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Identification and Quantification of Betalains from the Fruits of 10 Mexican Prickly Pear Cultivars by High-Performance Liquid Chromatography and Electrospray Ionization Mass Spectrometry

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Qualitative and quantitative analyses of betalain pigments in 10 cultivars/lines of prickly pear (*Opuntia* spp.) fruit grown in Mexico were conducted with reverse phase high-performance liquid chromatography−diode array detection (HPLC-DAD) coupled with electrospray mass spectrometry (ESI-MS). Betacyanins and betaxanthins were identified by comparison with the UV/vis and mass spectrometric characteristics as well as the retention times of semisynthesized reference betaxanthins. Data revealed that the ratio and concentration of betalain pigments are responsible for the color in the different cultivars, showing the highest betalains content in the fruit of purple colored Camuesa (*O. robusta Wendl.*) (8.1 mg/g dry fruit), which is comparable to that found in red beet (*Beta vulgaris* L. ssp. Var. Pablo) (8.6 mg/g dry tissue). Yellow betalains were absent in Reyna (*O. alba-carpa*) prickly pear cultivar. A total of 24 known/unknown betalains were present in the prickly pear fruit samples studied, including 18 betaxanthins and 6 betacyanins. Our results indicate that prickly pear cultivars can be considered as a potential source of yellow and red natural colorants.

KEYWORDS: Cactacea; *opuntia*; cactus pear; betalains; betaxanthins; betacyanins; mass spectrometry

INTRODUCTION

Betalains are water-soluble, nitrogen-containing vacuolar pigments. These compounds replace the anthocyanins in flowers and fruits of plants of most families of the Caryophyllales (*1*), representing a very interesting pigment class. The term betalain was introduced to describe these pigments as derivatives from betalamic acid (*2*). Betalain pigments were first isolated from the red roots of *Beta vulgaris*, which gave this class of pigments its name, and is used to color a wide range of processed food products. They are also found in some species of the fungal genera *Amanita* and *Hygrocybe* (*3, 4*). In addition to *Beta vulgaris* (family Chenopodiaceae), they are found in amaranth seeds (Amaranthaceae) (*5*), *Bougainvillaea* bracts (Nyctaginaceae), and flowers (*6–9*) or other plant parts within the Aizoaceae (*10*), Basellaceae (*11*), Didieraceae, Phytolaccaceae (*12*), and Portulaceae (*13*). Betalains may be purple to red (base forms, betacyanins) and orange to yellow (acid forms, betaxanthins), and often the color is bright. The knowledge of the chemistry of betalain started with the work of Dreiding and coworkers (*14*). Betalains have a number of health properties. Infusions of betalains from the bracts of *Bougainvillaea* mixed with honey, for example, are used to treat coughs in some regions of Mexico (*15*). Some antiviral and antimicrobial activity has been attributed to betalains (*16*). The main focus of interest, however, has recently been on betalain pigments as natural antioxidants (*17*).

Despite being afflicted with an earthy smell due to high levels of the odorant geosmin (*18*) and 2-*sec*-butyl-2-methoxypyrazine (*19*), high nitrate levels, microbial contamination (*20*), and a very limited range of color, red beet roots are currently exploited exclusively for betalain extraction (*21*). Because some of the artificial colorants are known to be a risk to human health and natural colorants have potential health benefits and have been popular, purple, red, and yellow cactus pear fruit could be a promising crop as a betalain source, especially because of their fresh odor and flavor, showing better nutritional properties compared to those of red beet root (*22*).

Prickly pear (*Opuntia* spp.) fruit, also known as tuna in Mexico and nochtli in the Nahuatl language, is one of the most representative fruits in Mexican culture and has recently gained attention for its nutritional and potential technological values (*23*). Evidences of its use have existed for more than 9000 years (*24*). *Opuntia* plants are characterized as tolerant to varied soils, temperatures, and moisture levels (*25*). Approximately 200 species are known in the world, and Mexico possesses a great genetic variability, with a diversity of fruit pulp tonalities (red, white, and yellow) and with a wide harvesting period including fruits of early (May), intermediate (August) and late maturation (November). Because of these particular characteristics and the fact that

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Table 1. Betaxanthins[a], Betacyanins[b], and Total Betalain Content in the Fruits of 10 Prickly Pear Cultivars/Lines and Red Beet

	betacyanins content (mg/g dry pulp)		betaxanthins content (mg/g dry pulp)		total betalains (mg/g dry pulp)	
cultivar	water	buffer	water	buffer	water	buffer
Camuesa	5.29 ± 0.35	5.01 ± 0.60	2.86 ± 0.24	2.56 ± 0.42	8.15	7.57
Roja Pelota	2.06 ± 0.06	1.86 ± 0.28	0.99 ± 0.03	0.84 ± 0.12	3.04	2.71
Cardona	2.04 ± 0.20	1.83 ± 0.00	1.04 ± 0.09	0.80 ± 0.00	3.08	2.63
2142	0.71 ± 0.04	0.66 ± 0.01	0.44 ± 0.03	0.38 ± 0.01	1.16	1.04
Liria	0.39 ± 0.03	0.34 ± 0.02	0.14 ± 0.01	0.11 ± 0.00	0.53	0.45
Roja Lisa	0.27 ± 0.01	0.22 ± 0.02	0.23 ± 0.02	0.18 ± 0.00	0.50	0.40
Naranjona	0.065 ± 0.01	0.04 ± 0.01	0.16 ± 0.02	0.12 ± 0.00	0.23	0.16
2651	0.072 ± 0.00	0.04 ± 0.01	0.14 ± 0.02	0.09 ± 0.01	0.21	0.13
21441	0.071 ± 0.00	0.05 ± 0.01	0.41 ± 0.02	0.35 ± 0.04	0.48	0.40
Reyna	0.05 ± 0.02	0.03 ± 0.03	0.12 ± 0.01	0.23 ± 0.20	0.17	0.26
red beet	5.41 ± 0.02	4.98 ± 0.00	3.21 ± 0.01	3.12 ± 0.00	8.60	8.10

[a] Pigment extraction using water or citrate−phosphate buffer. The results are the mean for three replicates ± standard error. [b] Pigment extraction using water or citrate−phosphate buffer. The results are the mean for three replicates ± standard error.

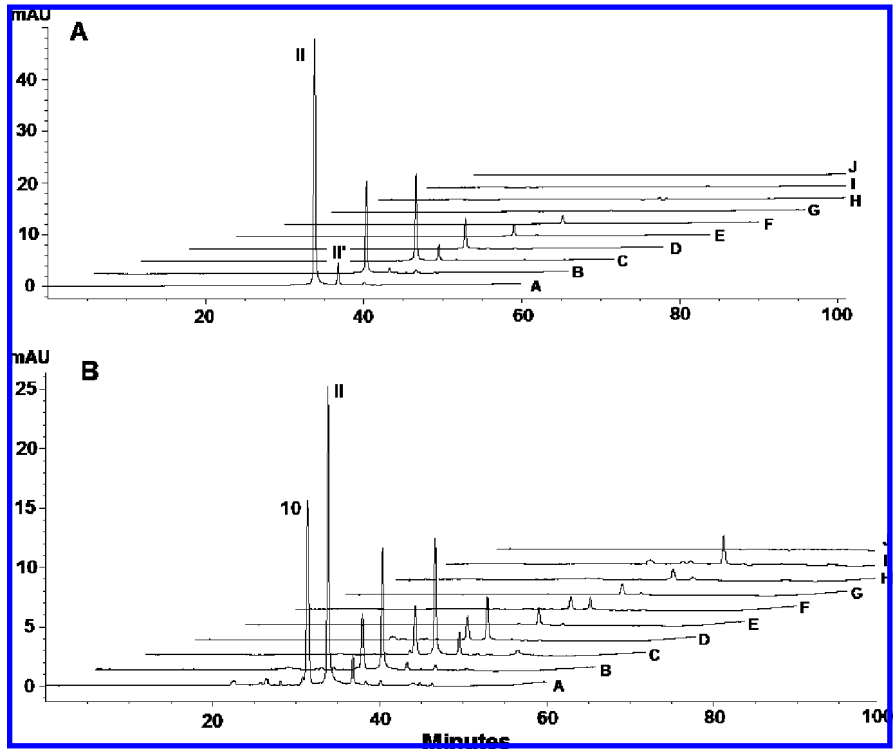

Figure 1. HPLC profile of (**A**) betacyanins (at 535 nm) and (**B**) bexanthins (at 482 nm) in aqueous extracts from 10 prickly pear cultivars and lines: (**A**) Camuesa, (**B**) Roja Pelota, (**C**) Cardona, (**D**) 2142, (**E**) Liria, (**F**) Roja Lisa, (**G**) Naranjona, (**H**) 2651, (**I**) 21441, (**J**) Reyna.

alternative uses for *Opuntia* plantations previously exploited for cochineal production are being searched, the use of prickly pear fruit as a source of valuable components presents a viable option. In addition to the betalain pigments present in prickly pear fruit, vitamins, minerals (*26*), amino acids, and sugars in the mucilage fraction appear to be highly valuable. Not only the fruit but also the green cladode may be used as food and for medicinal applications (*27*), and therefore, the cactus pear represents a very valuable plant complying with a high sustainability for food market in several aspects. The objective of this study was to identify betalain patterns and content in the fruits (pulp without seeds) of 10 Mexican prickly pear cultivars/lines using HPLC/ESI-MS.

MATERIALS AND METHODS

Chemicals and Solvents. Formic acid, methanol, acetonitrile, and other solvents of analytical or HPLC grade were purchased from J. T. Baker (Baker Mallinckrodt, Mexico). HPLC grade water was obtained by a Milli-Q plus water purification system (Millipore Corp., Bedford, MA). Amino acids and amines used for the condensation reaction with betalamic acid were from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material. Ten cultivars/lines of prickly pear fruits of purple, red, orange, yellow, and white pulp colors were selected for this study. The cultivars were Camuesa (*O. robusta* Wendl), Roja Pelota (*O. robusta*), Cardona (*O. streptacantha* Lemaire), Liria (*O. ficus-indica*), Roja Lisa (*O. ficus-indica* (L.) Mill), Naranjona (*O. megacantha*), and Reyna (*O. albi-carpa*), and the lines were 2651 (*O. ficus-indica*), 2142(*O. ficus-indica*), 21441 (*O. ficus-indica*). Fruits were provided by Instituto Nacional de Investigaciones Forestales y Agropecuarias, Guanajuato, Mexico. After manual peeling and removal of the seeds, the fruit pulp was immediately frozen in liquid nitrogen and then freeze-dried in order to improve pigment stability, which is sensitive to high temperatures and light. The dried powder was stored for up to four months in the dark at −20 °C until pigment extraction and analysis were finalized.

Pigment Extraction. Dry fruit tissue (100 mg) was stirred for 10 min in darkness in citrate−phosphate buffer (pH 6.5) or water (20 mL) as solvents. After they were stirred, the samples were centrifuged at

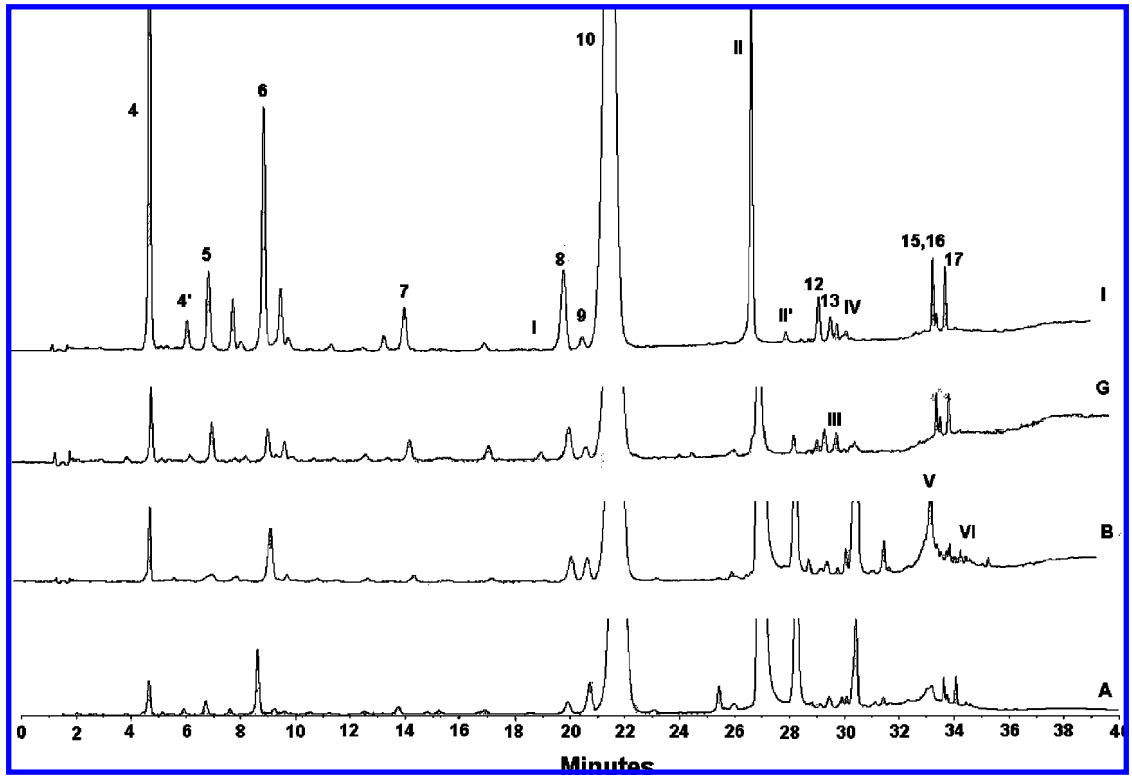

Figure 2. Peak assignment in the HPLC profile of betaxanthins and betacyanins, on the basis of that of **Table 2**, in purple (**A**) Camuesa, red (**B**) Roja Pelota, orange (**G**) Naranjona, and yellow (**I**) 21441 prickly pear fruit monitored at 482 and 535 nm.

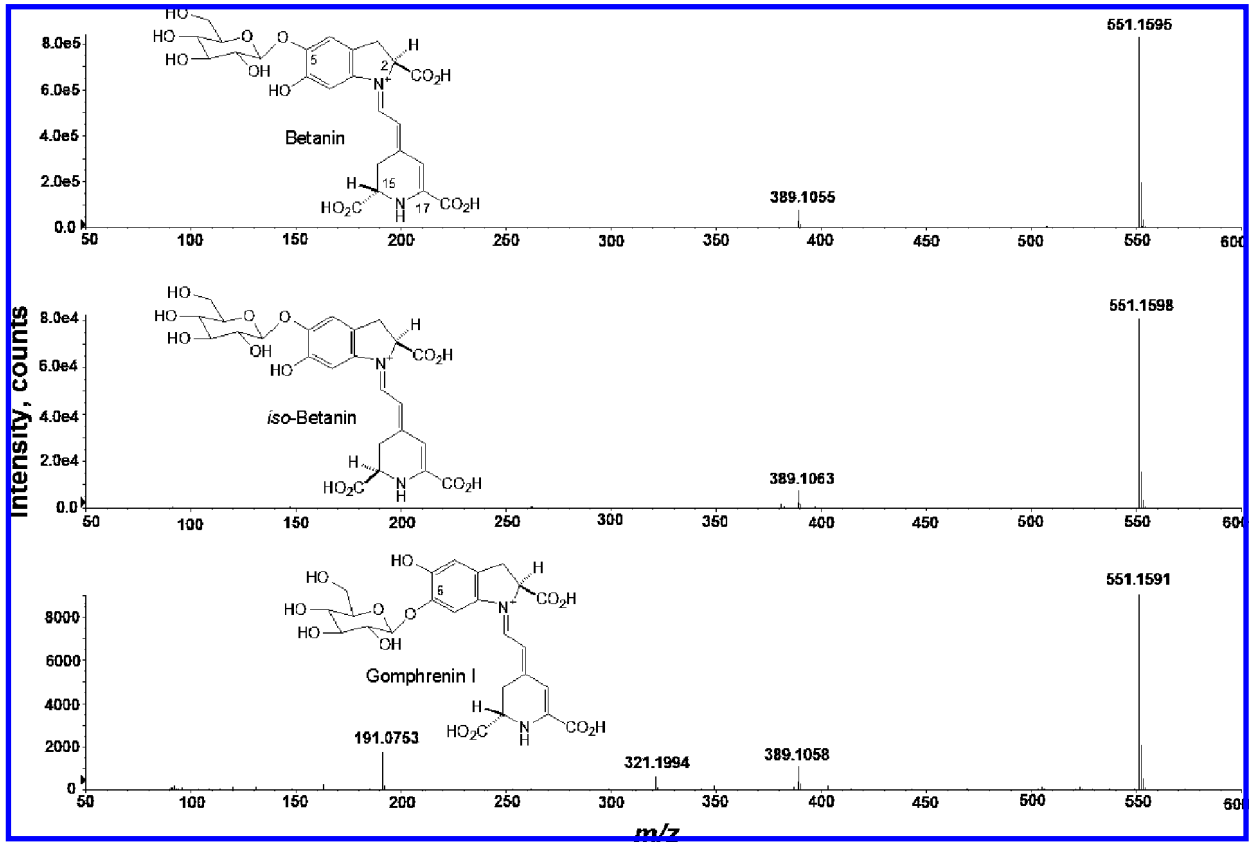

Figure 3. (**Top**) Positive ion spray mass spectra corresponding to betanin, (**middle**) *iso*-betanin, and (**bottom**) gomphrenin I as examples of the fragmentation pattern of betacyanin isomers. Parent ion scan of *m*/*z* 551 ($[M + H]^+$); daugther ion scan of *m*/*z* 389 [betanidin + H]$^+$.

12000 × *g* at 15 °C for 15 min in a Z323K Hermle centrifuge (Wehingen, Germany). Supernatants were filtered through a 0.45 μm nylon filter (Millipore Corp., Bedford, MA), and the extracts obtained were analyzed spectrophotometrically and by high-performance liquid chromatography (HPLC). Betanin was extracted for the partial synthesis of betaxanthin standards from the fresh roots of red beet (*Beta vulgaris* L. ssp. Var. Pablo). Beet roots were washed, peeled, and sliced before being freeze-dried. The dried powder was extracted using water as a

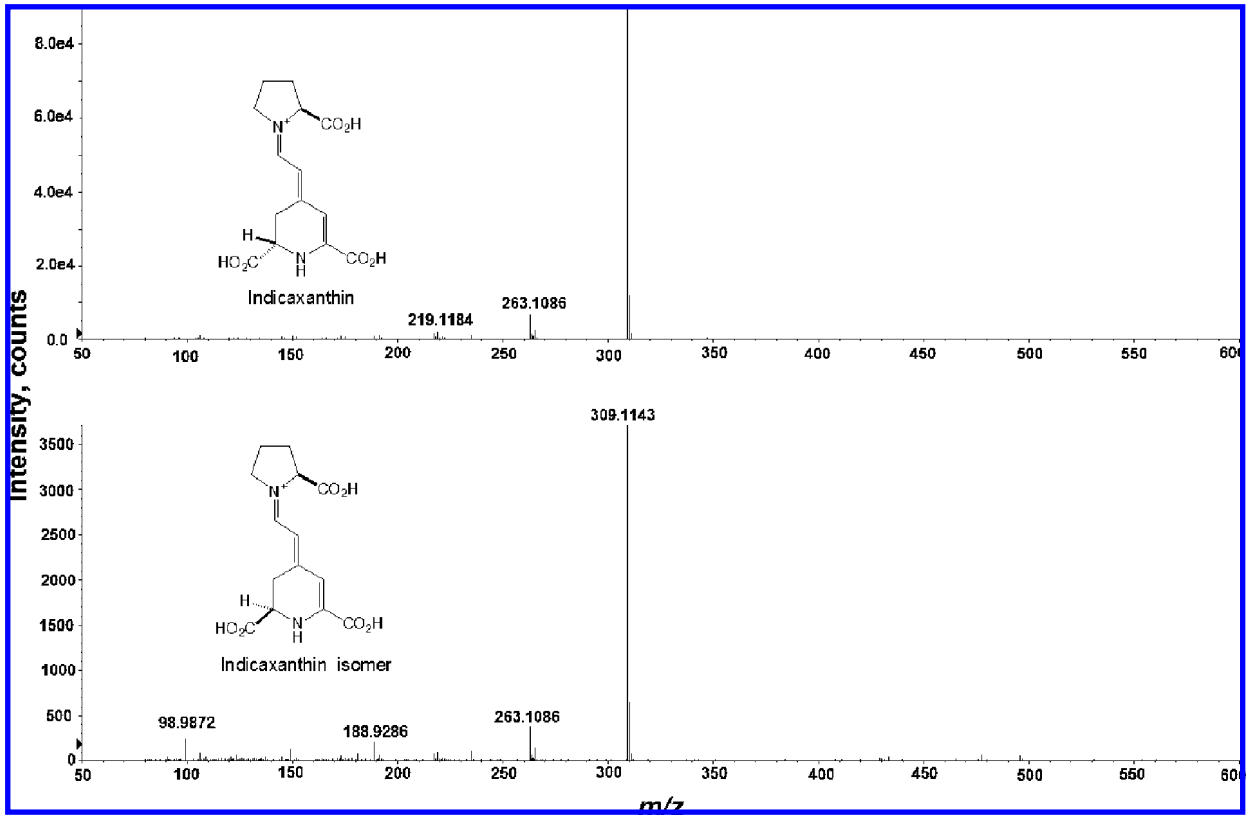

Figure 4. (**Top**) Ion spray mass spectrum corresponding to indicaxanthin, (**bottom**) indicaxanthin isomer. Parent ion scan of *m*/*z* 309 ($[M + H]^+$); daughter ion scan of *m*/*z* 263 $[M + H\text{-}CO_2]^+$.

Table 2. Qualitative and Quantitative Data of Betaxanthins and Betacyanins in the Fruits of 10 Prickly Pear Cultivars and Lines by HPLC-ESI-MS[a]

peak	trivial name	amino acid	λ_{max}(nm)	*m*/*z*[M+H]$^+$ (daughter ions)	R_t[b] (min)	cultivar A	B	C	D	E	F	G	H	I	J
				betaxanthin											
1	portulacaxanthin i	hydroxiproline	483	325 (309)	1.6	tr	tr	tr	−	−	+	−	−	−	−
2	portulacaxanthin iii	glycine	470	269 (225)	1.8	−	−	tr	−	−	−	−	−	+	−
3	vulgaxanthin iii	asparagine	474	326 (295,149)	5.1	−	−	−	−	−	tr	tr	−	tr	−
4	muscaaurin	histidine	478	349 (215,124)	5.2	−	+	−	+	−	−	+	+	+	−
4′	unknown	histidine derivative	478	305 (172,149)	6.5	−	−	−	−	−	−	−	−	+	−
5	unknown	serine	472	299 (268,136)	7.3	tr	−	−	+	−	+	−	−	+	−
6	vulgaxanthin i	glutamine	475	340 (323)	9.4	+	−	−	−	−	+	−	−	+	−
7	vulgaxanthin ii	glutamic acid	474	341 (325,149)	14.5	−	−	−	−	−	−	tr	−	tr	−
8	unknown	-amino butiric acid	470	297 (253,149)	20.1	+	tr	tr	tr	−	tr	tr	tr	+	−
9	unknown	proline isomer	483	309 (263,188)	21.0	+	−	−	−	tr	−	tr	tr	tr	−
10	indicaxanthin	proline	483	309 (263,219)	22.0	+++	+	+	+	tr	+	+	+	+	−
11	unknown	methionine	478	329 (295,297)	27.2	+	tr	−	tr	−	−	tr	tr	tr	−
12	unknown	valine	472	311 (175,137)	30.2	+	tr	+	−	−	+	tr	−	−	−
13	unknown	valine (isomer)	470	311 (299,137)	30.3	+	−	−	−	−	−	−	−	−	−
14	unknown	tryptophan	475	398 (353,311)	32.0	−	−	−	−	−	−	tr	−	−	−
15	unknown	*iso*-leucine	*472*	325 (308,219)	33.9	−	−	−	−	−	−	tr	−	+	−
16	vulgaxanthin iv	leucine	473	325 (209)	34.1	+	−	tr	−	−	−	tr	−	+	−
17	unknown	phenylalanine	467	359 (312,225)	34.4	+	−	tr	tr	−	+	+	tr	−	−
18	unknown	phenethylamine	475	315 (270)	36.0	−	−	tr	tr	tr	tr	tr	−	−	−
				betacyanin											
I	betanidin 5-*O*- β-sophoroside	535	713 (551,389)	18.9	−	−	tr	tr	−	tr	−	−	tr	−	
II	betanin	538	551 (389,149)	27.3	+++	++	++	++	+	+	+	+	+	tr	
II′	*iso*−betanin	538	551 (389,149)	28.5	+	+	+	+	tr	tr	tr	−	−	−	
III	betanidin	540	389 (345,150)	27.30	+	+	+	+	+	+	+	−	tr	tr	
IV	gomphrenin i	539	551 (389,321)	30.7	+	+	tr	+	tr	tr	−	−	tr	−	
V	*neo*-betanin	480	549 (387)	33.5	tr	tr	tr	tr	tr	tr	−	−	−	−	
VI	unknown	535	459 (443,413)	34.1	tr	tr	+	tr	−	−	tr	tr	−	−	

[a] (+++), present in high amount; (++), present in moderate amount; (+), present in low amount; (tr), present in trace amounts, not quantified; (−), not detectable, that is, no mass signals could be obtained. (**A**) Camuesa, (**B**) Roja Pelota, (**C**) Cardona, (**D**) 2142, (**E**) Liria, (**F**) Roja Lisa, (**G**) Naranjona, (**H**) 2651, (**I**) 21441, (**J**) Reyna. [b] Retention times of betalains according to mass spectrometric data.

solvent. The crude magenta colored extract was transferred to a Sephadex LH-20 column (10 g), and separated by elution with water, and this procedure was done three times for each 2 mL portion of extract. Betacyanins (red-violet fractions), which showed maximum

absorbance at 535 mn, were collected and then freeze-dried to obtain partially purified betalain powder. No internal standard was used during pigment extraction.

Photometric Quantification of Betalains. All determinations were performed on a UV/vis spectrometer (Beckman, USA) equipped with UVWinLab V 2.85.04 software. The pigments were extracted using two solvents, McIlvaine buffer (pH 6.5, citrate−phosphate) and water. The UV/vis absorption spectra were recorded from 200 to 700 nm to obtain absorption values of $0.9 \leq A \leq 1.1$ at their respective absorption maxima. Measurements were performed in triplicate, and the betalain content (BC) was calculated according to literature with a slight modification (*28*).

$$\text{BC [mg/g]} = [(A(\text{DF})(\text{MW})Vd/\varepsilon LWd)]$$

where A is the absorption value at the absorption maximum of 535 and 483 nm for betacyanins and betaxanthins, respectively, DF is the dilution factor, Vd is the dried pulp solution volume (mL), Wd is the dried pulp weight (g), and L is the path-length (1 cm) of the cuvette. The molecular weight (MW) and molar extinction coefficient (ε) of betanin [MW = 550 g/mol; ε = 60,000 L/(mol cm) in H_2O] were applied in order to quantify the betacyanins. Quantitative equivalents of the major betaxanthins (Bx) were determined by applying the mean molar extinction coefficient [ε = 48,000 L/(mol cm) in H_2O]. In all cases, water extracted the highest level of pigments.

High-Performance Liquid Chromatography (HPLC). Identification of betalains by HPLC-DAD analysis was performed in an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with ChemStation software, a G1322A degasser, a G1311A quaternary pump, a G1316A column oven, and a G1315A diode array detector. Analyses were performed using an analytical scale of 150 × 4.6 mm i.d. and a Symmetry C_{18} reversed phase column with a particle size of 3.5 μm (Waters, Milford, MA), operating at a temperature of 25 °C. The betaxanthin and betacyanin compositions of different colored extracts were studied using water (eluent A) and methanol (eluent B) mixture at a flow rate of 1 mL/min. Betalains were separated starting isocratically with 100% A in 10 min followed by a linear gradient from 0% B to 30% B in 30 min, and finally a linear gradient from 30% B to 100% B in 20 min, before re-equilibration to the starting conditions. The injection volume for all extract samples was 20 μL. Betaxanthins and betacyanins were monitored at 482 and 535 nm, respectively. The identities of the different chromatographic peaks were confirmed by their visible spectral characteristics in comparison with standards and retention times.

High-Performance Liquid Chromatography-Electrospray Mass Spectrometry (HPLC-MS) Analysis. HPLC-DAD was coupled to a 6210 time-of-flight (TOF) mass spectrometer (Agilent, Palo Alto, CA) equipped with an electrospray ionization (ESI) source and Mass Hunter manager software (A.02.01) operating in the positive ionization mode. Nitrogen was used as the dry gas at a flow rate of 12 L/min with nebulizing (35 psi). The spectra were taken in the presence of formic acid to promote $[M + H]^+$ ion production (electrospray voltage 3.5 kV), and nebulizer temperature was set at 350 °C. The betaxanthin and betacyanin composition of the different colored extracts was studied with 1% formic acid in water (v/v, eluent A) and methanol (eluent B) mixture. Betalains were separated starting isocratically with 100% A, followed by a linear gradient from 0% B to 10% B in 20 min, and then a linear gradient from 10% B to 30% B in 10 min, and finally a linear gradient from 30% B to 100% B in 5 min before re-equilibration to the starting conditions. The injection volume for all extract samples was 20 μL. For the identification of the yellow betaxanthins, semisynthetic standards were synthesized, and structures were confirmed by mass spectrometry.

Semi-Synthesis of Betaxanthins. Betaxanthin reference compounds were synthesized by modified partial synthesis according to a method described previously (*29–31*). Betanin powder (10 mg) was hydrolyzed in 1 mL of NH_4OH (pH 11) for 45 min, hydrolysis was followed spectrophotometrically at 420 nm to obtain betalamic acid, and amino acid or amine was added to the alkaline solution of betalamic acid in a 10-fold molar excess. After vortexing, condensation was allowed for 20 min to yield the respective betaxanthin, which was freeze-dried, and the yellow crude semisynthetic standards were kept frozen (−20 °C) in the dark until analysis.

RESULTS AND DISCUSSION

Photometric Betalain Quantification and Color Measurements. Table 1 shows the results obtained for betalain (betacyanin and betaxanthin) extraction using two solvents. In all cases, water extracted the highest amount of pigments. The highest betacyanin content was found in Camuesa (5.29 ± 0.35 mg/g dry pulp), followed by Roja Pelota (2.06 ± 0.06 mg/g dry pulp), Cardona (2.04 ± 0.20 mg/g dry pulp), and 2142 (0.71 ± 0.04 mg/g dry pulp), with the lowest contents found in Reyna (0.05 ± 0.02 mg/g dry pulp). In agreement with previous studies (*26, 32*), the prickly pear fruit proved to be a rich source of red−violet betacyanins as betanin equivalents. Betaxanthin content in Cardona, Roja Pelotaand Camuesa was much higher than that in the yellow cultivars/lines (1.04 ± 0.09, 0.99 ± 0.03, and 2.86 ± 0.24 mg/g dry pulp, respectively). These fruit are characterized by an intense reddish purple color caused by the presence of betacyanin pigments. Other cultivars/lines such as 2142, Liria, and Roja Lisa showed less betanin content but betacyanin content was higher than in other cultivars. However, the yellow/orange cultivars/lines (Naranjona, 2651 and 21441) had higher levels of betaxanthins (0.16 ± 0.01, 0.14 ± 0.02, 0.41 ± 0.02, and 0.12 ± 0.01 mg/g dry pulp, respectively) than betacyanin content, which is in agreement with results reported earlier for yellow cactus pear fruit showing higher levels of betaxanthins than betacyanins, whereas Reyna was virtually devoid of betalains (*33*). The amount of betacyanin found in Camuesa fruit is comparable with the amount found in red beet roots used in this study (5.41 ± 0.02 mg/g) and higher than that shown in some commercial red beets (40−60 mg/100 g (*34*), 71−77 mg/100 g (*35*)). These results are of great interest because this is the first time that the betalain content in Camuesa cultivar has been investigated. Thus, the quantitative determinations demonstrated that the different colors of prickly pear fruit are the result of variations in betaxanthin and betacyanin content.

Separation and Identification of Betalains from Prickly Pear Pulp. The HPLC chromatographic betalain pattern corresponding to the extract of all 10 prickly pear cultivars/lines, monitored at 535 nm (betacyanins) and 482 nm (betaxanthins), is shown in **Figure 1**. Betalain patterns of most of the Mexican prickly pear fruits have not been described previously. Therefore, in the present study, their characterization was performed on the basis of UV/vis and mass spectrometry, as well as by the comparison of retention times with those of semisynthesized reference compounds. Several solvent systems were used in previous studies for betalain analysis; the best results were obtained when the system described above (water/methanol) was used. Other methods for betalain analysis by HPLC also existed, and most of them used acetic acid in water/acetic acid in acetonitrile (*36, 37*) or phosphoric acid solution buffer. The betacyanin patterns (**Figure 1A**) was dominated by betanin (**II**) and the C15 diastereomer isobetanin (**II′**) as the principal red pigment (betacyanins), while indicaxanthin (peak 10 in **Figure 1B**) was the principal yellow pigment (betaxanthins) group (**Figure 1B**) in reddish purple fruit.

HPLC/ESI-MS Analysis. Figure 2 shows the chromatographic pattern and peak assignment of the aqueous extract of some betaxanthins and betacyanins in red−purple (Camuesa), red (Roja Pelota), orange (Naranjona), and a yellow (21441) cactus pear cultivars/lines for betaxanthin and betacyanin pigments monitored at 482 and 535 nm, respectively. The

identity of these compounds was confirmed by HPLC coupled with electrospray mass spectrometry and provided molecular mass and structural information. Betaxanthins and betacyanins from prickly pear fruit were characterized using 10% aqueous formic acid, and the analysis was done in positive ionization mode according to previous results obtained for studies on betalains (*38*). The presence of betanin (peak **II**) and isobetanin, its 15R-isoform (peak **II′**), were confirmed first by their identical spectral properties (maximum absorbance at 535 nm), by the presence of their protonated molecular ions $[M + H]^+$ with *m*/*z*551, and by the prominent secondary ion at *m*/*z*389 due to the presence of the protonated aglycones [betanidin + H]$^+$ or [isobetanidin + H]$^+$. Both molecules differ only in the absolute configuration of their C15 chiral center (*39*). The maximum absorbance (535 nm) and molecular ion ($[M + H]^+$ at *m*/*z*551) of peak **IV** (**Figure 2**) suggested that this peak should correspond to a betacyanin structure very close to betanin and isobetanin. Gomphrenin I showed a different HPLC retention time compared to that of betanin because of the difference in the attachment of the glucose at C-6 or C-5, respectively. Betanin eluted earlier than gomphrenin I. All betacyanins produced a daughter ion at *m*/*z* 389, corresponding to [betanidin + H]$^+$ (**Figure 3**).

We have shown here that indicaxanthin is the major betaxanthin compound in yellow, orange, red, and purple colored prickly pear fruit. **Figure 4** presents the mass spectra of indicaxanthin and an isomer. It can be appreciated that they are almost identical, with the same molecular ion of *m*/*z* 309 and the same molecular structure, and since fragmentation provided a very similar spectra, according to previous studies, we assigned a possible stereoisomer structure of indicaxanthin (*40*).

The yellow fruit of 21441 showed the most complex HPLC profile (**Figure 2**). A variety of betaxanthins present in this fruit provided information to identify most of the betaxanthins present in trace quantities in other cultivars/lines. Vulgaxanthin I, vulgaxanthin II, muscaaurin VII, miraxanthin II, serine Bx, and γ-amino butyric acid Bx were the most common betaxanthins present in the other yellow prickly pear cultivars (**Table 2**).

Betaxanthins were identified by comparison with the UV/vis and mass spectrometric characteristics as well as by comparison of retention times with those of semisynthesized reference betaxanthins obtained according to a method described previously (*41*). The identities of all betaxanthin standards were checked by LC-MS analysis. Betanin, isobetanin, betanidin, and isobetanidin were identified by comparison with the retention times of the respective betacyanins in an extract from red beet root prepared as described previously. No reference compounds were available for the remaining betacyanins. Quantification of betalains was done on the basis of relative abundance in ESI-MS.

Cardona, 2142, Roja Lisa, 21441, and Naranjona cultivars/lines contain traces of a new betacyanin identified as betanidin 5-*O*-β-sophoroside (*42*). To our knowledge, this is the first time that this betacyanin has been identified in prickly pear fruit. The identification of this compound was based on its molecular mass, determined by HPLC-ESI-MS.

Therefore, with regard to different colored phenotypes, our study has confirmed that the variations in the content of the yellow−orange betaxanthins and the red−violet betacyanins as well as the variations in the levels of certain structurally different betacyanins are responsible for the prickly pear fruit color. The diversity of betalains found in these prickly pear cultivars and lines, including the new betacyanins (betanidin 5-*O*-β-sophoroside), indicate the potential value of *Opuntia* cactus pear fruit, especially the Camuesa cultivar, which showed the highest betalain content, as a good source of pigments, and their potential industrial exploitation for drinks and food products. Therefore, consumption of cactus pear fruit may provide nutritional and health benefits.

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