



Betalains and phenolic compounds profiling and antioxidant capacity of pitaya (*Stenocereus* spp.) fruit from two species (*S. Pruinusus* and *S. stellatus*)



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ABSTRACT

Pitaya fruits (*Stenocereus* spp.) contain betalains and phenolic compounds that have not been completely characterized. Fruits of two ecotypes of *S. pruinusus*, red-fleshed (*SpR*) and orange-fleshed (*SpO*), and two of *S. stellatus*, red-fleshed (*SsR*) and white-fleshed (*SsW*), were characterized in their betalains and phenolic compounds. The chromatographic profile of betalains was similar in *SpR*, *SpO*, and *SsR*, where indicaxanthin, gomphrenin I, phyllocacthin, and their isomers predominated. Betaxanthins content was higher than betacyanins and ranged from $17,706.7 \pm 1128.1$ to $22,053.6 \pm 328.1$ $\mu\text{g/g}$ dry sample. The identified phenolics mostly corresponded to hydroxycinnamoyl derivatives, flavonols and flavanones. The phenolics content was higher in *S. stellatus* than in *S. pruinusus*. The ecotype *SsW*, that had flesh without color, showed the highest concentration. The antioxidant capacity varied within 9.21 ± 0.84 and 2.41 ± 0.36 $\mu\text{mol Trolox/g}$ fresh sample, as determined by the ABTS assay. By its phytochemical composition, the fruits of pitaya can be a good source of antioxidant compounds.

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1. Introduction

The genus *Stenocereus* includes a part of the columnar cactus species, among which *S. pruinusus*, *S. stellatus*, and *S. queretaroensis*, are endemic of Mexico, and the most cultivated species due to the economic value of their fruit (Bravo-Hollis & Sánchez-Mejorada, 1991; Luna-Morales, Aguirre-Rivera, & Peña-Valdivia, 2001). These species produce fruits named *pitayas*, which are poly-spermatocytic berries that have spherical or ovoid shape, with thorny and deciduous areoles, small and edible seeds, and juicy flesh of different colors, that vary from white, yellow, pink, orange, red or purple

Abbreviations: ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); CE, collision energy; CES, collision energy spread; DAD, diode array detector; DP, declustering potential; EMS, enhanced mass spectrometer; EPI, enhanced product ion; HPLC-DAD-ESI/MS, high-performance liquid chromatographic with diode array detector and electrospray ionization and mass spectrometry; IP, input potential; *SpR*, *Stenocereus pruinusus* red fleshed fruit; *SpO*, *Stenocereus pruinusus* orange fleshed fruit; *SsR*, *Stenocereus stellatus* red fleshed fruit; *SsW*, *Stenocereus stellatus* white fleshed fruit; TFA, trifluoroacetic acid; TE, trolox equivalent.

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(Casas, Cruse-Sanders, Morales, Otero-Arnaiz, & Valiente-Banuet, 2006). The species *S. pruinusus* is also known as *pitaya de mayo*, due to the harvest season, and it produces fruits with ovoid shape, low acidity, and total soluble solids that vary from 9 to 10 °Brix (García-Cruz, Valle-Guadarrama, Salinas-Moreno, & Joaquín-Cruz, 2013). On the other side, fruit of *S. stellatus*, known in Mexico as *pitaya de agosto* or *xoconochtli*, have spherical shape, a slightly acidic flesh, and similar total soluble solids to those of *S. pruinusus* (Beltrán-Orozco, Oliva-Coba, Gallardo-Velázquez, & Osorio-Revilla, 2009). The fruits of *S. queretaroensis* have similar characteristics, although they are dehiscent, what make them very perishable (Pimienta-Barrios & Nobel, 2004). In several references, the name *pitaya* has also been given to fruit of the species *Hylocereus* spp. (Stintzing, Schieber, & Carle, 2002; Montoya-Arroyo et al., 2014). However, there are notable differences between plants and fruits of these two genera. In particular, the fruits of *Hylocereus*, named *pitahayas*, do not have spines, possess certain type of bracts, and their size is higher than that of the fruits of *Stenocereus* spp.

Color in flesh of *pitaya* fruit, as occurs in other cactaceae species of genera *Opuntia* and *Hylocereus*, is due to the presence of betalains (Ochoa-Velazco & Guerrero-Beltrán, 2013), which are

hydrosoluble pigments with nitrogen in their structure, for which about 55 different molecules have been described. Betalains are divided in two groups, betacyanins and betaxanthins, which impart red-purple and yellow-orange tonalities, respectively (Stintzing & Carle, 2007). These pigments possess various biological activities, among which stand out their anti-inflammatory (Vidal, López-Nicolás, Gandía-Herrero, & García-Carmona, 2014), antioxidant (Gengatharan, Dykes, & Choo, 2015), and anti-cancer properties (Wu et al., 2006; Zou et al., 2005). Another important group of phytochemicals found in the flesh of *pitaya* fruit are the phenolic compounds (Beltrán-Orozco et al., 2009; García-Cruz, Salinas-Moreno, & Valle-Guadarrama, 2012), which, like betalains, have been associated with biological activities that are beneficial to the organism, as antiallergenic, antimicrobial, antioxidant, antithrombotic, and cardioprotective (Balasundram, Sundram, & Samman, 2006).

The antioxidant capacity of *pitaya* has been attributed to betalains and phenolic compounds, both present in the flesh of fruit. However, the characterization of these groups of compounds in *pitaya* has only been done partially (Beltrán-Orozco et al., 2009; García-Cruz et al., 2012), and such information would be valuable to conduct future works related to extraction of pigments and nutraceutical compounds, which could add value to the *pitaya* fruit. In this context, the objectives of the work were to characterize betalains and phenolic compounds present in the flesh of fruit of *S. pruinosa* and *S. stellatus* by HPLC-DAD-ESI/MS, and also to determine the antioxidant capacity of these materials.

2. Materials and methods

2.1. Plant material

Fruits of two ecotypes of *S. pruinosa*, red-fleshed (*SpR*) and orange-fleshed (*SpO*), and two of *S. stellatus*, red-fleshed (*SsR*) and white-fleshed (*SsW*) that came from homewards at Tepexi de Rodríguez, Puebla, Mexico (18°35'46" N, 97°55'48" W; 1644 masl) were studied. Fruits were harvested at consumption maturity, which occurs when thorns break off easily and the skin becomes shiny. A batch of 10 fruits of each variant was used, from which the skin was manually removed to obtain the flesh and this was homogenized and lyophilized.

2.2. Flesh color

The color of fruit flesh was determined with a colorimeter (Hunterlab, Reston, USA), using illuminant D65 and 10° observer angle. Color was expressed as L^* , a^* , b^* , chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$h^\circ = \arctan b^*/a^*$] as described by McGuire (1992).

2.3. Betalains analysis

2.3.1. Extraction

Five hundred milligrams were weighed from each sample and mixed with 25 mL of a solution of methanol:TFA (trifluoroacetic acid) 1% in water (80:20, v/v). Thereafter, the mixture was placed in an ultrasonic bath (Brason, model 5510, Danbury, USA) for 30 min and allowed to stand at -20°C during 3 h. After this time, the sample was centrifuged at $28,000\times g$ for 10 min at 5°C (Sorvall RC-5B, Newtown, USA), the supernatant was recovered, and the residue was submitted to two similar extractions, following the same conditions. The supernatants were pooled and concentrated to evaporate the methanol on a rotary evaporator (Büchi, model B-480, Flawil, CH). The extracts were re-suspended in 10 mL of distilled water and placed under freezing conditions until further analysis.

2.3.2. Analysis by HPLC-DAD ESI/MS

A high performance liquid chromatograph (HPLC, Hewlett-Packard 1100, Agilent Technologies, Santa Clara, USA) was used, which was equipped with a quaternary pump, autosampler, column thermostating system, diode array detector (DAD), and a flow cell to be used with high pressures, which had connection to a mass spectrometer (MS), coupled to a HP ChemStation. The separation was carried out using a Phenomenex $5\ \mu\text{m}$ column ($150 \times 4.6\ \text{mm}$), thermostated at 35°C . Mobile phases were: (A) 0.1% of trifluoroacetic acid in water, and (B) pure acetonitrile. The following gradients were optimized to separate compounds: 0 to 10% B in 15 min, 10 to 15% B in 5 min, 15 to 18% B in 5 min, 18 to 50% B in 10 min, 50 to 0% B in 10 min, followed by equilibration of the column. A flow rate of 0.5 mL/min was used. The preferred wavelengths for detection in the DAD system were 530 and 480 nm. In addition, the UV-Vis spectra of peaks were recorded in the range from 220 to 600 nm.

The analysis of mass spectrometry was carried out with an API 3200 Qtrap (Applied Biosystems, Darmstadt, DE), equipped with an electrospray ionization probe (ESI) and a triple quadrupole mass analyzer that could act also as an ion trap, coupled to the software Analyst 5.1. The detection was carried out in positive ionization mode, registering mass spectra between m/z 100 and m/z 1000. Zero air at 40 psi was used as nebulizer, turbo gas at 600°C and 50 psi to the elimination of eluent, and nitrogen at 100 psi as curtain and high collision gas. The method of *full scan* at high sensibility (*Enhanced MS*, EMS) was used for data acquisition, followed by an analysis in *Enhanced Product Ion* (EPI) mode to obtain the characteristic fragmentation of the majority ion obtained during the first experiment. The parameters used with EMS were: capillary voltage of 5000 V; as potentials: *declustering potential* (DP), $-41\ \text{V}$; *input potential* (EP), 7.5 V, and *collision energy* (CE), 10 V. The conditions in the EPI mode were: DP, 41 V; EP, 7.5 V; CE, 10 V, and *collision energy spread* (CES), 0 V.

The identification of betalains was made taking into account their retention behavior, UV-Vis spectrum, molecular ion, and mass spectrometry fragmentation pattern, which were compared with data reported previously in the literature. For quantification of betacyanins and betaxanthins standard curves were constructed with indicaxanthin and gomphrenin, both isolated in the laboratory. The indicaxanthin was isolated from flesh of *SsR* fruit, while gomphrenin was isolated from beetroot. To do this, an extraction with methanol:TFA 1% in water (80:20, v/v) was performed, followed by fractionation with column chromatography, using silica gel C18 (Octadecyl-functionalized silica gel, Sigma Aldrich) as stationary phase and distilled water as eluent. The collected fractions containing compounds were subjected to semipreparative HPLC purification and purity was further checked by HPLC-DAD-MS. The purity of the isolated betalains was 98% and 90%, for indicaxanthin and gomphrenin, respectively.

2.4. Analysis of phenolic compounds

2.4.1. Extraction

Five hundred milligrams from each sample were weighed and mixed with 25 mL of a solution of methanol:TFA 1% in water (80:20 v/v). Thereafter, mixtures were placed in an ultrasonic bath during 30 min and left at -20°C during 3 h. Subsequently they were centrifuged at $28,000g$ during 10 min at 5°C . The supernatant was recovered and the solid phase was submitted to a second similar extraction. The supernatants were pooled and concentrated to dryness on a rotary evaporator. The residue was re-dissolved in 2 mL of distilled water and centrifuged at $16,800g$ (EpendursHeraeusmod. Fresco 17, Waltham, USA). The extract was deposited in a cartridge (WatersSep-Pak[®] Vac), which was previously activated with 2 mL of methanol HPLC grade, followed by 4 mL of

water. In order to remove sugars, 20 mL of water were passed through and after that the phenolic compounds were eluted with 8 mL of methanol HPLC grade. This eluate was concentrated to dryness on a rotary evaporator and re-suspended in 250 μ L of distilled water in the case of samples of *SpO* and *SsW*, or in 500 μ L in the case of samples of *SsR* and *SpR*.

2.4.2. Analysis by HPLC-DAD-ESI/MS

The analysis of phenolic compounds was carried out with the same HPLC-DAD-ESI/MS equipment used to characterize betalains. The separation was performed through a column Waters Spherisorb of 3 μ m (150 \times 4.6 mm), which was thermostated at 35 $^{\circ}$ C. Mobil phases were: (A) 0.1% formic acid in water, and (B) pure acetonitrile. The following gradient was established: isocratic at 15% B for 5 min, 15 to 20% B in 5 min, 20 to 25% B in 10 min, 25 to 35% B in 10 min, 35 to 10% B in 10 min, followed by column equilibration. A flow of 0.5 mL/min was applied. The preferred wavelengths for detection in the DAD were 280 and 370 nm, and the UV–Vis spectra of peaks in the range 220 to 600 nm were recorded (Barros, Dueñas, Carvalho, Ferreira, & Santos-Buelga, 2012).

The analysis by mass spectrometry was performed under the same conditions described for betalains, except that detection was used in negative ionization mode. The identification of phenolic compounds was made according to their retention times, UV–Vis spectra, pseudomolecular ions, and the mass spectrometry fragmentation patterns. The quantification of individual phenolic compounds was based on standard curves of caffeic acid, ferulic acid, *p*-coumaric acid, taxifolin, naringenin, quercetin 3-*O*-rutinoside, isorhamnetin 3-*O*-glucoside, and kaempferol-3-*O*-glucoside.

2.5. Antioxidant capacity

To evaluate the antioxidant capacity (AC) of pitaya fruits, the extraction of betalains was done according to Wu et al. (2006). Briefly, two grams of macerated fruit pulp and 20 mL of 80% aqueous methanol were placed in an Erlenmeyer flask and sonicated for 10 min in an ultrasonic bath. Afterwards the sample was stirred for 20 min, and centrifuged at 2200g for 10 min, to recover the supernatant. The residue was extracted again under the same conditions. The supernatants were combined and adjusted to a defined volume with the extraction solvent. The ABTS method was chosen since it has been used to measure the AC of several fruits from the Cactaceae family such as fruits of *Hylocereus* (Wu et al., 2006) and *Opuntia* (Butera et al., 2002). The AC assay consisted of a 7 mM solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) in distilled water and another one of 2.45 mM of potassium persulfate ($K_2S_2O_8$; PP) were prepared and combined in 2:1 ratio. The mixture was left in darkness during 16 h to allow generation of free radicals. Thereafter, dilution with a 7.4-pH phosphate buffer was made until an absorbance of 0.7 ± 0.001 at 734 nm was obtained (Wu et al., 2006). In order to determine the antioxidant capacity, 200 μ L of betalains extract were placed in a quartz cell together with 2800 μ L of the mixture ABTS-PP, and the absorbance was measured with a spectrophotometer (PerkinElmer Lambda 25

UV/Vis) each minute along 7 min. The reduced ABTS was evaluated with the calculation $[\%ABTS_{reduced}] = [(Abs_0 - Abs_t) / Abs_0] \times 100$, where Abs_0 is the absorbance of the blank (200 μ L of phosphate buffer plus 2800 μ L of ABTS-PP) and Abs_t is the absorbance of the sample at the time *t*. In addition, a standard curve of Trolox (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was prepared and results were expressed as μ mol Trolox equivalents per gram of fresh pulp (μ mol TE/g FP).

2.6. Data analysis

An analysis of variance complemented with routines of comparison of means (Tukey, $p < 0.05$) was performed to determine whether significant differences exist between ecotypes studied. Data were analyzed with the SAS statistical package.

3. Results and discussion

3.1. Color characteristics

The ecotype with the highest luminosity was *SsW*, which was attributed to the lack of betalains. Such ecotype was followed by *SpO* and *SpR*, and finally by *SsR*. The fruit of *pitaya* with white flesh (*SsW*) was distinguished by low values in a^* , b^* and C^* , and high value in h_{ab} . The low value in C^* could be related with the presence of seeds with black color in the flesh of this fruit. The fruit of *SpO* showed a bright color with red-orange tonality, according to the observed values of L^* , C^* , and h_{ab} . Values of a^* , b^* , h_{ab} , and C^* were different (Table 1). The *pitaya SpR* showed a red-pink tint, with higher value in C^* than *SsR*, which is associated with higher color purity. On the other side, *SsR* has red-purple flesh, with low value in C^* and, therefore, the color purity is lesser than that of *SpR*.

3.2. Betalains identification

The analysis by HPLC allowed to separate up to 11 betalains in the analyzed samples (Fig. 1). The highest diversity was observed in the group of betacyanins (Fig. 1A), to which nine compounds were associated, and these could be tentatively identified from their mass and UV–Vis spectra, while the other two compounds (peaks 1 and 2) were identified as betaxanthins (Fig. 1B). The three analyzed ecotypes of fruit showed similar profile, with differences only in the relative proportion of each betalain. A higher diversity has been reported in fruit of *Hylocereus polyrhizus* (Esquivel, Stintzing, & Carle, 2007; Zou et al., 2005). In the case of fruit of *Opuntia* spp., Castellanos-Santiago and Yahia (2008) and Cejudo-Basante, Chalaal, Louaileche, Parrado and Heredia (2014) reported higher diversity of betaxanthins than betacyanins. However, Stintzing et al. (2005) reported higher number of betacyanins than betaxanthins. The proportion of these two groups of betalains and the particular type inside each group has impact in the color of fruit of the *Opuntia* genus (Castellanos-Santiago & Yahia, 2008).

Peak 1 and 2 presented λ_{max} at 478 nm and an $[M + H]^+$ ion at m/z 309 releasing an MS^2 fragment at m/z 263, which was coherent

Table 1

Color parameters in fruits of the ecotypes orange (*SpO*) and red (*SpR*) of *S. pruinosus*, and red (*SsR*) and white (*SsW*) of *S. stellatus*.

Ecotypes	L^*	a^*	b^*	h_{ab} ($^{\circ}$)	C^*
<i>SpO</i>	27.85 \pm 7.31 b	35.30 \pm 4.74 a	24.72 \pm 1.54 a	35.28 \pm 4.83 b	43.21 \pm 3.62 a
<i>SpR</i>	20.82 \pm 2.51 b	28.54 \pm 7.00 b	11.81 \pm 1.97 b	22.87 \pm 3.12 c	30.92 \pm 7.058 b
<i>SsR</i>	15.73 \pm 1.44 c	9.70 \pm 2.75 c	2.74 \pm 0.94 c	15.50 \pm 1.47 d	10.08 \pm 2.90 c
<i>SsW</i>	41.98 \pm 2.76 a	-0.35 \pm 0.18 d	4.08 \pm 0.91 c	95.29 \pm 2.51 a	4.10 \pm 0.98 c

L^* : luminosity (%); h_{ab} : hue; C^* : chroma. Means with the same letter inside the same row indicate that there is no significant difference, according to the means comparison routines (Tukey, $p < 0.05$).

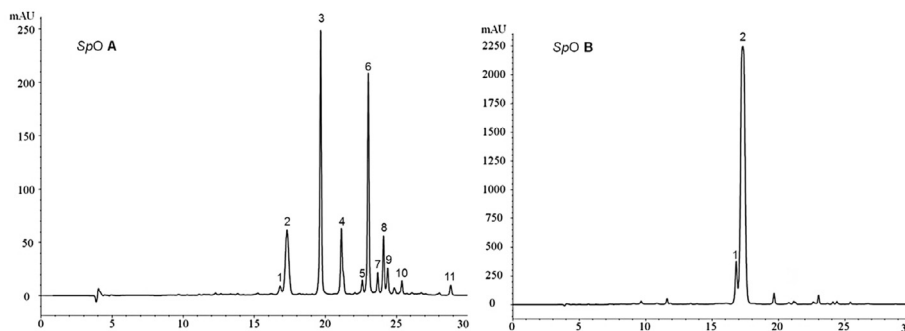


Fig. 1. HPLC profiles of betalains from the flesh of orange ecotype fruit of *Stenocereus pruinosus* (SpO) recorded at 530 nm (A) and 480 nm (B). Peaks identities are in Table 2.

with the compounds proline-betaxanthin (indicaxanthin) and its isomer, isoindicaxanthin. Indicaxanthin is a very common betalain that is present in cactus pear (*Opuntia ficus-indica*) (Castellanos-Santiago & Yahia, 2008; Cejudo-Bastante, Chalaal, Louaileche, Parrado, & Heredia, 2014).

Peaks 3 and 4 presented λ_{\max} at 536–538 nm and an $[M + H]^+$ ion at m/z 551 releasing an MS^2 fragment at m/z 389 (–162 mu, loss of a hexosyl moiety), pointing to they corresponded to betanidin-glucosides, a type of dopamine-derived betalains (Kobayashi, Schmidt, Wray, & Schliemann, 2001; Strack, Vogt, & Schliemann, 2003). An identity as (iso)betanin [i.e., (iso)betanidin-5-*O*- β -glucoside] was discarded by comparison with a betalain extract from beetroot. Thus, these compounds were assigned as gomphrenin-I [betanidin-6-*O*- β -glucoside] and isogomphrenin-I [isobetanidin-6-*O*- β -glucoside], respectively, which have also been reported in purple *pitahaya* fruit (i.e., *Hylocereus* spp.) (Cai, Xing, Sun, & Corke, 2006; Esquivel et al., 2007) and cactus pear (*Opuntia* spp.) (Castellanos-Santiago & Yahia, 2008; Stintzing et al., 2005).

Peaks 5, 9 and 11, showing λ_{\max} at 498–516 nm, were associated as 2-descarboxy-betanidin derivatives (Kobayashi et al., 2001; Strack et al., 2003). Peak 5 presented an $[M + H]^+$ ion at m/z 507, 44 mu lower than betanin, i.e., less a carboxylic group, and released an MS^2 fragment at m/z 345 (–162 mu, loss of a hexosyl moiety). This allowed its tentative identification as 2-descarboxy-betanin, reported in beetroot (Kobayashi et al., 2001; Strack et al., 2003), but also in some Cactaceae (Morales, Barros, Ramirez-Moreno, Santos-Buelga, & Ferreira, 2015). Peak 9 and 11 showed $[M + H]^+$ at m/z 593, 86 mu greater than peak 5, suggesting the presence of an additional malonyl residue, and produced MS^2 fragments at m/z 549 (–44 mu, loss of CO_2 ; carboxylic moiety), 507 (–86 mu, loss of a malonyl residue) and 345 (–162–86 mu, loss of a malonylhexoside residue), which allowed their identification as malonyl-2-descarboxy-betanin isomers. Although, the linkage of the malonyl residue cannot be attributed from the mass spectra, substitution at the 6'-*O*-position of the glucose was assumed, as previously has been described (Kobayashi et al., 2001; Strack et al., 2003). Thus, the compounds were tentatively assigned as 6'-*O*-malonyl-2-descarboxy-betanin [2-descarboxy-betanidin 5-*O*-(6'-*O*-malonyl- β -D-glucoside)] and 6'-*O*-malonyl-2-descarboxy-isobetanin, respectively, considering their relative order of elution with isobetanin eluting later than betanin [e.g., 7, 20, 22, 25, 27]).

Peaks 6, 7 and 8 showed similar absorption spectra with λ_{\max} at 536–538 nm and the same m/z value $[M + H]^+$ at 637, 86 mu greater than betanin. These features are coherent with malonyl-betanin isomers, pigments that are characteristic of flowers and fruits of Cactaceae (Kobayashi et al., 2001; Strack et al., 2003), and that have been reported in fruits from red-purple *pitahaya* (*Hylocereus polyrhizus*) (Stintzing et al., 2002; Cai et al., 2006;

Wybraniec, Nowak-Wydra, Mitka, Kowalski, & Mizrahi, 2007). Based on their abundance in the extracts and relative elution order, peaks 6 and 8 were tentatively identified as phylloactin [betanidin 5-*O*-(6'-*O*-malonyl- β -D-glucoside)] and isophylloactin [isobetanidin 5-*O*-(6'-*O*-malonyl- β -D-glucoside)], respectively. Minority peak 7 might correspond to betanidin-5-*O*-(6'-*O*-3-hydroxy-butyl)- β -glucoside (Stintzing et al., 2002) or 4'-*O*-malonyl-betanin (Wybraniec, Stalica, Spórna, & Mizrahi, 2010), betacyanins that have also been reported in purple pitaya. Peak 10 ($[M + H]^+$ at m/z 651) would correspond to a betanidin derivative, as revealed by the MS^2 fragment ion produced at m/z 389, although no further support could be obtained to propose an identity.

3.3. Quantification of betalains by HPLC

The quantification of betalains is commonly performed by spectrophotometry and using a calculation where the absorbance is taking into account, besides to the molar extinction coefficient, and the molecular weight of betanin, in the case of betacyanins, or indicaxanthin, in the case of betaxanthins (Castellanos-Santiago & Yahia, 2008). In the present work, each of the identified betaxanthins and betacyanins were quantified from the area under the curve of peaks obtained in chromatograms of HPLC-DAD detected at 480 nm and 530 nm, respectively, by comparison with standard curves of indicaxanthin (betaxanthins) and gomphrenin I (betacyanins). The content of betacyanins was lower than betaxanthins in the three ecotypes analyzed (Table 2). Largest diversity and content of betacyanins were found in the red fruit samples. Values ranged from 165.1 ± 7.0 to 5423.4 ± 135.3 mg/g dry weight (DW), with the greatest ($p \leq 0.05$) quantities found in the ecotype SpR. The main difference between red ecotypes was observed in the contents of gomphrenin I and isogomphrenin I, which were higher ($p \geq 0.05$) in SpR in relation to SsR. In the orange fruit (SpO) only three betacyanins (isogomphrenin I, phylloactin and isophylloactin) were detected, with contents that were significantly lower than those observed in red fruits, and with dominance of phylloactin.

Betaxanthins were found in much greater levels than betacyanins, with values ranging between $17,706.7 \pm 1128.1$ and $22,053.5 \pm 329.1$ μ g/g DW, but without statistical differences between the analyzed ecotypes (SpO, SpR, and SsR). The content of betacyanins obtained for the fruits of the three analyzed *Stenocereus* spp. ecotypes were comparable to those reported by Castellanos-Santiago and Yahia (2008) in the flesh of 10 cultivars of *Opuntia* spp., who determined values that varied from 50 to 5290 μ g/g of dry flesh, using water as extraction solvent. These authors also reported values of betaxanthins between 120 and 2860 μ g/g of dry pulp, well below than the ones obtained in the present study. Cejudo-Bastante et al. (2014) analyzed betalains in the fruit of three cultivars of *O. ficus indica* [L.] Mill using aqueous

Table 2

Identification and quantification of betalains by HPLC-DAD-MS in the flesh fruit of the ecotypes, red (*SpR*) and orange (*SpO*) of *S. pruinus*., and of red fruits of *Stenocereus stellatus* (*SsR*). Concentrations are in $\mu\text{g/g DW}$.

Peak	Rt (min)	λ_{max} (nm)	[M + H] ⁺ (m/z)	MS ² (m/z)	Tentative identity	References ^a	SpO	SpR	SsR
<i>Betacyanins</i>									
3	19.6	536	551	389	Gomphrenin I	1, 2, 4, 5, 8, 9	t	3276.08 ± 9.24a	509.09 ± 19.60b
4	21.1	538	551	389	Isogomphrenin I	1, 2, 4, 5, 8, 9	36.92 ± 7.4b	972.57 ± 69.25a	59.70 ± 5.65b
5	22.7	498	507	345	2-descarboxy-betanin	5, 6, 9	t	65.69 ± 26.18a	t
6	23.1	536	637	593,551,389	Phyllocactin	1, 5, 7, 9, 10	109.11 ± 0.33b	798.38 ± 57.6ab	1449.66 ± 273.24a
7	23.8	536	637	–	4'-O-malonyl-betanin or betanidin-5-O-(6'-O-3-hydroxy-butyryl)- β -glucoside	1, 5, 7, 9, 10, 11	t	9.20 ± 4.54b	53.95 ± 13.98a
8	24.2	538	637	593,551,389	Isophyllocactin	1, 5, 7, 9, 10	16.05 ± 0.7b	190.60 ± 7.02ab	269.97 ± 63.82a
9	24.5	512	593	549,507,345	6'-O-malonyl-2-descarboxy-betanin	5, 9	t	42.19 ± 2.74a	35.69 ± 5.32a
10	25.4	514	651	389	Betanidin derivative		t	55.66 ± 18.35a	68.65 ± 46.74a
11	28.9	516	593	549,507,345	6'-O-malonyl-2-descarboxy-isobetanin	5, 9	t	t	79.08 ± 2.52a
Total betacyanins							162.07 ± 7.03c	5423.38 ± 135.32a	2525.82 ± 326.76b
<i>Betaxanthins</i>									
1	16.9	478	309	263,235	Isoindicaxanthin	2, 3	1158.64 ± 147.34a	1143.38 ± 148.42a	1026.44 ± 179.65a
2	17.4	478	309	263,235	Indicaxanthin	2, 3	20894.82 ± 181.76a	16563.32 ± 979.68a	20970.47 ± 2363.25a
Total betaxanthins							22053.46 ± 329.09a	17706.72 ± 1128.12a	21681.66 ± 1097.06a

Means with the same letter inside the same row indicate that there is no significant difference, according to the means comparison routines (Tukey, $p < 0.05$). t: traces.

^aReferences used to support compounds identification: (1) Cai et al., 2006; (2) Castellanos-Santiago and Yahia, 2008; (3) Cejudo-Bastante et al., 2014; (4) Esquivel et al., 2007; (5) Kobayashi et al., 2001; (6) Morales et al., 2015; (7) Stintzing et al., 2002b; (8) Stintzing et al., 2005; (9) Strack et al., 2003; (10) Wybraniec et al., 2007; (11) Wybraniec et al., 2010.

methanol as solvent and reported levels of betaxanthins well above of betacyanins. Nevertheless, they do not actually quantified the compounds, as they just expressed the level of the different betalains identified through the area under the curve of each peak and, due to this, it is not possible to make a strict comparison with the results obtained herein. However, the ratio betaxanthins/betacyanins resulted in values that ranged from 34.03 in the case of red cultivar, to 170.9 in yellow-fleshed cultivars. In the case of fruit of *Stenocereus* of the present work the ratio between both groups (betaxanthins/betacyanins) was 136.07, 3.26, and 8.8, for *SpO*, *SsR*, and *SpR*, respectively. Fathordoobady, Mirhosseini, Selamat, and Abd Manap (2016) showed that the amount of betalains extracted from *H. polyrhizus* doubled when the solvent was modified from water to a mixture of ethanol:water (90:10 v/v). Pérez-Loredo, García-Ochoa, and Barragán-Huerta (2015) reported contents of betacyanins in fruits of *Stenocereus stellatus* with orange and red flesh of 135.2 ± 3.5 and $1457.2 \pm 55.2 \mu\text{g}$ of betanin per gram of dry sample, respectively. In addition, the contents of betaxanthins in these materials were 928.7 ± 11.9 and $1511.3 \pm 62.2 \mu\text{g}$ of indicaxanthin per gram of dry flesh. Data for betacyanins are similar to those obtained in the present work, but those of betaxanthins are far below. Indeed, it becomes clear that the solvent used for the extraction played an important role in the recovery of betalains from cactus fruit. It is to say that in most of the reported studies, betalains have been extracted with water and they have been quantified by spectrophotometry, due to the lack of standards.

The predominance of any betalain in fruit of cactus varies among genera. The fruits of *H. polyrhizus* have betanin and phyllocactin as predominant betalains (Wybraniec et al., 2001). In *Opuntia* spp. the dominant betalain depends on the color of the fruit; in materials with purple color in flesh, as cultivar Camuesa, the predominant betalains are betanin and indicaxanthin (Castellanos-Santiago & Yahia, 2008).

3.4. Analysis of phenolic compounds

Identification and quantification of individual phenolic compounds of *S. pruinus* and *S. stellatus* were performed by HPLC-

DAD-ESI/MS. Up to thirteen compounds of five different phenolic classes could be detected and tentatively identified based on their retention time and absorption and mass spectra (Table 3). Among them, quercetin 3-*O*-rutinoside (peak 8) and isorhamnetin 3-*O*-glucoside (peak 12) were positively identified by comparison with commercial standards.

Peaks 1, 3, 4, and 5 showed UV spectra that coincided with hydroxycinnamoyl derivatives. Compounds 1 and 3 possessed the same pseudomolecular ion $[\text{M}-\text{H}]^-$ at m/z 341, yielding MS² product ions at m/z 179 ($[\text{caffeic acid}-\text{H}]^-$, from the loss of a hexosyl moiety, 162 mu) and 135 ($[\text{caffeic acid}-\text{H}-\text{CO}_2]^-$), which allowed assigning them as caffeoyl hexosides. Similarly, peak 4 ($[\text{M}-\text{H}]^-$ at m/z 517) was identified as a feruloyl dihexoside based on the production of the fragment ions at m/z 193 mu ($[\text{ferulic acid}-\text{H}]^-$; loss of two hexosyl moieties, 324 mu) and 149 ($[\text{ferulic acid}-\text{H}-\text{CO}_2]^-$). Peak 5 showed a precursor ion $[\text{M}-\text{H}]^-$ at m/z 337 and MS² product ions at m/z 191 ($[\text{quinic acid}-\text{H}]^-$), 173 ($[\text{quinic acid}-\text{H}-\text{H}_2\text{O}]^-$), 163 ($[\text{coumaric acid}-\text{H}]^-$) and 145 ($[\text{coumaric acid}-\text{H}-\text{H}_2\text{O}]^-$), which allowed its identification as a coumaroylquinic acid.

Compounds 9 ($[\text{M}-\text{H}]^-$ at m/z 447) and 11 ($[\text{M}-\text{H}]^-$ at m/z 623) showed absorption spectra characteristic of flavonols and were identified as kaempferol and isorhamnetin hexosides based on the aglycone fragment ions observed at m/z 285 and 315, respectively, from the loss of a hexosyl residue in both cases. The pseudomolecular ion of peak 11 was the same as isorhamnetin-3-*O*-glucoside, suggesting that peak 12 might be the corresponding galactoside, typically eluting before the equivalent glucoside in RP-HPLC (Santos-Buelga, García-Viguera, & Tomás-Barberán, 2003); nonetheless, the possibility of being a glucoside substituting other position of the isorhamnetin cannot be discarded either. Peaks 6 ($[\text{M}-\text{H}]^-$ at m/z 449), 11 ($[\text{M}-\text{H}]^-$ at m/z 491) and 13 ($[\text{M}-\text{H}]^-$ at m/z 475) showed UV spectra characteristic of flavanones and were assigned as eridictyol-*O*-hexoside, eridictyol-*O*-acetylhexoside, and naringenin-*O*-acetylhexoside, respectively, according to their pseudomolecular ions and MS² fragments produced from the loss of hexosyl (–162 mu) or acetylhexoside (–162 + 42 mu) moieties. Compound 7 was identified as taxifolin acetylhexoside, owing to its UV spectrum typical of a dihy-

Table 3
Retention time (Rt), wavelengths of maximum absorption in visible region (λ_{\max}), mass spectral data, tentative identification, and quantification of phenolic compounds in fruit of *S. pruinosus* (SpR: red flesh ecotype, SpO: orange flesh ecotype) and *S. stellatus* (SsR: red flesh ecotype and SsB: white flesh ecotype). Concentrations are in $\mu\text{g/g DW}$.

Peak	Rt (min)	λ_{\max} (nm)	[M + H] ⁺ (m/z)	MS ² (m/z)	Tentative identity	References ^a	SpO	SpR	SsR	SsW
<i>Hydroxycinnamoyl derivatives</i>										
1	7.4	316	341	179,135	Caffeoyl hexoside I	1, 2, 4	17.48 ± 2.31b	35.4 ± 3.61a	18.09 ± 0.28b	14.07 ± 0.72b
3	8.8	316	341	179,135	Caffeoyl hexoside II	1, 2, 4	7.91 ± 0.83b	23.94 ± 2.2a	13.93 ± 2.04b	12.69 ± 0.68b
4	11.1	316	517	193,149	Feruloyl dihexoside	1, 2, 3, 4	8.80 ± 1.13b	nd	28.37 ± 7.92a	45.24 ± 5.55a
5	12	300	337	191,173,163,145	<i>p</i> -coumaroyl quinic acid	1, 4	7.51 ± 0.10d	12.14 ± 0.08c	15.61 ± 0.54b	19.97 ± 0.52a
Total hydroxycinnamoyl derivatives							41.7 ± 1.09	71.48 ± 1.96	76.0 ± 2.69	91.97 ± 1.86
<i>Flavonols</i>										
8	19.4	352	609	301	Quercetin 3- <i>O</i> -rutinoside		4.16 ± 0.17 b	2.91 ± 0.13 b	7.09 ± 0.17a	7.19 ± 0.54a
9	20.7	348	447	285	Kaempferol hexoside		nd	nd	3.50 ± 0.24a	4.06 ± 0.25a
11	23.4	356	623	315	Isorhamnetin hexoside		1.31 ± 0.13 a	1.17 ± 0.14 a	nd	nd
12	24	356	623	315	Isorhamnetin 3- <i>O</i> -glucoside		1.29 ± 0.01	nd	nd	nd
Total flavonols							6.76 ± 0.10	4.08 ± 0.13	10.59 ± 0.20	11.25 ± 0.39
<i>Flavanones</i>										
6	17.2	284	449	287	Eriodictyol hexoside		nd	nd	8.98 ± 1.33a	nd
10	22.4	284	491	287	Eriodictyol acetylhexoside		nd	nd	12.31 ± 0.99a	16.25 ± 1.77a
13	27.6	282	475	271	Naringenin acetylhexoside		nd	nd	5.91 ± 0.07a	nd
Total flavanones									14.89 ± 0.79	16.25 ± 1.77
<i>Others</i>										
2	8	278	137	119	Tyrosol		Not quantified			
7	18.2	296	507	303	Taxifolin acetylhexoside		5.25 ± 0.55b	nd	8.02 ± 1.43b	21.08 ± 2.56a
Total phenolic compounds							53.75 ± 2.95b	75.58 ± 1.61b	121.86 ± 8.11a	140.59 ± 9.06a

Means with the same letter inside the same row indicate that there is no significant difference, according to the means comparison routines (Tukey, $p < 0.05$). nd: not detected.

^aReferences used to support compounds identification: (1) Herrera-Hernández et al., 2011; (2) Morales et al., 2015; (3) Morales et al., 2014; (4) Osorio-Esquivel et al., 2011.

droflavonol and its pseudomolecular ion [M-H]⁻ at m/z 507, releasing a MS² fragment at m/z 303 ($-162 + 42$ mu). Finally, absorption and mass characteristics spectra of peak 2 and its comparison with a commercial standard allowed its identification as tyrosol, a widespread phenolic alcohol. The remaining peaks that can be observed in the HPLC chromatograms (Fig. 2) could not be assigned, although they might not correspond to phenolic compounds.

Phenolic acid derivatives and flavonols were present in the four ecotypes fruit of *Stenocereus*, which did not occur with flavanones that were present only in the fruits of *S. stellatus* (Table 3). The derivatives of caffeic acid (caffeoyl hexoside I and II) and *p*-coumaroylquinic acid were common in the four type of fruit analyzed, while feruloyl dihexoside was present only in three (SpO, SsR, and SsW). Caffeoyl hexoside has been reported by Morales et al. (2015) to be present in *joconostle* (*Opuntia matudae* Scheinvar) cv. Rosa. Among flavonoids, quercetin 3-*O* rutinoside was present in the four ecotypes of fruit, but in smaller amounts in SpO and SpR. This flavonol, besides isorhamnetin 3-*O*-glucoside and isorhamnetin hexoside, have been identified in *Opuntia joconostle* (Morales, Barros, Ramirez-Moreno, Santos-Buelga, & Ferreira, 2014). Flavanones were present only in the fruit of *S. stellatus* species (SsR and SsW). There are no reports about the presence of flavanones in fruit of cacti species. In fruits of *O. joconostle*, *O. macrorhiza* and *Myrtillocactus* sp. both phenolic acids and flavonols have been identified (Osorio-Esquivel, Ortiz-Moreno, Álvarez, Dorantes-Álvarez, & Giusti, 2011; Herrera-Hernández, Guevara-Lara, Reynoso-Camacho, & Guzmán-Maldonado, 2011). Particularly, in the mesocarp and endocarp of *O. matudae*, many flavonols have been identified, but only one phenolic acid derivative (ferulic

acid glucoside) (Morales et al., 2015; Morales et al., 2014). Other phenolic acids that were identified in *O. joconostle* were protocatechuic, vanilic, 4-hydroxybenzoic, and hydroxycinnamic acids (Osorio-Esquivel, Ortiz-Moreno, Álvarez, Dorantes-Álvarez, & Giusti, 2011). In *Myrtillocactus* sp. caffeic and gallic acids (Guzmán-Maldonado et al., 2010), as well as protocatechuic acid and ellagic acid (Herrera-Hernández et al., 2011) have been described.

Among the four ecotypes studied, the white fruit of *S. stellatus* had the highest numerical value in total phenolic compounds, although it was statistically equal ($p > 0.05$) to the fruit with red flesh (SpR), which in turn was equal to SsR. The fruit with orange flesh (SpO) had the lowest content. In the *Hylocereus* genus, higher content of total phenolics in a species with intense red color (*H. polyrhizus*) than the white species (*H. undatus*) have been reported (Suh et al., 2014). However, those authors performed the quantification of total phenolics with the Folin-Ciocalteu method, so in the red fruit the value could have been overestimated, since aromatic rings of betalains can react with the Folin-Ciocalteu reagent, as well as with other reducing substances present in the extracts.

3.5. Antioxidant capacity

The antioxidant capacity (AC) among the four ecotypes of fruit analyzed was different. The fruit of *S. stellatus* with red flesh (SsR) had the highest AA (9.21 ± 0.84 $\mu\text{mol TE/g}$ fresh pulp) followed by SpR (6.80 ± 0.33). The lesser AC was observed in fruits of SpO and SsW (Fig. 3). The AC of pitaya fruit is due to the presence of both, betalains and phenolic compounds, which have the ability

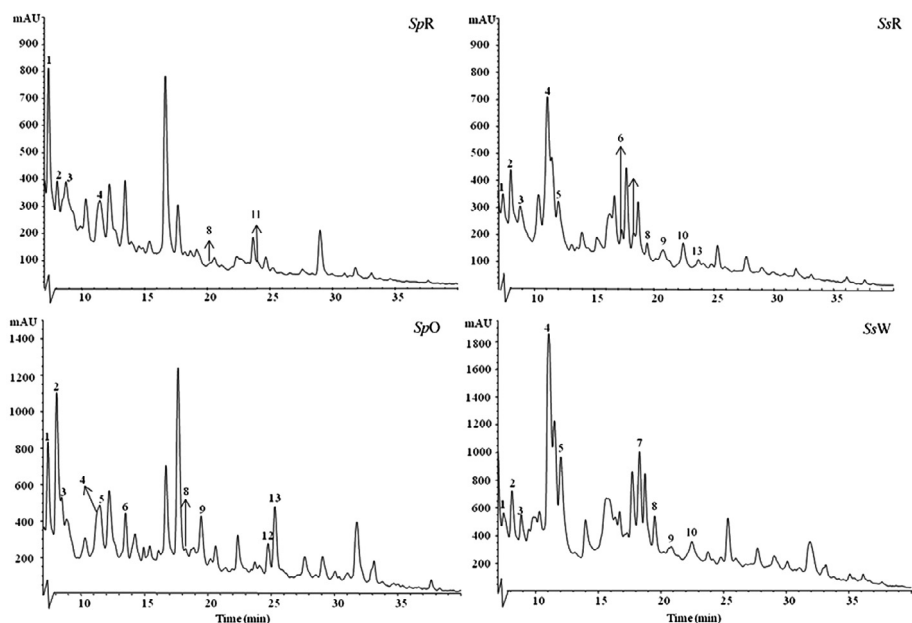


Fig. 2. HPLC profile in fruits of two ecotypes of *Stenocereus pruinosus* (SpR: red ecotype and SpO: orange ecotype) and two ecotypes of *S. stellatus* (SsR: red ecotype and SsW: white ecotype) recorded at 280 nm.

to donate their electrons and to scavenge the ABTS cation radical as Trolox does (Rice-Evans, Miller, & Paganga, 1997; Butera et al., 2002). However, the interactions that occur between different types of compounds within each group, to express a particular antioxidant capacity, are not fully understood. In prickly pears of *Opuntia ficus indica* with yellow, red and white flesh, the AC reported with ABTS assay was of 5.31 ± 49 , 4.20 ± 0.51 and 4.36 ± 0.41 $\mu\text{mol TE/g}$ fresh pulp, respectively. Of the two betalains predominant in the fruits, betanin showed a much higher AC than indicaxanthin, however, the red fruit, with the highest betanin content, did not present the highest AC (Butera et al., 2002). The AC of the most abundant betalain present in the fruit does not always correlate with the AC expected for the whole fruit, but rather is the result of the interaction of all the antioxidant compounds it contains.

Both, betalains and phenolic compounds could influence the AC of pitaya fruit. According to the Pearson correlation analysis, the content of total betalains was the variable that showed the highest

positive correlation ($r = 0.7664$, $p < 0.01$) with the AC of the analyzed samples; while the phenolics content showed a non-significant correlation (data not shown). The non-significant correlation between phenolic compounds and AC may be due to the marginal contribution of polyphenols in relation to the total amount of betalains.

The content of betalains was similar in the samples SpR, SpO, and SsR (Table 2); however, the total content of phenolic compounds was higher in fruit of SsR, which may be influencing its antioxidant capacity. It should be noted that although the SsW ecotype had the highest content of phenolic compounds (Table 3) this was not reflected on its AC value. One plausible explanation could be that since this particular ecotype lacks betalains the contribution to the AC of the total phenolics present did not outweigh the AC of ecotypes with these pigments. Similarly, the extracts from pitaya fruit can contain other reducing substances that could also influence in the antioxidant capacity. In this regard, it has been reported that in fruit where the concentration of total soluble phenolics is higher, the antioxidant capacity is mostly influenced by such compounds, despite other potent antioxidants such as vitamin C and E, and β -carotene could be present (Jimenez-Aguilar, Lopez-Martinez, Hernandez-Brenes, Gutierrez-Urbe, & Welti-Chanes, 2015).

4. Conclusions

The betalains pattern of *Stenocereus* species fruit was different to that reported for *Hylocereus* spp. and *Opuntia* spp. The profiles and concentrations of both betalains and phenolic compounds in fruits of the two species of *Stenocereus* studied varied by color of flesh and among species. In red varieties, betalains were present in much higher levels than phenolic compounds, however, the contribution of these latter to the antioxidant potential of pitaya fruits seemed evident. Thus, in samples with similar betalain concentrations (i.e., SpO and SsR), significantly higher antioxidant capacity was observed in those with greater phenolic content. Furthermore, white fruits (SsW), despite lacking betalains, yielded similar antioxidant values as the orange sample SpO with lower phenolic content. The presence of flavanones in *Stenocereus stellatus*, a class

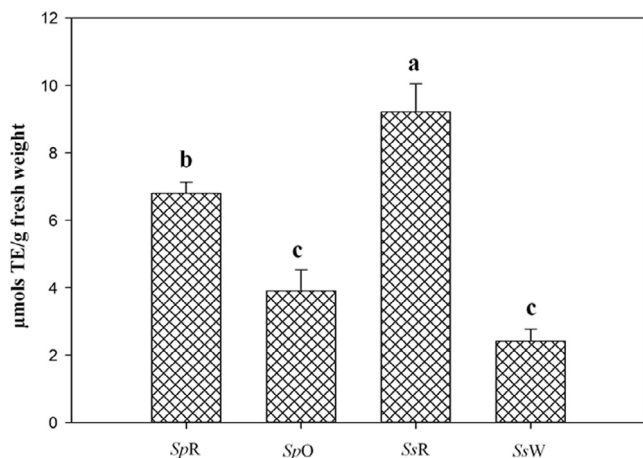


Fig. 3. Antioxidant capacity in the fruits of *S. pruinosus* (ecotypes SpR, with red flesh and SpO, with orange flesh) and of *S. stellatus* (ecotypes SsR, with red flesh, and SsW, with white flesh) determined by the ABTS assay. Means with the same letter indicate that values are not significantly different (Tukey, $p < 0.05$).

of phenolic compounds that has been not previously reported in cacti fruits, showed the enormous wealth of phytochemicals that this genus possesses and that could be exploited in benefit to human health.

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