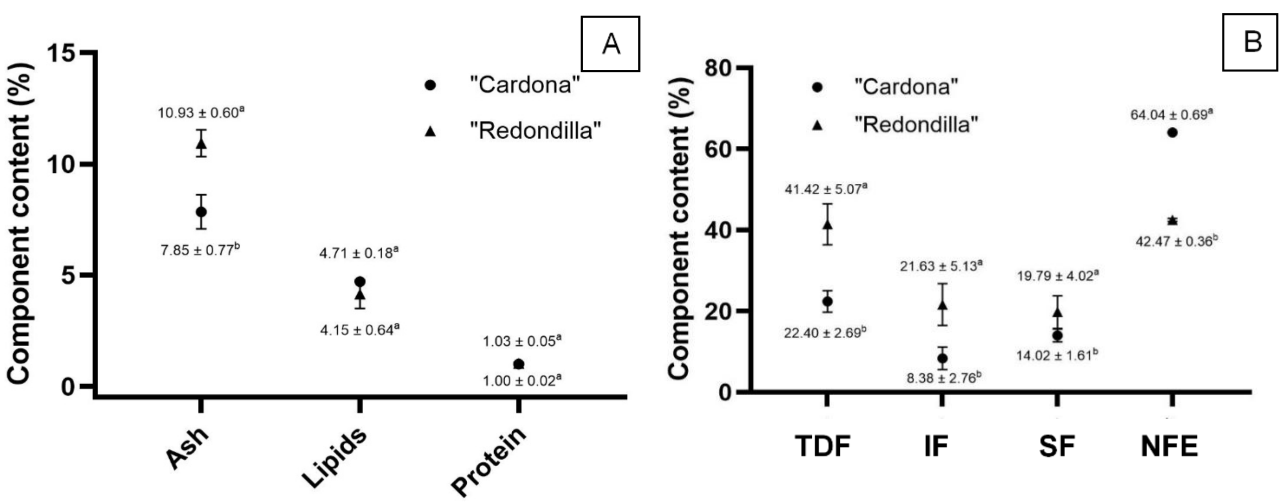

Figure 3. The proximate composition of the dry fruit peels from two *Opuntia streptacantha* cultivars. Figure “A” shows ash, lipids, and protein contents. Carbohydrate-type components, total dietary fiber (TDF), insoluble fiber (IF), soluble fiber (SF), and nitrogen-free extract (NFE) are represented in Figure “B”. Data are expressed as the mean ± standard deviation. Statistical significance ($p<0.05$) represented by different superscripts.

In agreement with the present results, other studies reported that the moisture content in *Opuntia* fruit peels ranged from 83.8 to 90.9% (Jiménez-Aguilar *et al.*, 2015; Aparicio-Fernández *et al.*, 2017), while ash contents have been reported in the range from 8.0 to 20.56% (Chahdoura *et al*., 2024; Issami *et*

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al., 2024). It has been interesting to note that the protein content quantified in both *O. streptacantha* fruit peels (around 1%) were lower than those quantified in OFI fruit peels of different colors, in the range of 4.5 to 8.91% (Hernández-Carranza *et al*., 2019; Chahdoura *et al*., 2024; Issami *et al*., 2024). Similarly, the TDF results quantified here were lower than those reported by Jiménez-Aguilar *et al.* (2015), from 43.2% to 58.1%, and Hernández-Carranza *et al*. (2019), who found 74.5% of TDF in the dry peel from OFI fruits. The NFE content was 1.5 times higher in “Cardona's” peel; the value approximates the total available carbohydrates and includes chemical species not quantified in the other parameters.

Carbohydrates, including NFE and TDF, are the major components in the dry matter of the analyzed CPPs. Cellulose, hemicellulose, pectin, lignin, mucilage, and other gums are indigestible constituents of DF; while different monomer sugars have been identified in OFI CPP polysaccharides, including glucose, galactose, xylose, arabinose, mannose (Albergamo *et al*., 2022; Madrigal-Santillán *et al*., 2022), stachyose, and sucrose (Murad *et al*., 2023).

Both polysaccharides and their constituent sugars have been shown to have positive health effects. The mechanisms of action are different; first, their abundant water binding capacity, which increases stomach volume and generates satiety; the dilution effect of nutrients in the intestinal lumen, which reduces the speed of digestion and absorption of sugars and lipids; and the demonstrated antioxidant and anti-inflammatory effects of the monomer sugars (Madrigal-Santillán *et al*., 2022). Through these mechanisms, DF consumption modulates food ingestion and its metabolism. This, in turn, reduces the risk of metabolic diseases such as hyperlipidemia, hypercholesterolemia, and hyperglycemia; even its consumption is related to weight regulation and risk reduction for type 2 diabetes, cardiovascular disease, and colon cancer, among others (Ioniță-Mîndrican *et al*., 2022; Madrigal-Santillan *et al*., 2022; Xu *et al*., 2022). Therefore, the high TDF content in CPPs of both *O. streptacantha* cultivars found in this study suggests that these by-products have the potential to be utilized, either as a fiber supplement or in the food industry to enrich low-fiber products, such as jam, meat products, and bakery products.

Qualitative Phytochemical profile

The qualitative phytochemical profiles of the studied CPPs were very similar (Table 3). The polar extracts, obtained using ethanol and water, exhibited a higher abundance and variety of phytochemicals: sterols, tannins, and flavonoids in the ethanolic extract, and alkaloids, tannins, flavonoids, and saponins in the aqueous extract. Sterols, tannins, and flavonoids were detected in greater abundance, while saponins and alkaloids were detected in lower amounts. Conversely, a low abundance of tannins was present in both extracts with lower polarity. Hexane and EA extracts showed a low abundance of saponins and sterols, respectively. Quinones were not detected in the extracts from both peel cultivars, which does not necessarily imply the absence of this class of compounds in CPPs, but that they were not extracted under the applied conditions.

The phytochemicals are secondary metabolites synthesized by plants to accomplish specific functions related to the protection and survival of species. The class of compounds and quantities produced depend on genetic factors in conjunction with the environmental conditions during cultivation; as well as the maturity stage at harvest (Wink, 2011).

Table 3. Phytochemical profile of different polarity extracts from peels of two *Opuntia streptacantha* cultivars.

Phytochemical group	*Opuntia streptacantha*							
	"Cardona"				**"Redondilla"**			
	H	EA	E	W	H	EA	E	W
Alkaloids	-	-	-	+	-	-	-	+
Sterols	-	+	+++	-	-	-	+++	-
Tannins	+	+	+	++	+	+	+	+
Flavonoids	-	-	++	+++	-	-	+++	+++
Quinones	-	-	-	-	-	-	-	-
Saponins	+	-	-	++	+	-	-	+

Positive test (+): + low-, ++ medium-, +++ high-abundance of compounds. Negative test (-): absence of compound. H: Hexane, EA: Ethyl acetate, E: 80% Ethanol, W: Water

On the other hand, the detection of these chemical compounds is related to the techniques used and the solvents employed for their extraction. To determine the phytochemical profile of the studied CPPs, successive extractions were carried out using solvents of increasing polarity, hexane (nonpolar), ethyl acetate (moderately polar), 80% ethanol (polar), and water (highly polar). The qualitative tests were used to detect the phytochemical groups present. Thus, the greater variety of compounds detected in ethanolic, and aqueous extracts relates to the abundance of phytochemicals with polar characteristics in the peels of both cultivars. The hydroxyl groups attached to aromatic structures, as well as their union to sugar moieties, give polyphenols their polar characteristics. The flavonoids, quercetin, luteolin, kaempferol, myricetin, isorhamnetin, hesperidin, and naringenin, and some of their glycosides have been identified in peels and pulp of cactus pears (Amaya-Cruz *et al*., 2019; Albergamo *et al*., 2022; Madrigal-Santillán *et al*., 2022). The tannins, polyphenolic compounds of high molecular weight, have also been detected and quantified in OFI and *O. streptacantha* CPPs (Amaya-Cruz *et al*., 2019; Zourgui *et al*., 2020). Regarding the presence of sterols, practically non-polar compounds, in the polar ethanolic extracts, this may be attributed to the extraction of glycosylated forms, as well as the forms esterified to phenolic acids, which increases their polarity. The high temperature applied during the reflux also favors the extraction of these compounds. Small amounts of β-sitosterol, lanosterol, fucosterol, and campesterol, have been detected in CPPs (Amaya-Cruz *et al*., 2019; Albergamo *et al*., 2022; Madrigal-Santillán *et al*., 2022).

Small amounts of saponins were detected in the hexane and water extracts, those with the lowest and the highest polarities, respectively. The explanation for this could be related to the class of saponins extracted in each case, as these compounds are classified into two groups, the non-polar triterpenoids (extracted in hexane) and the sterol glycosides with higher polarity (extracted in water) (Wink, 2011). The low amounts of alkaloids detected in the aqueous extracts may be related to the abundance of betalains in the vegetable material, which are water-soluble pigments structurally related to alkaloids (chromo alkaloids) and may produce false positives in qualitative tests (Wink, 2011). The contents of betalains will be described in the next section.

A wide range of beneficial health effects from consuming *Opuntia* species have been studied and reported. The mechanisms of action are attributed to the wide variety of phytochemicals with antioxidant and anti-inflammatory effects, which are capable of activating detoxification pathways and maintaining DNA stability (Madrigal-Santillán *et al*., 2022). Hence, the present results are considered valuable since they demonstrate the richness of phytochemicals present in the by-products of *O. streptacantha* fruits, providing an opportunity for further studies to analyze their biological potential and thereby giving these agricultural wastes applied value as a source of phytochemicals for use in healthcare.

Betalain contents

The betalains are water-soluble pigments synthesized in plants of the order Caryophyllales; they are classified into two main structural groups, the red violet betacyanins and the yellow betaxanthins (Wink, 2011; Sadowska-Bartosz and Bartosz, 2021). The contents of betacyanins quantified (Figure 4A) in the peels of "Cardona" and "Redondilla" (131.86 and 94.22 mg/100g of dry peel (DW), respectively), are among the highest reported in research carried out on CPPs of red OFI cultivars, grown in Mexico (Jiménez-Aguilar *et al*., 2015; Aparicio-Fernández *et al*., 2018; García-Cayuela *et al*., 2019; Hernandez-Carranza *et al*., 2019). Other countries, such as Tunisia (Giraldo-Silva *et al*., 2023), and Portugal (Graça Miguel *et al*., 2018) reported a range from 16.3 to 134.7 mg/100g DW. Betaxanthin contents in "Cardona" and "Redondilla" CPPs (102.81 and 64.46 mg/100g DW, respectively) were also in the range reported for OFI cultivars, from 12.4 to 191 mg/100 g DW, in the reports. In both cultivars, betacyanins contributed more than 50% of total betalains, which agrees with the red-purple hue displayed by the fruits. The total pigment content in “Cardona’s” peel was up to 48% higher than “Redondilla’s”. The variation in the betalain content among different species and cultivars of *Opuntia* may be attributed to genetic diversity and different environmental conditions (Jiménez-Aguilar *et al*., 2015).

In previous studies, the pigment contents in the pulp of "Cardona", "Redondilla", and other *Opuntia* fruit cultivars collected at Altos de Jalisco, México, have been communicated (Aparicio-Fernández *et al*., 2017); but, to our knowledge, this is the first time that the quantification of betalains in the peel of "Cardona" and "Redondilla" fruits is reported. Hence, this study offers novel information on the relatively high content of betacyanins and betaxanthins present in the studied by-products. The betalains are generally recognized as safe, they are relatively easy to obtain, their color is stable at neutral pH values and they pose antioxidant effects (Chougui *et al*., 2015; Sadowska-Bartosz and Bartosz, 2021); thus, the present results open an opportunity to explore the use of the CPPs as source of betalain pigments for their use as food additives (pigments and antioxidants), as well as, to further study their possible biological effects.

Total phenolics content

The phenolic compounds were abundant in the analyzed by-products (Figure 4B); indeed, CPPs have been reported to contain higher amounts of phenolics compared to the pulp and seeds (Jimenez-Aguilar *et al*., 2015; Issami *et al*., 2024). The total phenolics content (TPC) in peels from “Redondilla” (1,197.04 mg GAE/100 g) was 19.4% higher than in “Cardona’s” (1,002.82 mg GAE/100 g). Both values are among the highest in the wide range that has been reported in the TPC quantification in peels of red and purple OFI varieties harvested in Mexico and other countries, which range from 306 to 1,430 mg GAE/100 g of dried peel (Chougui *et al.,* 2015; Jiménez-Aguilar *et al*., 2015; Aparicio-Fernández *et al.*, 2018; Amaya-Cruz *et al*., 2019; García-Cayuela *et al*., 2019; Hernandez-Carranza *et al*., 2019).

https://www.jpacd.org Electronic ISSN: 1938-6648

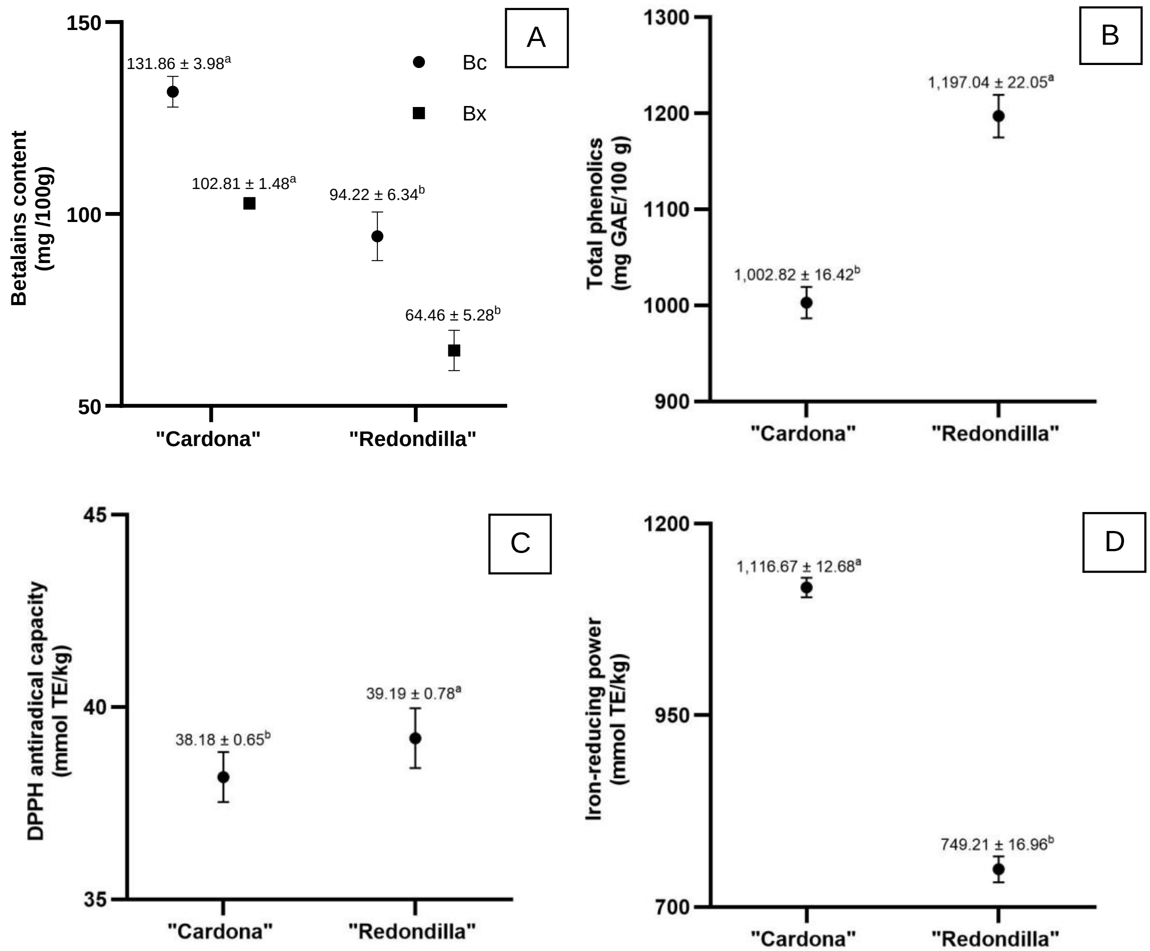

Figure 4. Betalains (A), total phenolic compounds (B), and antioxidant capacities assessed by DPPH (C) and iron-reducing power (D) of dry peel from two *Opuntia streptacantha* cultivars. Statistical significance ($p < 0.05$) represented by different superscripts. GAE, gallic acid equivalents; TE, Trolox equivalents.

Some of the polyphenolics in cactus pear peels have been characterized; in the comprehensive phytochemical profile carried out by Amaya-Cruz *et al*. (2019) using UPLC-ESI-QTOF MS^E, they discovered the presence of sixty-eight extractable phenolic compounds in peels of OFI varieties, including hydroxybenzoic and hydroxycinnamic acids, and different classes of flavonoids such as flavanols, flavonols and flavanones. In addition, for the first time, fifteen polyphenols linked to fiber (hydrolysable polyphenols) were identified, which gives fiber an antioxidant effect. According to the authors, cactus pear peels can be considered a good source of antioxidant fiber, rich in non-extractable polyphenols, and biologically active throughout the digestive system. The phenolic compounds are recognized for their biological activities, including antioxidant, anti-inflammatory, antimutagenic, antidiabetic, and antimicrobial effects, which translate into health benefits for consumers (Madrigal-Santillán *et al*., 2022; Chahdoura *et al*., 2024). The dietary polyphenols are poorly absorbed; however, the observed health benefits derive from their interaction with gut microbiota. It has been described that polyphenols selectively enrich the proportion of beneficial microorganisms and modulate the synthesis of bioactive metabolites such as short-chain fatty acids and dopamine. Furthermore, the microbiota metabolizes polyphenols into simpler, more bioactive, and absorbable molecules (Cheng *et al*., 2023). Therefore, the abundance of polyphenols makes "Cardona's" and "Redondilla's" by-products valuable sources of those compounds for their extraction and/or use as antioxidant fiber.

Antioxidant capacities

The scientific evidence attributes an antioxidant effect to phenolic compounds (Prior *et al*., 2005; Madrigal-Santillán *et al*., 2022; Rana *et al*., 2022) and betalains (Belhadj Slimen *et al*., 2017; Sadowska-Bartosz and Bartosz, 2021) from different plant sources. The DPPH and iron-reducing power assays, two methods based on the electron transfer (ET) mechanism, were employed to assess the antioxidant capacities in CPP samples. The antiradical capacity of "Redondilla", by the DPPH method, was found to be slight but significantly higher than that of "Cardona" (39.19 and 38.18 mmol Trolox equivalent (TE)/kg, respectively) (Figure 4C). The effective concentration (EC_{50}) of "Redondilla's" peel (1.21 g L^{-1}) is lower than "Cardona's" (EC_{50}=1.28 g L^{-1}), which confirms its greater capacity to stabilize 50% of the DPPH radicals present in the reaction tube. The results reported for the antiradical capacity by the DPPH method in CPPs of different colors present significant variations. For example, Zourgui *et al*. (2020) reported an EC_{50} value of 0.61 g L^{-1}, for the aqueous extract of *O. streptacantha* skin from Tunisia; while in the peel of an OFI variety from Algeria, Chougui *et al*. (2015) found an EC_{50} value of 77.81 g L^{-1}; and in the extracts, obtained with different solvents, of freeze-dried CPPs from South Africa, Aruwa *et al*. (2019) reported comparatively higher EC_{50} values, in the range of 16.89 to 145.14 g L^{-1}.

On the other hand, the iron-reducing power (IRP) results showed an opposite trend to those of DPPH antioxidant capacity; "Redondilla's" peel had significantly lower IRP (749.21 mmol TE kg^{-1}) than "Cardona's" (1,116.67 mmol TE kg^{-1}) (Figure 4D); which is confirmed by the value of the EC_{50}, at which the absorbance of the reduced iron equals 0.50, of "Cardona" (2.05 g L^{-1}), lower than that of "Redondilla" (2.40 g L^{-1}). The iron-reducing power results are in the range reported by Gouws *et al*. (2019) in the methanolic extract from the peel of purple-colored cactus pear dried by different methods (658-1,377 µM TE); but they are higher than data reported by Bensadón *et al*. (2010) in the whole OFI fruit of green (40.39 mmol TE kg^{-1} dw) and red (47.35 mmol TE kg^{-1} dw) varieties; as well as, those reported by Aruwa *et al*. (2019) in the range of 40 to 192 mmol TE kg^{-1} dw, in extracts, obtained with different solvents, of freeze-dried cactus pear peel from South Africa. The EC_{50} values reported here are higher than those reported by Chougui *et al.* (2015) of 1.03 g L^{-1} in the peel extract from an orange-colored OFI variety from Algeria.

The studies on peels from *Opuntia* fruits have been increased, and a lot of information about their antioxidant capacities is available. However, the results are not always concordant. The differences among the results of diverse studies lie in the diversity of existing techniques for antioxidant capacity evaluation based on the same chemical principle, as well as in the extraction method and differences in the form to express the results (µM TE, µmol TE g^{-1} or kg, EC_{50}, dry weight, fresh weight, peel, extract, among others). In addition to the plethora of *Opuntia* varieties analyzed, these factors yield different research results that are not numerically comparable.

An antioxidant is a substance with the capacity to inhibit, stop, or control the oxidation of a substrate. The main assays for measuring the antioxidant capacities are based on two principal chemical reaction fundaments: hydrogen atom transfer (HAT) and electron transfer (ET). In the HAT-based assays, the antioxidant donates a hydrogen atom to neutralize free radicals. In contrast, in ET-based assays, the antioxidant transfers an electron to reduce an oxidant, typically a metal ion or a radical cation, resulting in a color change or shift in absorbance (Christodoulou *et al*., 2022). The use of the DPPH and iron-reducing power methods for evaluating antioxidant capacity significantly contributes to understanding of the potential of peels from "Redondilla" and "Cardona" *Opuntia* cultivars as sources of phytochemicals to protect against free radicals and other oxidant species (Prior *et al*., 2005). However, it is important to note that the crude aqueous extracts of CPPs as analyzed. Therefore, the observed antioxidant effects are attributable to both the phenolic compounds and betalains, as well as to other phytochemicals that may be present in the extracts.

Correlation analysis of antioxidant parameters

The results of the relationship between betalains, TPC, and the antioxidant capacities of the CPPs from both *Opuntia* cultivars are shown in Table 4. The highest significant positive correlation was observed between TPC and DPPH antiradical capacity ($r = 0.97$; $p < 0.0001$), which is expected since both quantification methods are based on the ET mechanism for scavenging radicals. The Folin-Ciocalteu method, employed here for polyphenol quantification, is commonly used to determine the antioxidant capacity of organic samples (Prior *et al*., 2005), which is generally attributed to phenolic compounds. Conversely, although the contents of both betalain pigments have a significant correlation with each other ($r = 0.98$; $p < 0.0001$), they showed lower Pearson coefficient values versus antioxidant capacity measured by the DPPH method ($r < 0.14$). These compounds are best correlated ($r > 0.8$) with antioxidant capacity measured by the iron-reducing power technique. This last relationship could be attributed to their chemical structure since it has been described that betalamic acid (the central structure of both betalain pigments) can donate 2 electrons to oxidizing agents; therefore, it can reduce two Fe^{3+} to Fe^{2+} ions, which are detected in the IRP test (Belhadj Slimen *et al*., 2017). These results align with previous reports (Stintzing *et al*., 2005; Melgar *et al*., 2017; Aparicio-Fernández *et al*., 2018) on the presence of phenolics and betalains in the peel and pulp of various cactus pear varieties as well as their high correlation with antioxidant capacities.

Table 4. Pearson correlation between betalain pigments, total phenolic compounds, and antioxidant capacities of peel from two *Opuntia streptacantha* cultivars.

			Antioxidant capacities	
	Betacyanins	**Betaxanthins**	**DPPH**	**Reducing power**
Total phenolic compounds	0.33[b]	0.23	0.97[a]	0.57[a]
Betacyanins	-	0.98[a]	0.14	0.89[a]
Betaxanthins	-	-	0.03	0.81[a]
DPPH	-	-	-	0.39[b]

[a]$p < 0.0001$. [b]$p < 0.05$. (n = 60)

The effectivity of phenolic compounds and betalains as antioxidants is well known (Prior *et al*., 2005; Belhadj Slimen *et al*., 2017), The present results demonstrate the capacity of antioxidants (including phenolic compounds and betalains) present in CPPs of "Cardona" and "Redondilla", from western Mexico, to exert an effect through the ET mechanism. However, further studies are required to determine the antioxidant activity through the hydrogen atom transfer (HAT) mechanism, thereby getting a better understanding of the mechanisms of the phytochemicals present in CPPs, as well as a possible synergistic effect between them.

Taken together, the results show that although the fruits of both *O. streptacantha* cultivars analyzed did not show significant differences in their dimensions, their weights are significantly different. The peel accounts for 54% of the fruit weight in both cultivars, and their qualitative phytochemical profile is very similar. However, the results of chemical analyses show that “Redondilla” CPP has a significantly higher DTF content, with a soluble-to-insoluble fiber ratio slightly greater than 1. Furthermore, this higher fiber content is associated with a higher amount of extractable minerals (ash) and phenolic compounds, as well as a slightly but significantly higher antioxidant effect, measured by the DPPH free radical technique. The higher fiber and phenolic content in “Redondilla” could be attributed to its status as a wild variety, which makes it more susceptible to environmental stress and, therefore, requires more protection. These results suggest that “Redondilla” CPP could be a good source of antioxidant fiber. On the other hand, “Cardona” CPP is distinguished for its NFE content (digestible carbohydrates) and both types of pigments (betacyanins and betaxanthins), as well as for its significantly higher IRP; therefore, its use could focus on the extraction of pigments for application in the food and pharmaceutical industries, as coloring additives and natural antioxidants. Several studies have demonstrated the health benefits of consuming red prickly pears, as well as the nutraceutical potential of the fruit by-products due to the presence of fiber and antioxidant phytochemicals (Madrigal-Santillán *et al*., 2022). However, it is worth conducting a more in-depth and detailed study of the components to explore the utilization of “Cardona’s” and “Redondilla’s” by-products in the search for viable alternatives for health preservation. Therefore, it is necessary to thoroughly study the variations in secondary metabolites throughout different seasons for the use of these byproducts.

Conclusions

For the first time, this study contributes to the knowledge of the phytochemical profile and the antioxidant properties of underutilized by-products from fruits of *Opuntia streptacantha* cv. Dojä, "Redondilla", and *O. streptacantha* cv. Jocoquillo, “Cardona", from Altos Norte, Jalisco, Mexico. The peels from “Cardona” and “Redondilla” represent more than 50% of the fruit weight and contain valuable phytochemical components with antioxidant properties, including phenolic compounds and betalains, as well as dietary fiber. These components add value to the by-products, allowing them to be revalued as promising sources of phytochemicals for use in food and pharmaceutical industries. The results obtained in this study may contribute to promoting the consumption and commercialization of these *Opuntia* cultivars in the region, as well as to the utilization of their CPPs. Future research should focus on evaluating the stability and acceptance of CPPs for direct use as a source of antioxidant fiber in dietary supplements or enriched processed foods such as confectionery, baked goods, and meat products. The medium-scale extraction and stability of components, such as betalains, and their use as natural colorants and antioxidants in foods represent another opportunity to exploit these by-products.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

All data generated or analyzed during this study are included in this published article.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Not applicable.

AUTHOR CONTRIBUTIONS

Conceptualization, X.A.-F., S.L.-C. Methodology, X.A.-F., S.L.-C., C.E.O-C. Validation, X.A.-F., C.E.O-V. Formal analysis, C.E.O.-V., C.E.O.-C. Investigation, C.E.O.-C., H.D.J.G.-V. Resources, X.A.-F., S.L.-C. Visualization, X.A.-F., S.L.-C. Writing—original draft, X.A.-F., C.E.O.-C.; Review and editing of the final version of the manuscript before sending it, H.D.J.G.-V., C.E.O.-V., S.L.-C.

ACKNOWLEDGMENTS

The authors thank to SECIHTI for the graduate fellowship granted and CEMAI course to C.E.O.-C.; to Mr. Fernando Torres Romo and Mr. Daniel Villa Becerra for providing the plant material; and to Alma and Dario Gómez Jiménez for their collaboration in the development of the study. This research was supported by the University of Guadalajara.

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