Pigment composition and antioxidant capacity of betacyanins and betaxanthins fractions of *Opuntia dillenii* (Ker Gawl) Haw cactus fruit

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Abstract

A purification and fractionation of *Opuntia dillenii* (Ker-Gawl) Haw extracts followed by betalainic and phenolic characterization by HPLC-DAD-ESI-MS were carried out. Several betalains and polyphenols have been identified, the majority of them not previously described in *Opuntia dillenii*: 17-decarboxybetanin and 17-decarboxyisobetanin, 6′-O-sinapoyl-O-gomphrenin and 6′-O-sinapoyl-O-isogomphrenin, 2’-O-apiosyl-4-O-phyllocactin and 5’’-O-E-sinapoyl-2’-apiosyl-phyllocactin as betacyanins, tryptophan-betaxanthin and tyrosine-betaxanthin (portulacaxanthin II) as betaxanthins and isoramnethin-3-glucuronide and quercetin-3-*O*-glucoside as polyphenolics. Moreover, the antioxidant activity of extracts of *Opuntia dillenii* (Ker-Gawl) Haw fruit followed the ensuing decreasing order (crude extract (CE) > purified extract (PE) > red fraction (RF) > yellow fraction (YF)), being in concordance with the total reducing power (TRP). These evidences suggest that *Opuntia dillenii* is a rich source of bioactive compounds, with a high amount of total betacyanins (16.63 mg betanin/100 g fresh fruit) and betaxanthins (7.55 mg indicaxanthin/100 g fresh fruit).

Keywords: *Opuntia dillenii; betacyanins; betaxanthins; polyphenols; antioxidant activity; HPLC-DAD-ESI-MS.*

1. Introduction

Opuntia ss. is the most abundant genus in the Cactaceae family, growing in semi-desert climate with water-scarce conditions and an optimal low pluviometry for its growth (150-200 mm median annual precipitation) (Cejudo-Bastante, Hurtado, & Heredia, 2015). Their yellow flowers and red fruits are characteristic from *Opuntia* genus, together with thick, flat and stiff stems. Among this genus, *Opuntia ficus-indica* is the most widespread and investigated fruit (Cejudo-Bastante, Chaalal, Louaileche, Parrado, & Heredia, 2014; Stintzing, Schieber, & Carle, 2002), but there are further many species of *Opuntia* which deserve special attention for their potential beneficial effects. That is the case of *Opuntia dillenii* (Ker-Gawl) Haw.

The origin, distribution and taxonomy of *Opuntia dillenii* have been previously described by Böhm (2008) and Sharma (2015). It is considered native from India and America, but it could be also grown in the Mediterranean area, Asia or Australia. In Colombia, *Opuntia dillenii* is wildly found in the arid zones of Santander, Nariño and Guajira departments. Their fruits are characterized by the red colour and contain cladode spines, an average length and width around 4.0 and 3.2 cm, respectively, an acidic taste and a great number of seeds (Medina, Rodríguez, & Romero, 2007). This feedstock has been studied from different points of view: the analysis of polysaccharides (Li, Yuan, Zhou, Zeng, & Lu, 2016), the physicochemical characterization (total fibre, proteins, fat, minerals and ascorbic acid, among others) (Medina, Rodríguez, & Romero, 2007; Méndez, Flores, Martín, Rodríguez Rodríguez, & Díaz Romero, 2015), and the analysis in terms of health amelioration (such as the protective effect of the pulp, skin and seeds against the low-density lipoprotein peroxidation) (Chang, Hsieh, & Yen, 2008). Concretely, our research groups have contributed to increase the knowledge of *Opuntia dillenii* by deepening in the stability

of the colorimetric characteristics, betalains and antioxidant activity under different conditions of pH and temperature (Cejudo-Bastante, Hurtado, & Heredia, 2015; Montes-Lora, Hurtado, Mosquera, Heredia, & Cejudo-Bastante, 2016), being pioneers in the study of betalains in this raw material.

Betalains are water-soluble compounds present in a restricted number of families of the plant order of Caryophyllales and of the genus *Amanita* of the Basidiomycetes. Their basic structure consists of a moiety of betalamic acid, and, depending on the residue, betalains could be classified as red/purple betacyanins and yellow-orange betaxanthins (when *cyclo*-Dopa and hydroxycinnamic acid derivatives or sugars, and amines or amino acids residues are linked to betalamic acid, respectively) (Herbach, Stintzing, & Carle, 2006). Betacyanins do not only vary by the degree and type of glycosylation in C_5 and C_6 positions of *cyclo*-Dopa, but also by the acylation of sugars with organic acids such as ferulic, cinnamic or malonic acids (Herbach, Stintzing, & Carle, 2006). They are present in a large extent number of plants, vegetables, flowers and fruits such as *Opuntia.* Although individual betalains have been well-described in other species of *Opuntia*, to the best of our knowledge, any accurately characterization study on individual betalains have not been already reported in *Opuntia dillenii*.

In terms of biological activity, betalain-rich products have been described as important sources of free radical-scavenging activity. Although red beet and red pitahaya fruits have been assigned as two important sources of antioxidant activity (Sawicki, Bączek, & Wiczkowski, 2016; Vaillant, Perez, Davila, Dornier, & Reynes, 2005), other raw materials, such as ulluco tuber (Campos, Noratto, Chirinos, Arbizu, Roca, & Cisneros-Zevallos, 2006; Cejudo-Bastante, Hurtado, Mosquera, & Heredia, 2014) or *amaranthus* flowers (Li, Deng, Liu, Zhu, Draves, Marcone, et al., 2015), showed remarkable antioxidant ability related to betalains. With regard to *Opuntia* genus, several studies

have been conducted about determining the antioxidant capacity, such as Jiménez-Aguilar, López-Martínez, Hernández-Brenes, Gutiérrez-Uribe, & Welti-Chanes (2015) in Mexican commercial varieties of cactus pear, Gandía-Herrero, Escribano, & García-Carmona (2016), Osorio-Esquivel, Alicia Ortiz, Álvarez, Dorantes-Álvarez, & Giusti (2011) and Tamer E. Moussa-Ayoub, Jaeger, Knorr, El-Samahy, Rohn, & Kroh (2011) in *Opuntia ficus-indica*, *Opuntia joconostle* and *Opuntia macrorhiza* fruits, respectively. However, up to the present, scarce studies about the antioxidant capacity of *Opuntia dillenii* have been developed. Among them, Kumar, Ganesh, Peng, & Jang (2014) demonstrated that Indian *Opuntia dillenii* flowers showed effective antioxidant properties against DPPH free radicals. Another example is Montes-Lora, Hurtado, Mosquera, Heredia, & Cejudo-Bastante (2016), who studied the influence of technological treatments on the antioxidant activity of *Opuntia dillenii* extracts, and Ghazi, Ramdani, Tahri, Rmili, Elmsellem, El Mahi, et al. (2015) studying the antioxidant activity of seeds oils and fruit juice of *Opuntia dillenii* from Morocco. However, the antioxidant activity of *Opuntia dillenii* extracts after purification and fractionation has not been previously carried out.

Overall, this research study sought to develop a great chemical characterization of *Opuntia dillenii* extracts, in the light of any detailed study concerning individual betalains and phenolic compounds of this fruit have been performed up to now. To bring something to fruition, a purification and fractionation based on the colour of the extracts was carried out, followed by the identification of each compound by HPLC-DAD-ESI-MS. The study was complemented with the determination of the antioxidant capacity against $ABTS^{+}$ radical of each fraction, in order to value the under-utilized *Opuntia dillenii* as source of bioactive compounds.

2. Material and methods

2.1. Chemical and solvents

All solvents (Folin-Ciocalteau reagent and HPLC-grade methanol, acetonitrile and water) and standards (gallic acid and Trolox) were supplied by Sigma-Aldrich (St. Louis, MO, USA).

2.2. Vegetal material

Prickly pear samples (*Opuntia dillenii*) were collected in the village of Chachagüí (Nariño, Colombia), which is located at approximately 25 Km north of the city of San Juan de Pasto. It is situated at 1° 21′ N latitude and 77° 16′W longitude at an altitude of 1982 m above the mean sea level.

A specimen of the plant reposes in the herbarium of the Universidad de Nariño (code No 13691). A representative set of samples of up to a weight of about 2 kg was harvested, collecting mature fruits according to visual characteristics and similar size. A simple random sampling model of ten plants was underwent. After homogenization, only 1450 g of fruits was considered, with an average weight of each fruit around 11 g. They were carefully washed and dried, and prickles were manually removed. Fruits were kept under refrigeration at 4 ºC until their analysis.

2.3. Preparation of extracts

The crude extract (CE) was obtained according to the method described by Fernández-López, Giménez, Angosto, & Moreno (2012). Fruits were cut into small pieces (1 cm^2) and extracted with 1.5 L of methanol:water (60:40) for 24 h at 10 ºC (chemical maceration). The procedure was developed several times until the complete discoloration of the plant material. After vacuum filtration (Whatman filter No 1), the organic solvent was evaporated at 35 ºC using a rotary evaporator (Heidolph, Schwabach, Germany) and the re-dissolved extract with distilled water (relation 1

g/mL) was lyophilized (Labconco, MO, USA). Lyophilized samples were stored at 4 ºC until their analysis.

2.4. Precipitation of hydrocolloids and proteins

In order to remove hydrocolloids and proteins which could interfere in the determination of betalains, the methods described by Stintzing, Schieber, & Carle (2002) was followed. 20 mL of ethanol at 96% were added to 10 g of CE previously dissolved in 10 mL of water. The solution was stirred and then was reposed for 20 min. The mucilages were separated from the aqueous phase by vacuum filtration (Whatman No 1 filters), and the organic solvent was evaporated at reduce pressure using a rotary evaporator (Heidolph, Schwabach, Germany). The purified extract (PE) was then lyophilized and kept at 4 ºC until its further analysis.

2.5. Fractionation on C18 column

Column chromatography was carried out in order to separate different fractions of pigments from the PE by means a differential fractionation based on the hydrophobicity. A C_{18} column (28 x 2 cm) (Sigma-Aldrich, USA) was previously conditioned by passing, separately, 3 volumes of 100 % methanol and then rinsed with 3 volumes of deionized water acidified with formic acid (pH 3), according to the method proposed by Stintzing, Schieber, & Carle (2002). 0.3 g of PE dissolved in 1 mL of deionized water was then added to the column. For fractionation, two fractions were distinguished based on their colour. The less hydrophobic compounds (yellow fraction, YF) were eluted from the column by passing 2 volumes of 100% methanol. The red fraction (RF) were eluted with two volumes of a mixture of methanol:acidified water (pH 3) (95:5, v/v). Both fractions were concentrated at reduce pressure and then lyophilized.

2.6. Total reducing power (TRP)

Total reducing power (TRP) of the extracts of *O. dillenii* was evaluated by Folin–Ciocalteau method (Singleton, 1999). An aliquot of 100 µL of the methanolic extract was mixed with 900 µL of Folin-Ciocalteau reagent and was maintained for 5 min at room temperature. Subsequently, after the addition of 750 µL of sodium bicarbonate, the solution was stirred for 30 seconds and left to stand for 90 min at room temperature. Absorbance was measured at 765 nm with a spectrophotometer (Merck, Spectroquant® Pharo 300, USA), and the results were expressed as milligrams of gallic acid per litre (mg GAE/L). Subsequently, total reducing power was expressed as milligrams per 100 gram of dry weight.

2.7. Spectrophotometric quantification of betalains

A spectrophotometric quantification of betalains was also carried out, following the method proposed by Stintzing & Carle (2004) (Merck, Spectroquant® Pharo 300, USA). The UV-vis spectra were recorded from 360-800 nm and all measurements were carried out in duplicate. Two different absorbances at maxima were reported (480 nm and 538 nm) on the basis of the quantification of betaxanthins and betacyanins, respectively. Betalain content (B) was developed by the following equation:

$$[B]\ (mg/g) = [(Abs)(DF)(V)(MW)/(\varepsilon)(L)(W)]$$

where Abs is the value of maximum absorbance at 480 or 538 nm, DF is the dilution factor, V is the volume (mL) of the extracts, MW and ε are the molecular weight and the molar extinction coefficient of betanin (550 g/mol and 60,000 L/(mol cm) in H_2O) and indicaxanthin (308 g/mol and 48,000 L/(mol cm) in H_2O), the major betacyanins and betaxanthin presented in *Opuntia*, L is the path-length (0.2 cm) and W the dried weight of the sample (g). All analyses were carried out in duplicate.

2.8. HPLC-DAD-ESI-MS analysis of betalains and polyphenols

HPLC separation and identification of the compounds (betalains and polyphenols) were performed in a Shimadzu LC-MS 2010 chromatographic system (Shimadzu, Tokio, Japan), that was equipped with a quaternary pump, an UV−vis diode array detector, coupled to a Shimadzu LC-MS software for data processing. Prior to direct injection, the samples were filtered through a 0.45 µm nylon filter (Sigma Aldrich, USA). All analyses were made in triplicate.

The identification was performed following the method proposed by Fernández-López, Castellar, Obón, & Almela (2002), using 1% formic acid in water (v/v, eluent A) and a mix of acetonitrile:water:formic acid (80:19:1) (eluent B). Compounds were separated in a Kinetex Phenomenex C_{18} (150 x 2.1 mm, 2.6 µm particle size) maintained at 25 ºC, at a flow rate of 0.4 mL/min. The volume injection was 5 µL. The chromatographic method started with 100% of A, followed by a linear gradient from 0 to 20% of B in 35 min and then a linear gradient from 20 to 100% of B in 5 min. To re-establish the initial conditions, a linear gradient from 100% of B to 100% of A was used during 10 min. UV-vis detection were performed at 280, 484 and 535 nm according to Fernández-López, Castellar, Obón, & Almela (2002). The identity of individual betalains and polyphenols was confirmed by mass spectrometry (Shimadzu LC 2010, Tokio, Japan). The instrument was operated in positive and negative ion mode using a scan range from *m/z* 50 to 1000. Nitrogen was used as the dry gas at a flow rate of 4.5 mL/min, the temperature was set at 250 ºC and the detector voltage was 1.8 kV.

2.9. Determination of Trolox equivalent antioxidant capacity (TEAC)

The antioxidant capacity was measured *in vitro* on the basis of the ability to scavenge the $ABTS^{\cdot+}$ radical (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999), which was produced by the oxidation of 7 mM ABTS with potassium persulfate (2.45

mM) in water. $ABTS^{\cdot+}$ solution was diluted with phosphate-buffered saline (PBS) at pH 7.4 after storage under dark conditions and room temperature for 16 h, until reaching an absorbance of 0.70 ± 0.02 at 734 nm. Then, 30 µL of each sample was mixed with 3 mL of the $ABTS^{\cdot+}$ diluted solution and vortexed for 1 min. After waiting for 6 min, the absorbance was measured at 734 nm in a spectrophotometer (UV-Vis Pharo, Merck, Germany). Results were obtained by interpolating the absorbance on the calibration curve of Trolox (0.5-3.0 µM). The results were expressed as µmol of Trolox equivalent/g of sample.

2.10. Statistical Analysis

All statistical analyses were performed using Statgraphics Centurion 16.1.15 software. Univariate analysis of variance (ANOVA) was applied using the general linear model program to establish whether mean values of the sample data differed significantly from each other. The means values of each set of samples (n = 3) were compared by the multiple ranges test at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Betalains and Polyphenol Compounds Separation, Identification and Quantification

Figures 1 and 2 show the chromatographic pattern and peak assignment of the two families of betalains (betacyanins and betaxanthins) and some polyphenols, in RF and YF, monitored at 538, 480 and 280 nm, respectively. These compounds are summarized in Table 1, which shows the retention times, UV-vis data, and mass spectra ((*m/z*) and MS ions) from each betalain and polyphenol identified in *Opuntia dillenii* by HPLC-DAD-ESI-MS.

The compounds achieved in the red fraction mainly consisted of betacyanins and some polyphenols. Among betacyanins, the presence of betanidin-*5*-*O*-β-glucoside (betanin)

and its isomer isobetanidin-*5*-*O*-β-glucoside (isobetanin) have been confirmed (551.15 *m/z* units ($[M+H]^+$, and a daughter ion at *m/z* 507.10) (compound 1 and 3). This fragmentation seemed to be the breaking of one carbon dioxide bond (loss of a fragment of *m/z* 44 units correspond to the CO_2 molecule) ($[M+H\text{-}CO2]^+$) (Table 1). As can be seen in Fig. 1a, these betacyanins were present at high levels in *Opuntia dillenii* extracts, according to other authors (Chang, Hsieh, & Yen, 2008; Montes-Lora, Hurtado, Mosquera, Heredia, & Cejudo-Bastante, 2016). These betacyanins have been also well-described as the major betacyanins in other species of *Opuntia*, such as *Opuntia ficus-indica* (Fernández-López, Almela, Obón, & Castellar, 2010) and *Opuntia stricta* (Castellar, Obón, Alacid, & Fernández-López, 2003; Fernández-López, Castellar, Obón, & Almela, 2002).

Moreover, two isomers of betacyanins were also identified, with similar UV-vis data compared to the compounds 1 and 3. These two compounds were observed in the extracted ion chromatogram (EIC) at the *m/z* value of its molecular ion ($[M-H]^-$ of 505.10). Taking into account that a daughter peak of 461.25 *m/z* units appeared as MS ion, these peaks could be attributable to the decarboxy forms of betanin and isobetanin ($[M-H-CO_2]^-$), called 17-decarboxybetanin and 17-decarboxyisobetanin (compounds 2 and 5, respectively). Those compounds have been already described in Cactaceae (Kugler, Stintzing, & Carle, 2007; Strack, Vogt, & Schliemann, 2003) and red beet (Stintzing, Schieber, & Carle, 2002), but this is the first attempt at accurately identifying them by HPLC-DAD-ESI-MS in *Opuntia dillenii*.

Other betacyanins identified in RF were derived from gomphrenin (betanidin-6-*O*-glucoside) (compounds 6 and 7) (Table 1, Fig. 1a). These compounds were assigned as isomers on the basis of UV-vis spectra and MS spectra, considering the similar molecular ion at 755.10 *m/z* units ($[M-H]^-$) (Table 1; Fig. 3a). They were tentatively

identified as 6′-*O*-sinapoyl-*O*-gomphrenin and 6′-*O*-sinapoyl-*O*-isogomphrenin, in agreement with the *m/z* values reported by Spórna-Kucab, Jagodzińska, & Wybraniec (2017) in *Gomphrena globosa*. To the best of our knowledge, these compounds have not been previously described in *Opuntia dillenii*.

The compound 8 showed a molecular ion at 767.15 *m/z* units ($[M-H]^-$) and a fragment at 551.35 *m/z* units (Table 1; Fig. 3b). The loss of a fragment of 215.8 *m/z* units could correspond to the joint contribution of one moiety of malonic acid (86 *m/z* units) and apiose sugar (132 *m/z* units) ([M-H-apiose-malonic acid]$^-$), confirming the presence of 2'-*O*-apiosyl-4-*O*-phyllocactin (2'-*O*-apiosyl-4-*O*-malonyl-betanin) (Table 1, Fig. 1a). In addition, taking into account the mass spectra 975.65 *m/z* units ($[M+H]^+$), and the *m/z* values ($[M+H]^+$) of betanin, malonyl, sinapoyl and apiosyl (551, 86, 206 and 132, respectively), previously described by Wybraniec, Stalica, Spórna, & Mizrahi (2010), another betacyanin derived from betanin was also identified in *Opuntia dillenii* (5''-O-E-sinapoyl-2'-apiosyl-phyllocactin or 5''-O-E-sinapoyl-2'-apiosyl-6-*O*-malonyl-betanin) (compound 9; Table 1). These compounds has been described in several cacti from the Botanical Garden of the University Institute of Botany (Cracow, Poland) (Wybraniec, Stalica, Spórna, & Mizrahi, 2010), but any bibliography has been found in *Opuntia dillenii*.

Regarding polyphenols, isorhamnetin-3-glucuronide and quercetin-3-glucoside were identified in the RF of *Opuntia dillenii* (compounds 10 and 15) (Table 1, Fig. 1b). This attribution was developed based on the UV-vis spectra and *m/z*, with maximum absorption values of 352 and 354 nm, and a mass spectra of 493.05 ($[M+H]^+$) and 463.10 ($[M-H]^-$) *m/z* units, respectively. Those polyphenols have been previously found in *Myrtillocactus geometrizans* by Correa-Betanzo, Jacob, Perez-Perez, & Paliyath (2011), who affirmed that quercetin-3-glucoside was one of the major polyphenols

found in that plant material. Moussa-Ayoub, Jaeger, Youssef, Knorr, El-Samahy, Kroh, et al. (2016) identified isorhamnetin-3-*O*-rutinoside in *Opuntia dillenii* juice, but, to our knowledge, the polyphenols identified in this study have not been previously reported in that feedstock. Other polyphenols kept remained unknown (peaks 11-14, tentatively assigned as flavonols-*O*-substituted taking into account the UV-vis spectra data).

Betaxanthins were found in YF and they were accurately identified (Table 1; Fig. 2). Among them, the presence of tryptophan-betaxanthin (compound 16) and portulacaxanthin II (the betaxanthin derived from tyrosine) (compound 17) were confirmed according to the UV-vis and mass spectra, with a molecular ion at 399.15 and 377.5 *m/z* units ($[M+H]^+$), respectively. Compound 18 was identified as proline-betaxanthin (also called indicaxanthin), with a molecular ion at 309.15 *m/z* units. These compounds have been previously identified in *Opuntia ficus-indica* (Mata, Ferreira, Semedo, Serra, Duarte, & Bronze, 2016) and *Myrtillocactus geometrizans* fruits (Correa-Betanzo, Jacob, Perez-Perez, & Paliyath, 2011). In *Opuntia dillenii*, only indicaxanthin have been previously described (Montes-Lora, Hurtado, Mosquera, Heredia, & Cejudo-Bastante, 2016), but, to the best of our knowledge, it is the first report identifying betaxanthins derived from tryptophan and tyrosine in *Opuntia dillenii* fruits.

It is also worth mentioning that RF contained traces of the compounds 17 and 18, evidencing that an elongation of the column is needed to improve the separation of betaxanthins. Apart from the explained fact, the proposed method of fractionation supposed an important advance in the betalain and phenolic determination, in the light of the scarce reported methods of fractionation of these kind of compounds.

In quantitative terms, the total amount of betacyanins in RF were altogether higher (and significant, $p < 0.05$) compared to that of betaxanthins in YF (16.63 ± 0.20 mg

betanin/100 g fresh fruit and 7.55 ± 0.04 mg indicaxanthin/100 g fresh fruit, respectively). To draw a comparison with other *Opuntia* species, *Opuntia ficus-indica* and *Opuntia undulata* displayed greater content of total betaxanthins and similar order of magnitude of betacyanins (Fernández-López, Almela, Obón, & Castellar, 2010), whereas Sepúlveda (2003) exhibited that betacyanins content of Chilean *Opuntia ficus-indica* cacti ranged between 16.6 and 62.4 mg of betanin/100 g of fresh fruit. With regard to *Opuntia dillenii*, only Cejudo-Bastante, Hurtado, & Heredia (2015) previously provided a concentration of total betacyanins and betaxanthins (around 7 and 6 mg betanin and indicaxanthin/100 g of dry fruit, respectively).

3.2. Total reducing power (TRP) and Antioxidant Activity

Table 2 summarizes the values of total reducing power and antioxidant activity of the different fractions of *Opuntia dillenii*. The values of antioxidant activity of ascorbic acid used as positive control was in agreement with other authors (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999) (1.08 mmol Trolox/mmol ascorbic acid).

The results evidenced a diminution of the antioxidant activity along the purification process (CE > PE > RF > YF), with significant differences ($p < 0.05$) between samples. The fact that PE had lesser antioxidant activity than CE might be from the elution of some uncoloured compounds (such as organic acids), responsible for supplying antioxidant capacity. These results were in accordance with the values of total reducing power (TRP) (Table 2), as the crude extract (CE) evidenced the highest antioxidant activity and TRP. In fact, univariate lineal correlation was applied to these data and a very strong correlation ($R^2 = 0.9684$) was observed between the TRP and the antioxidant activity measured by TEAC, which indicate that *Opuntia dillenii* represent a good source of natural antioxidants with high added value. Similar relations were

observed by Moussa-Ayoub et al. (2016) in *Opuntia dillenii* juices when compared antioxidant activity, measured by electron paramagnetic resonance spectrometry, and total phenolic content.

It is also remarkable that RF manifested significantly ($p < 0.05$) higher values of antioxidant activity and TRP compared to YF. Similar results were found by Butera, Tesoriere, Di Gaudio, Bongiorno, Allegra, Pintaudi, et al. (2002), who demonstrated that the radical-scavenging capacity of purified betanin and indicaxanthin from *Opuntia ficus-indica* were highly effective, but betanin was more active than indicaxanthin.

4. Conclusions

It is demonstrated that *Opuntia dillenii* is a rich source of betalain compounds. Apart from the well-known betanin, isobetanin and indicaxanthin previously described in *Opuntia dillenii*, a large extent of betalains and polyphenols have been identified for the first time in this fruit: 17-decarboxybetanin and 17-decarboxyisobetanin, 6′-O-sinapoyl-O-gomphrenin and 6′-O-sinapoyl-O-isogomphrenin, 2’-O-apiosyl-4-O-phyllocactin and 5’’-O-E-sinapoyl-2’-apiosyl-phyllocactin as betacyanins; tryptophan-betaxanthin and portulacaxanthin II as betaxanthins; and isorhamnetin-3-glucuronide and quercetin-3-*O*-glucoside as polyphenols. This fact evidenced that the purification and subsequent fractionation of the extract (scarcely studied in betalain-rich products), make possible to identify a greater number of betalains and phenolic compounds. Moreover, it is also proved that *Opuntia dillenii* exhibited a potent antioxidant activity against $ABTS^{+}$ radical, strongly related to the total reducing power. Therefore, the described properties could make interesting the use of this low-exploited natural resource in terms of nutrition and future industrial applicability. The commercialization and industrialization of fruits with high functional value could generate competitive advantages and economic opportunities for the producers.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

We are indebted to Vicerrectoría de Investigaciones Postgrados y Relaciones Internacionales – VIPRI – UDENAR (Universidad de Nariño, Colombia). Authors are also grateful to Biology Service (SGI, Universidad de Sevilla) for technical assistance.

Conflict of interest. None.

References

Butera, D., Tesoriere, L., Di Gaudio, F., Bongiorno, A., Allegra, M., Pintaudi, A. M., Kohen, R., & Livrea, M. A. (2002). Antioxidant activities of sicilian prickly pear (*Opuntia ficus indica*) fruit extracts and reducing properties of its betalains: Betanin and indicaxanthin. *Journal of Agricultural and Food Chemistry, 50*, 6895-6901.

Böhm, H. (2008). *Opuntia dillenii* - an interesting and promising cactaceae taxon. In *Journal of the professional association for cactus development* (pp. 148-170).

Campos, D., Noratto, G., Chirinos, R., Arbizu, C., Roca, W., & Cisneros-Zevallos, L. (2006). Antioxidant capacity and secondary metabolites in four species of Andean tuber crops: Native potato (*Solanum* sp.), mashua (*Tropaeolum tuberosum* Ruiz & Pavón), Oca (*Oxalis tuberosa* Molina) and ulluco (*Ullucus tuberosus* Caldas). *Journal of the Science of Food and Agriculture, 86*, 1481-1488.

Castellar, R., Obón, J. M., Alacid, M., & Fernández-López, J. A. (2003). Color properties and stability of betacyanins from *Opuntia* fruits. *Journal of Agricultural and Food Chemistry, 51*, 2772-2776.

Cejudo-Bastante, M. J., Chaalal, M., Louaileche, H., Parrado, J., & Heredia, F. J. (2014). Betalain profile, phenolic content, and color characterization of different parts and varieties of *Opuntia ficus-indica*. *Journal of Agricultural and Food Chemistry, 62*, 8491-8499.

Cejudo-Bastante, M. J., Hurtado, N., & Heredia, F. J. (2015). Potential use of new Colombian sources of betalains. Colorimetric study of red prickly pear (*Opuntia dillenii*) extracts under different technological conditions. *Food Research International, 71*, 91-99.

Cejudo-Bastante, M. J., Hurtado, N., Mosquera, N., & Heredia, F. J. (2014). Potential use of new Colombian sources of betalains. Color stability of ulluco (*Ullucus tuberosus*) extracts under different pH and thermal conditions. *Food Research International, 64*, 465-471.

Chang, S. F., Hsieh, C. L., & Yen, G. C. (2008). The protective effect of *Opuntia dillenii* Haw fruit against low-density lipoprotein peroxidation and its active compounds. *Food Chemistry, 106*, 569-575.

Correa-Betanzo, J., Jacob, J. K., Perez-Perez, C., & Paliyath, G. (2011). Effect of a sodium caseinate edible coating on berry cactus fruit (*Myrtillocactus geometrizans*) phytochemicals. *Food Research International, 44*, 1897-1904.

Fernández-López, J. A., Almela, L., Obón, J. M., & Castellar, R. (2010). Determination of Antioxidant Constituents in Cactus Pear Fruits. *Plant Foods for Human Nutrition, 65*, 253-259.

Fernández-López, J. A., Castellar, R., Obón, J. M., & Almela, L. (2002). Screening and mass-spectral confirmation of betalains in cactus pears. *Chromatographia, 56*, 591-595.

Fernández-López, J. A., Giménez, P. J., Angosto, J. M., & Moreno, J. I. (2012). A process of recovery of a natural yellow colourant from *Opuntia* fruits. *Food Technology and Biotechnology, 50*, 246-251.

Gandía-Herrero, F., Escribano, J., & García-Carmona, F. (2016). Biological Activities of Plant Pigments Betalains. *Critical Reviews in Food Science and Nutrition, 56*, 937-945.

Ghazi, Z., Ramdani, M., Tahri, M., Rmili, R., Elmsellem, H., El Mahi, B., & Fauconnier, M. L. (2015). Chemical composition and antioxidant activity of seeds oils and fruit juice of *Opuntia ficus-indica* and *Opuntia dillenii* from Morocco. *Journal of Materials and Environmental Science, 6*, 2321-2327.

Herbach, K. M., Stintzing, F. C., & Carle, R. (2006). Betalain stability and degradation - Structural and chromatic aspects. *Journal of Food Science, 71*, R41-R50.

Jiménez-Aguilar, D. M., López-Martínez, J. M., Hernández-Brenes, C., Gutiérrez-Uribe, J. A., & Welti-Chanes, J. (2015). Dietary fiber, phytochemical composition and antioxidant activity of Mexican commercial varieties of cactus pear. *Journal of Food Composition and Analysis, 41*, 66-73.

Kugler, F., Stintzing, F. C., & Carle, R. (2007). Characterisation of betalain patterns of differently coloured inflorescences from *Gomphrena globosa* L. and *Bougainvillea* sp. by HPLC-DAD-ESI-MSn. *Analytical and Bioanalytical Chemistry, 387*, 637-648.

Kumar, A. S., Ganesh, M., Peng, M. M., & Jang, H. T. (2014). Phytochemical, antioxidant, antiviral and cytotoxic evaluation of *Opuntia dillenii* flowers. *Bangladesh Journal of Pharmacology, 9*, 351-355.

Li, H., Deng, Z., Liu, R., Zhu, H., Draves, J., Marcone, M., Sun, Y., & Tsao, R. (2015). Characterization of phenolics, betacyanins and antioxidant activities of the seed,

leaf, sprout, flower and stalk extracts of three *Amaranthus* species. *Journal of Food Composition and Analysis, 37*, 75-81.

Li, H., Yuan, Q., Zhou, X., Zeng, F., & Lu, X. (2016). Extraction of *Opuntia dillenii* Haw. polysaccharides and their antioxidant activities. *Molecules, 21*(12), E1612.

Mata, A., Ferreira, J. P., Semedo, C., Serra, T., Duarte, C. M. M., & Bronze, M. R. (2016). Contribution to the characterization of *Opuntia* spp. juices by LC-DAD-ESI-MS/MS. *Food Chemistry, 210*, 558-565.

Medina, E. M. D., Rodríguez, E. M. R., & Romero, C. D. (2007). Chemical characterization of *Opuntia dillenii* and *Opuntia ficus-indica* fruits. *Food Chemistry, 103*, 38-45.

Montes-Lora, S., Hurtado, N., Mosquera, N., Heredia, F. J., & Cejudo-Bastante, M. J. (2016). Effect of technological practices on individual betalains and antioxidant activity of Columbian betalain-rich raw materials. *International Journal of Food Science and Technology, 51*, 1041-1047.

Moussa-Ayoub, T. E., Jaeger, H., Knorr, D., El-Samahy, S., Rohn, S., & Kroh, L. W. (2011). Impact of traditional and innovative technologies on some characteristics and bioactive compounds of *Opuntia macrorhiza* juice. *Procedia Food Science, 1*, 1410-1416.

Moussa-Ayoub, T. E., Jaeger, H., Youssef, K., Knorr, D., El-Samahy, S., Kroh, L. W., & Rohn, S. (2016). Technological characteristics and selected bioactive compounds of *Opuntia dillenii* cactus fruit juice following the impact of pulsed electric field pre-treatment. *Food Chemistry, 210*, 249-261.

Méndez, L. P., Flores, F. T., Martín, J. D., Rodríguez Rodríguez, E. M., & Díaz Romero, C. (2015). Physicochemical characterization of cactus pads from *Opuntia dillenii* and *Opuntia ficus-indica*. *Food Chemistry, 188*, 393-398.

Osorio-Esquivel, O., Alicia Ortiz, M., Álvarez, V. B., Dorantes-Álvarez, L., & Giusti, M. M. (2011). Phenolics, betacyanins and antioxidant activity in *Opuntia joconostle* fruits. *Food Research International, 44*, 2160-2168.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231-1237.

Sawicki, T., Bączek, N., & Wiczkowski, W. (2016). Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. *Journal of Functional Foods, 27*, 249-261.

Sepúlveda, E., Sáenz, C., Gómez, C. (2003). Determinación de betanina en ecotipos de tuna roja colectados en Chile. In *IX Congreso Nacional y VII Congreso Internacional Conocimiento y Aprovechamiento del nopal* (pp. 282-285).

Sharma, C., Rani, S., Kumar, B., Kumar, A., Vinit, R. (2015). Plant *Opuntia dillenii*: a review on its traditional uses, phytochemical and pharmacological properties. In *Pharmaceutical science*, vol. 1 (pp. 29-43).

Singleton, V. L., Orthofer, L., Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*, vol. 299 (pp. 152-178).

Spórna-Kucab, A., Jagodzińska, J., & Wybraniec, S. (2017). Separation of betacyanins from purple flowers of *Gomphrena globosa* L. by ion-pair high-speed counter-current chromatography. *Journal of Chromatography A, 1489*, 51-57.

Stintzing, F. C., & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science and Technology, 15*, 19-38.

Stintzing, F. C., Schieber, A., & Carle, R. (2002). Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography-electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 50,* 2302-2307.

Strack, D., Vogt, T., & Schliemann, W. (2003). Recent advances in betalain research. *Phytochemistry, 62,* 247-269.

Vaillant, F., Perez, A., Davila, I., Dornier, M., & Reynes, M. (2005). Colorant and antioxidant properties of red-purple pitahaya (*Hylocereus* sp.). *Fruits, 60,* 3-12.

Wybraniec, S., Stalica, P., Spórna, A., & Mizrahi, Y. (2010). Profiles of betacyanins in epidermal layers of grafted and light-stressed cacti studied by LC-DAD-ESI-MS/MS. *Journal of Agricultural and Food Chemistry, 58,* 5347-5354.

Figure captions

Fig. 1. HPLC chromatogram corresponding to the red fraction (RF) of *Opuntia dillenii*. a) 538 nm and b) 280 nm.

Fig. 2. HPLC chromatogram corresponding to the yellow fraction (YF) of *Opuntia dillenii* (480 nm).

Fig. 3. MS chromatogram in negative mode of the (a) 6'-*O*-sinapoyl-6-*O*-gomphrenin (compound 6) and (b) 2'-*O*-apiosyl-4-O-malonyl-betanin (compound 8).

Figure1

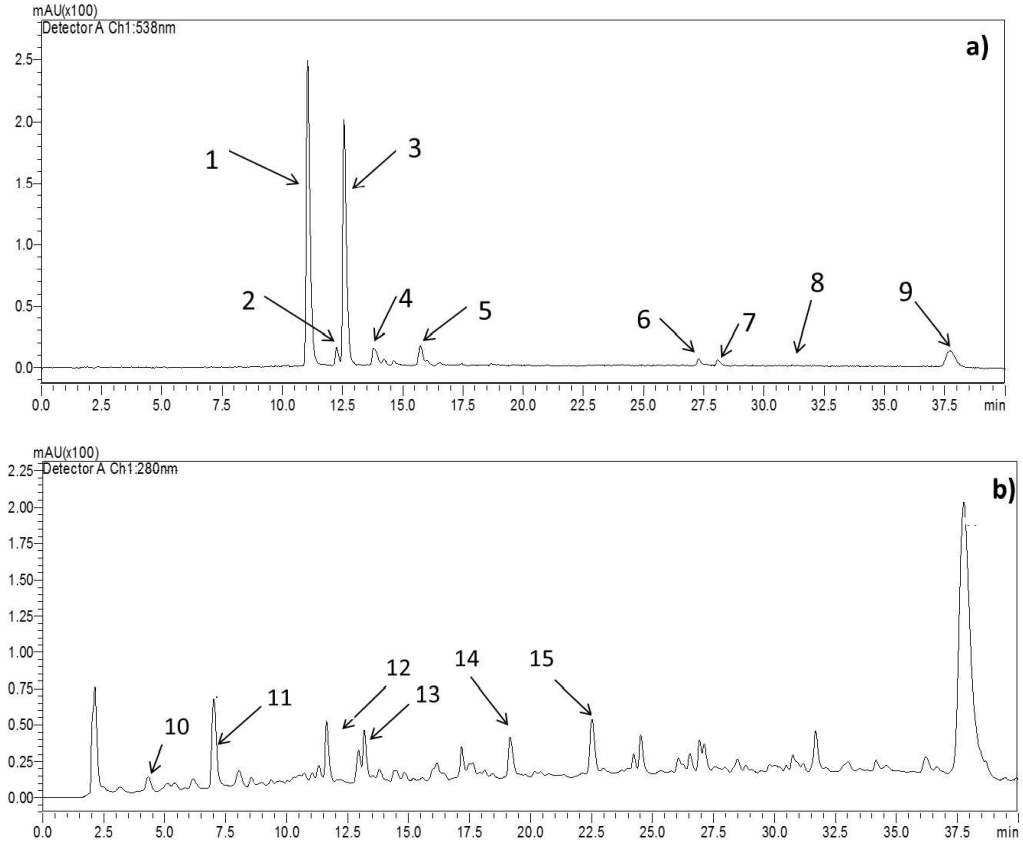


Figure 1.

Figure2

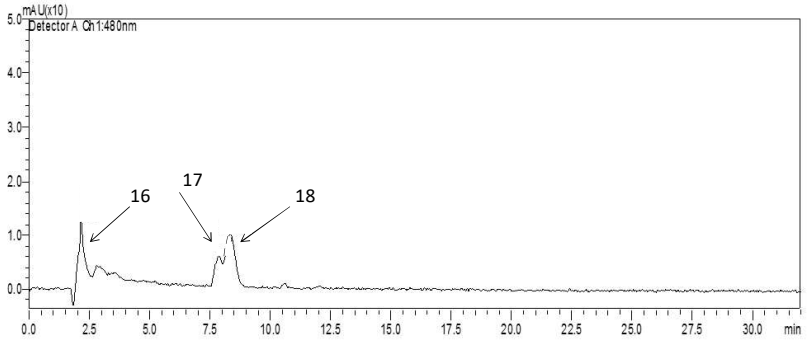


Figure 2.

Figure 3

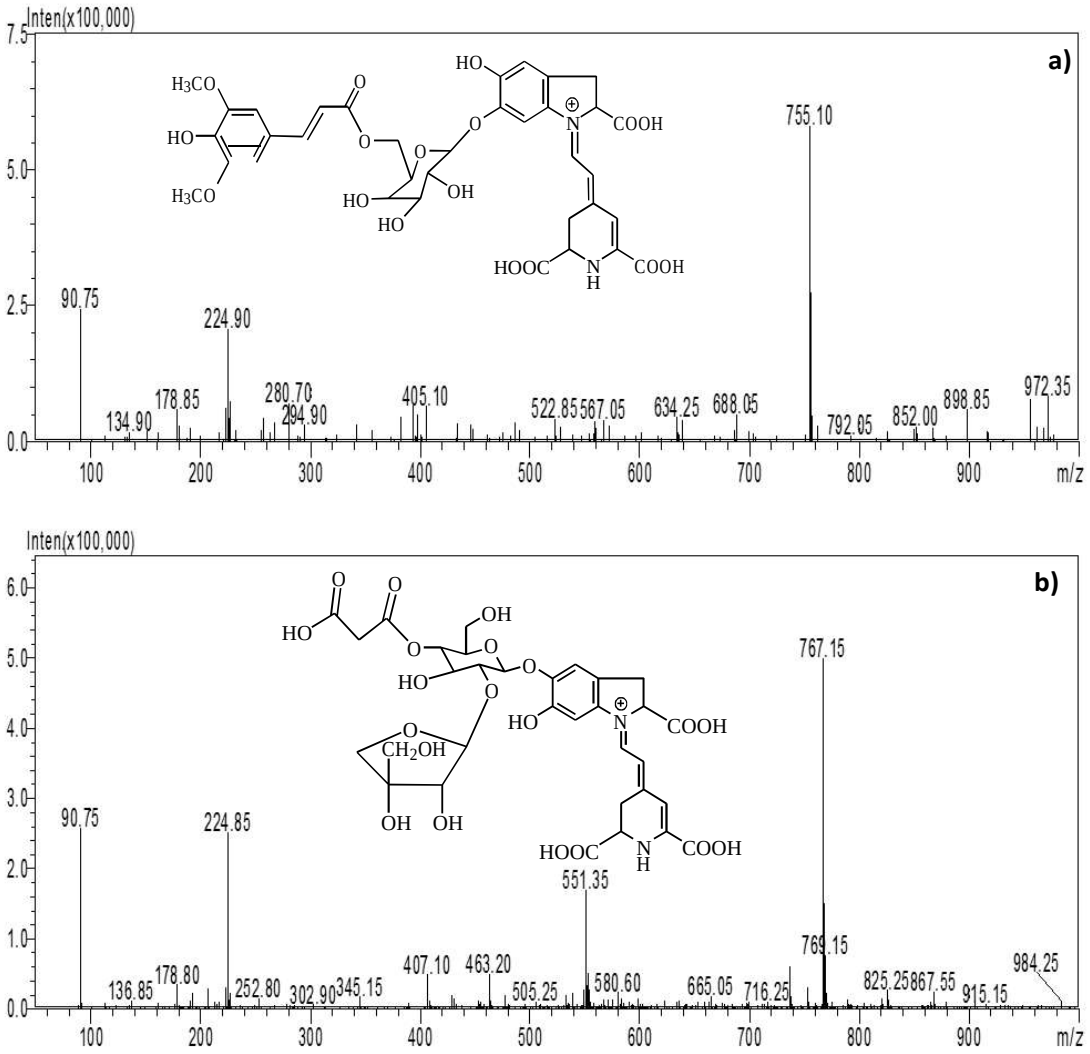


Figure 3.

Table 1

Table 1. Molecular formula, retention times, UV-Vis data and mass spectral of betalains and polyphenols identified in *Opuntia dillenii* by HPLC-DAD-ESI-MS.

Peak	Compounds	Molecular formula	t_r (min)	UV–vis maximum (nm)	*m/z* $[M + H]^+$	*m/z* $[M - H]^-$	MS ions
	Red fraction						
	Betacyanins						
1	Betanin	$C_{24}H_{26}N_2O_{13}$	11.021	536	551.15	-	507.10
2	17-decarboxy-betanin	$C_{23}H_{26}N_2O_{11}$	12.105	505	-	505.10	461.25
3	Isobetanin	$C_{24}H_{26}N_2O_{13}$	12.535	536	551.15	-	507.20
4	Unknown	-	13.210	525	623.05	-	441.20, 317.00
5	17-decarboxy-isobetanin	$C_{23}H_{26}N_2O_{11}$	15.511	532	-	505.10	461.25
6	6′-*O*-sinapoyl-O-gomphrenin	$C_{35}H_{36}N_2O_1$	27.215	538	-	755.10	224.90
7	6′-*O*-sinapoyl-O-isogomphrenin	$C_{35}H_{36}N_2O_{17}$	28.125	538	-	755.10	224.90
8	2’-*O*-apiosyl-4-*O*-phyllocactin	$C_{32}H_{36}N_2O_{20}$	31.683	-	-	767.15	551.35
9	5’’-*O*-E-sinapoyl-2’-apiosyl-phyllocactin	$C_{43}H_{46}N_2O_{24}$	37.762	553	975.65	-	[a]
	Polyphenols						
10	Isorhamnetin-3-glucuronide	$C_{22}H_{20}O_{13}$	4.272	352	493.05	-	[a]
11	Unknown	-	7.008	345	-	383.95	[a]
12	Unknown	-	11.632	348	663.75	-	513.10
13	Unknown	-	13.538	335	-	776.30	567.15
14	Unknown	-	19.158	348	663.75	-	513.10
15	Quercetin-3-*O*-glucoside	$C_{21}H_{20}O_{12}$	22.514	354	-	463.10	300.10
	Yellow fraction						
	Betaxanthins						

16	Tryptophan-betaxanthin	$C_{20}H_{20}N_3O_6$	2.131	473	399.15	-	381.05
17	Tyrosine-betaxanthin (portulacaxanthin II)	$C_{18}H_{20}N_2O_7$	7.861	472	377.05	-	[a]
18	Proline-betaxanthin (indicaxanthin)	$C_{14}H_{17}N_2O_6$	8.328	480	309.15	-	[a]

[a] Fragmentation was not achieved.

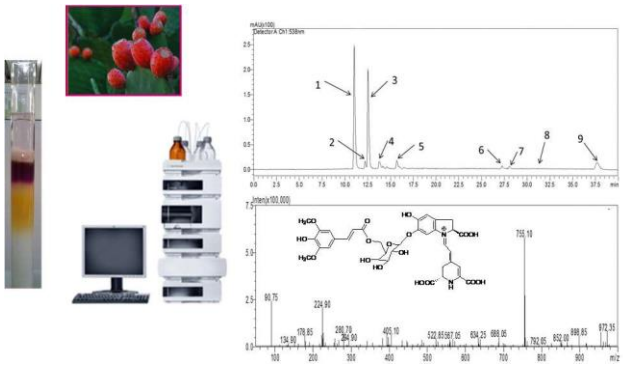
Table2

Table 2. Total reducing power (TRP) (mg/100 g fresh fruit) and antioxidant activity (TEAC) (μmol Trolox/g of fresh fruit) of each fraction of *Opuntia dillenii* fruit.

	TRP	TEAC
CE	311.61 ± 12.29	35.24 ± 0.08
PE	187.25 ± 8.64	15.70 ± 0.30
RF	49.82 ± 0.95	5.37 ± 0.03
YF	12.16 ± 0.06	1.74 ± 0.02

CE, crude extract; PE, purified extract; RF, red fraction; YF, yellow fraction.

*Graphical Abstract



Graphical abstract. Purification, fractionation and chemical characterization of *Opuntia dillenii* (Ker Gawl) Haw cactus fruit.