Color, Betalain Pattern, and Antioxidant Properties of Cactus Pear (Opuntia spp.) Clones

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Color, Betalain Pattern, and Antioxidant Properties of Cactus Pear (*Opuntia* spp.) Clones

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Total phenolics, ascorbic acid, and betalain contents of differently colored cactus pear clones (nine *Opuntia ficus-indica* [L.] Mill. clones and one *O. robusta* Wendl. clone) were investigated and related to their respective antioxidant potential assessed by Trolox-equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) assays. TEAC and ORAC values were very highly correlated with each other and also with values for total phenolics, betalain contents, and ascorbic acid concentrations. Total phenolics had the greatest contribution to ORAC and TEAC values. High-performance liquid chromatography (HPLC)−diode array detector (DAD)−tandem mass spectrometry (MS/MS) measurements of cactus pear juices permitted the differentiation of the clones based on variations in pigment patterns and betalain concentrations. The red and yellow betalains were absent in lime green colored cactus fruits. The ratio and concentration of these pigments were responsible for the yellow, orange, red, and purple colors in the other clones. Progeny of purple and lime green colored parents were characterized by 12% and 88% of plants bearing lime green and purple fruit, respectively. This implies that the genes for betalain production were lacking in the lime green fruits but could be provided by a parent with a complete set of genes, that is, purple fruits. Besides known pigments typical of Cactaceae, two unexpected betalains were identified. Whereas gomphrenin I was found for the first time in tissues of cactus plants, methionine-betaxanthin has never been described before as a genuine betalain. In addition to their alleged health-promoting properties, various combinations of yellow betaxanthins and red-purple betacyanins may allow the development of new food products without using artificial colorants.

KEYWORDS: Cactaceae; *Opuntia*; cactus pear; progeny; color; betalains; gomphrenin I; methionine-betaxanthin; antioxidant activity; ORAC; TEAC; phenolics; ascorbic acid

INTRODUCTION

Fruit weights of commercial cactus pear *Opuntia ficus-indica* (L.) Mill. typically range from 120 to 200 g with 45−60% of the fruit being edible. Their fruit color varies from lime green, yellow, orange, and red to purple (*1*, *2*) yielding juice of 12 to 15°Bx. In Mexico, their center of origin, cactus pears have been enjoyed for millennia (*3*). Only lately, there has been a surge in interest among the scientific community with respect to Opuntias' nutritional and health-promoting benefits, among others, improving platelet function (*4*), reducing blood lipid and total cholesterol, low-density lipids, and triglycerides (*4*−*6*), and lowering isoprostane concentrations in blood indicating lower oxidative injury (*7*). Its antiulcerogenic activity (*8*, *9*) has been proven, and various antioxidant compounds (*10*) are believed to improve human health after fruit consumption. Finally, cactus pears were suggested as promising sources for red and yellow food colorants for use at neutral pH (*11*, *12*).

The nutraceutical benefits of *Opuntia* fruits are believed to stem from their alleged antioxidant properties related to ascorbic acid, phenolics including flavonoids, and a mixture of yellow betaxanthin and red betacyanin pigments (*8*, *13*, *14*). Unfortunately, these antioxidant concentrations appear to be generally much more variable than the classic proximate values for fat, sugar, protein, etc. Not only is there a high variability of these compounds in plants, but considerable diversity of opinion exists on the appropriate method to assess these antioxidants in plant tissues. Among others, the Trolox-equivalent antioxidant capac-

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Table 1. Characterization of the Cactus Pear Clones

clone	morphological characteristics	additional information
no. 1240	spineless, round bluish pads, large dark purple round fruits	*O. robusta*
no. 1281	spineless, green elliptic pads, elliptic fruits, deeply red pulp	*O. ficus-indica*
no. 1288	spiny, green elliptic pads, ovoid fruits, white pulp	*O. ficus-indica,* "tuna blanca" (Mexico)
no. 1320	spineless, green elliptic pads, elliptic fruits, yellow-orange pulp	*O. ficus-indica*, originating from Chile
no. 1379	spineless, green ovate pads, round fruits, pink peel, red pulp	*O. ficus-indica*
no. 1458	spineless, green long elliptic pads, fruits with a curved neck, yellow peel, white pulp	*O. ficus-indica*, did not match with FAO descriptor list
cv. Purple	spineless, blue-green elliptic pads, elliptic fruits, purple pulp	*O. ficus-indica*
cv. Red	spineless, green elliptic pads, elliptic fruits, red pulp	*O. ficus-indica*
cv. Orange	spiny, green elliptic pads, elliptic fruits, orange peel, orange pulp	*O. ficus-indica*
cv. Green	spineless, green elliptic pads, elliptic fruits, lime green or yellow peel, lime green pulp	*O. ficus-indica*

ity (TEAC), the water-soluble and lipid-soluble oxygen radical absorbance capacity (ORAC), and the vitamin C equivalent antioxidant capacity (VCEAC) have been proposed (*15*−*17*). To compound matters further, a study comparing 927 samples of major freeze-dried vegetables with one antioxidant assay demonstrated that there were 3- to 10-fold differences in the ORAC values for the same vegetable depending on the provenance and harvest season (*18*).

The present work was conducted to study in more detail the relationships between ascorbic acid, total phenolics, and betalains and their respective antioxidant potential in a collection of differently colored cactus pear fruits. To have a more complete understanding of the heredity of pigment composition, data from hybridization experiments were included.

MATERIALS AND METHODS

Plant Material. The cacti were grown on D'Arrigo Bros experimental fields near Salinas (CA) at 36.6° N latitude under drip irrigation. From the same plants, fruits were selected at maximum full maturity without being overripe. Cactus pears were harvested on 3 November 2003 or on 20 January 2004 and sent to Germany by air freight for pigment and color analyses or to the University of Georgia for measurements of total phenolics, ascorbic acid, and TEAC. Since the coastal Pacific climate allows cactus pears to be harvested for commercial use from August through April, fruits picked in November were in the 4th month, while the January fruits were in the 6th month of the 9 month harvest period.

Recent molecular (*19*) and morphological work (*20*) suggested that all spiny and spineless commercial fruit types belong to the species *Opuntia ficus-indica*. Accordingly, all clones used in this trial were of the *O. ficus-indica* type except clone no. 1240, which was assigned to *O. robusta*. The latter was obtained from South Africa and was reported to be one of Burbank's selections known as "Chico". *O. robusta* is generally distinguished from *O. ficus-indica* by having round bluish pads and round larger dark purple fruits. Despite their large size, *O. robusta* fruits are not consumed fresh due to their poor sensorial quality.

The *O. ficus-indica* clones analyzed in the present study were both breeding lines and cultivars (cv.) and exhibited the characteristics listed in **Table 1** according to the FAO descriptor list (*21*). Whereas clones nos. 1240, 1281, 1288, 1320, 1379, and 1458 have already been described in previous publications (*22*, *23*), the *O. ficus-indica* AndyBoy cultivars have not been studied before. For co-injection experiments (see below), purple globe amaranth (*Gomphrena globosa* L., Amaranthaceae) was purchased from a local supplier (Germany).

Solvents and Reagents. Formic acid, acetonitrile, citric acid monohydrate, and disodium phosphate dihydrate were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout. Gallic acid, ascorbic acid, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Fluka (Milwaukee, WI).

Reference Substances. For co-injection experiments, betaxanthin reference compounds were synthesized as described earlier (*24*) from recondensation of betalamic acid obtained through alkaline betaxanthin hydrolysis with specific amino compounds. Betacyanin reference compounds were extracted from globe amaranth (*Gomphrena globosa* L., Amaranthaceae) as follows: The purple inflorescences were ground in a mortar after the addition of liquid nitrogen. The resulting powder was extracted with 80% aqueous methanol containing 50 mM sodium ascorbate at a solvent/tissue ratio of 10 mL/g. After continuous stirring for 2 h, the solid plant material was separated from the purple solution by paper filtration (Schleicher-Schuell, Dassel, Germany) and concentrated in vacuo at 30 °C. The residue was taken up in deionized water and filtered (0.45 μm) before analysis.

Sample Preparation. The samples from selected cactus pear clones (January harvest) for phenolic, ascorbic acid, and antioxidant determinations were treated as follows: After manual separation of the peel from the pulp, the latter was weighed and briefly homogenized in a kitchen-type blender. The seeds were strained with a colander (0.5-mm mesh size) and the volume of the resulting juice was determined. The conversion factors for the cultivars were 0.75, 0.73, 0.70, and 0.68 mL of juice per gram of pulp for cv. Orange, cv. Purple, cv. Red, and cv. Green, respectively. Aliquots were frozen for vitamin C, total phenolics, and antioxidant measurements by the TEAC assay at the University of Georgia. Frozen aliquots from the same homogenate were sent by overnight courier on dry ice to Brunswick Laboratories (Wareham, MA) for ORAC measurements. TEAC and ORAC assays were conducted on the pure juice without prior sample workup.

For pigment and color analyses, the peel and pulp of four to seven fruits (November harvest) of each *Opuntia* clone were manually separated and pooled. The juice was obtained from the pulp by paper filtration (Schleicher-Schuell, Dassel, Germany), flushed with nitrogen, and stored at −25 °C. Thawed samples were filtered (0.45 μm) before analyses.

Soluble Solid Contents, Density, and pH. Soluble solids content (°Bx) and pH were measured in pure cactus juice without prior solvent extraction. Density was determined in duplicate at 20 °C with a density meter DMA 48 (Anton Paar, Graz, Austria).

Spectrophotometric Measurements. *Photometric Determination of Total Phenolics.* Total phenolics were measured according to the Folin−Ciocalteu reagent method (*25*). Two hundred microliters of sample extract was applied in a test tube, 1.0 mL of Folin−Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) were added, and the contents were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured in a Shimadzu 300 UV−vis spectrometer (Shimadzu UV-1601, Norcross, GA), and the total phenolic contents was expressed as gallic acid equivalents (GAE) in milligrams per liter of cactus juice with a calibration curve using 100, 200, 300, and 400 mg/L of gallic acid, respectively.

Photometric Quantification of Betalains. The aqueous pigment extracts were diluted with McIlvaine buffer (pH 6.5, citrate-phosphate) to obtain absorption values of $0.9 \leq A \leq 1.0$ at their respective absorption maxima. The betalain content (BC) was calculated as described earlier (*12*): $\mathrm{BC\ [mg/L]} = [(A \times \mathrm{DF} \times \mathrm{MW} \times 1000/\epsilon \times l)]$ where A is the absorption value at the absorption maximum corrected by the absorption at 600 nm, DF the dilution factor, and l is the path length (1 cm) of the cuvette. For quantification of betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (ϵ) of betanin (MW = 550 g/mol; ϵ = 60 000 L/(mol cm) in H_2O; λ = 538 nm) and indicaxanthin (MW = 308 g/mol; ϵ = 48 000

Table 2. Total Phenolics, Betaxanthins, Betacyanins, and Ascorbic Acid Contents and Corresponding TEAC and ORAC Values (Fluorescein-Based) in Pure Cactus Juice and Edible Pulp[a] (January Fruit)[b]

							TEAC		ORAC hydrophilic		
cultivar	Brix [°Bx]	pH	total phenolics (as GAE) [mg/L][b]	betaxanthins (as indicaxanthin equiv) [mg/L]	betacyanins (as betanin equiv) [mg/L]	ascorbic acid [mg/L]	juice [mmol/L]	pulp [mmol/kg]	juice [mmol/L]	pulp [mmol/kg]	ORAC lipophilic [mmol/L]
Green	14.2	6.5	242 ± 13.4	0.4 ± 0.02	0.1 ± 0.01	51.1 ± 3.0	3.31 ± 0.13	2.24 ± 0.09	5.45	3.68	0
Orange	12.6	6.3	247 ± 23.1	76.3 ± 0.38	6.6 ± 0.04	70.2 ± 16.0	3.10 ± 0.04	2.32 ± 0.03	5.83	4.36	0
Red	14.8	5.6	335 ± 19.3	67.9 ± 0.19	120.0 ± 0.44	67.9 ± 16.5	3.71 ± 0.47	2.60 ± 0.33	6.35	4.44	0
Purple	12.8	6.3	660 ± 35.8	195.8 ± 0.46	431.0 ± 1.04	95.4 ± 0.6	4.99 ± 0.37	3.64 ± 0.27	11.20	8.16	0

[a] Data for cactus pear pulp were calculated from the values obtained for juices considering the respective conversion factors mentioned above (sample preparation). [b] Values expressed as means of duplicate determinations ± standard deviation.

L/(mol cm) in H_2O; $\lambda = 480$ nm) were applied. All measurements were performed in duplicate using a UV−vis spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with UVWinLab V 2.85.04 software (Perkin-Elmer Instruments, Norwalk, CT).

Color Analyses. For color analyses, a UV−vis spectrometer (Perkin-Elmer Instruments) was used applying UVWinLab V 2.85.04 and Wincol V 2.05 color softwares (Perkin-Elmer Instruments). To normalize absorption, the aqueous pigment extracts were diluted with McIlvaine buffer (pH 6.5, citrate-phosphate) to reach 0.95 ± 0.05 at the respective maxima. Spectral curves were recorded from 380 to 780 nm in 1 cm path length disposable cuvettes. Chroma [$C^* = (a^{*2} + b^{*2})^{0.5}$] and hue angle [$h° = \arctan(b^*/a^*)$] were calculated from CIE a^*- and b^*-values using illuminant D_{65} and 10° observer angle. All determinations were performed in duplicate.

High-Performance Liquid Chromatography (HPLC)−Diode Array Detector (DAD) Analyses. *Ascorbic Acid.* The quantification of ascorbic acid was conducted as previously reported (*26*) at a monitoring wavelength of 210 nm. HPLC was performed with a Hewlett-Packard (Avondale, PA) model 1100 liquid chromatograph with quaternary pumps and a diode array detector. The mobile phase was water adjusted to a pH between 2.10 and 2.15 with perchloric acid. The separation was carried out on a Hypersil ODS (100 mm × 4.0 mm i.d., Agilent Technologies, Foster City, CA) with a particle size of 3 μm by isocratic elution at 30 °C and a flow rate of 1.0 mL/min.

Betalains. Juice samples were analyzed on an HPLC system (Merck, Darmstadt, Germany) equipped with an autosampler L-7200, an interface module D-7000, a pump L-7100, a column oven L-7350 with a Peltier cooling module, and a diode array detector L-7450A. As described earlier (*27*), separation was achieved at 25 °C on an analytical scale Atlantis dC_{18}-column (250 mm × 4.6 mm i.d.) with 5 μm particle size (Waters, Wexford, Ireland) fitted with a C_{18} ODS security guard column (4 mm × 3.0 mm i.d.; Phenomenex, Torrance, CA). The mobile phase A was 0.2% (v/v) formic acid in water, MeCN was used as B. At a flow rate of 1 mL/min, the first 7 min were performed isocratically with 0% B, succeeded by a linear gradient from 100% A to 93% A in 3 min, then to 90% A in 17 min, followed by isocratic elution for 8 min and a linear gradient from 90% A to 80% A in 10 min. Simultaneous monitoring was performed at 476 and 538 nm for betaxanthins and betacyanins, respectively.

HPLC−Tandem Mass Spectrometry (MS/MS) Analyses of Betalains. Using the same chromatographic conditions, LC-MS analyses were performed on an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with a degasser G1322A, a binary gradient pump G1312A, an autosampler G1329/1330A, a column oven G1316A, and a diode array detector G1315A interfaced with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source operating in the positive ionization mode. Nitrogen was used as dry gas at a flow rate of 12 L/min and a pressure of 70 psi. The nebulizer temperature was set at 365 °C. Using helium as the collision gas (4.1×10^{-9} bar), collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.2 V (MS/MS).

Antioxidant Measurements. To assess the antioxidant potential of bioactive compounds, it has been recommended to apply at least two different assays varying in their mechanisms of antioxidant action (*17*). While the TEAC test directly yields the radical scavenging capacity of the respective compound reducing the ABTS radical, the ORAC assay monitors both the inhibition percentage and length of inhibition time toward the azo-induced oxidation of fluorescein (*16*, *17*).

TEAC. Based on the improved TEAC assay (*28*), antioxidant capacity was registered using a Shimadzu UV−vis spectrometer (Shimadzu UV-1601, Norcross, GA) running in the kinetic mode. ABTS radical cation was produced by reacting 7 mM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM (final concentration) potassium persulfate after incubation at room temperature in the dark for 16 h. The ABTS solution was diluted with ethanol to an absorbance (A) of 0.70 ± 0.02 at 734 nm. The filtered sample was diluted with ethanol to produce 20−80% inhibition of the blank absorbance with 40 μL of sample. An aliquot of 1960 μL of ABTS solution ($A = 0.70 \pm 0.1$) was monitored at 734 nm. After exactly 1 min, 40 μL of the sample was added and mixed thoroughly. Absorbance was continuously registered up to 7 min at 6 s intervals. Standards of final concentrations of 0−15 μM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a vitamin E analogue) in ethanol were prepared and assayed under the same conditions. The Trolox-equivalent antioxidant capacity (TEAC) of the sample was calculated on the basis of the inhibition exerted by standard Trolox solution in 6 min. Measurements of each sample were repeated three times.

ORAC. Hydrophilic and lipophilic oxygen radical absorbing capacities (ORAC) were assessed at Brunswick Laboratories (Wareham, MA) according to refs *16* and *29*, respectively. Using fluorescein as a target allowed direct measurement of chain-breaking antioxidant capacity against peroxyl radicals without the drawbacks of phycoerythrin such as photolability, nonspecific protein binding, and chemical inconsistency (*16*). For comparison, theoretical ORAC values were determined on the basis of the molecular weights of 170 g/mol for gallic acid, 176 g/mol for ascorbic acid, 308 g/mol for betaxanthins, and 550 g/mol for betacyanins.

Statistical Analyses. The means, standard deviations, and correlation matrices were calculated using Microsoft Excel 2000.

RESULTS AND DISCUSSION

Ripeness Determination. The Brix values for the four cultivars listed in **Table 2** were higher than those described for Italian (*12*, *30*) or Israeli summer or winter crops (*31*). Therefore, despite being harvested in January, the fruits were of commercial quality. Upon maturation, the pH usually rises from below 5 to values between 5.6 and 6.5 depending on the cactus pear variety (*23*). Since in this study pH values were in the 5.6 to 6.5 range, fruits were judged mature.

Ascorbic Acid. The ascorbic acid values of 51−95 mg/L (**Table 2**) corresponded to 3.5−6.5 mg/100 g of edible portion. These contents were considerably lower than those previously published for commercial varieties amounting to 28−30 mg/100 g of edible portion (*10*), 31−38 mg/100 g (*13*) or 44−81 mg/100 g of fruit (*32*), and 218−256 mg per liter of juice (*12*). While ascorbic acid contents in this study were generally lower than those reported earlier, they compared well with values found in the flesh of Californian peaches, nectarines, and plums (*33*).

Total Phenolics. There was nearly a 3-fold range in total phenolics, cv. Purple having the highest concentration (**Table**

Table 3. Betalain Contents of Juices from the Fruit Pulps of Different *Opuntia* Clones (November Fruit)[a]

	betaxanthins (Bx) ± SD (as indicaxanthin equiv)		betacyanins (Bc) ± SD (as betanin equiv)		sum (Bx + Bc)	
clone	mg/L	mg/kg	mg/L	mg/kg	mg/L	mg/kg
no. 1240	581.0 ± 9.2[b]	553.7 ± 8.7[b]	615.6 ± 9.7	586.7 ± 9.2	1196.6	1140.4
cv. Purple	151.4 ± 3.9[b]	143.7 ± 3.7[b]	303.3 ± 7.7	287.9 ± 7.3	454.6	431.6
no. 1281	98.8 ± 1.4	93.8 ± 1.3	106.9 ± 1.5	101.5 ± 1.4	205.8	195.3
cv. Red	52.2 ± 2.7	49.4 ± 2.6	68.3 ± 3.5	64.7 ± 8.2	120.5	114.1
no. 1379	34.3 ± 0.1	32.7 ± 0.1	34.9 ± 0.0	33.2 ± 0.0	69.2	65.9
cv. Orange	84.4 ± 0.3	80.1 ± 0.3	11.1 ± 0.0	10.5 ± 0.0	95.5	90.6
no. 1320	179.6 ± 1.8	169.8 ± 1.7	15.4 ± 0.3	14.6 ± 0.3	195.0	184.4

[a] No betalains could be determined for no. 1288, no. 1458, and cv. Green. [b] Due to the similar absorption maximum with betaxanthins, neobetanin contents are codetermined.

2). These values correlated again with those for peach, plum, and nectarine fruit flesh ranging from 91 to 1042 mg/kg (*33*) and were similar to 2.2−22.7 μmol per gram of tissue (*34*) from a survey where 80% of 20 selected fruits afforded less than 8.9 μmol of phenolics per gram.

In red, yellow, and orange cactus pears, only very small amounts of about 237 ng of polyphenolics per 100 g of pulp (quercetin equivalents) were reported (*10*). In another study, the presence of isorhamnetin, rutin, and kaempferol in cactus pear juice amounting to a total mean phenolic concentration of 74.6 g/100 mL (isorhamnetin 3-glucoside equivalents) was verified (*8*). However, these authors obtained the juice from the entire fruit including the nonedible peel portion of 40%. Kuti (*32*) stated that total flavonoids (total flavonoid aglycone equivalents) ranged from 0.98 to 9.35 mg per 100 g. Because there is some ambiguity in those methods with regard to pulp and peel preparation, it was assumed that peel was not completely separated to obtain the juice analyzed. In the present investigation, it was demonstrated that clones with the highest pigment contents tended to show the highest GAE, while identification of individual phenolics applying the method of ref *35* was not possible.

Betalain Contents. In accordance with previous reports (*12*, *36*, *37*), cactus pears proved to be a rich source of yellow-orange betaxanthins and red-violet betacyanins ranging from 65.9 to 1140.4 mg/kg in clone nos. 1379 and 1240, respectively (**Table 3**). In agreement with the general trend observed in this study, it has been reported earlier that the Italian yellow-orange cultivar Gialla contained much higher indicaxanthin than betanin levels. Contrarily, in red fruits, cv. Rossa, betacyanin levels were higher than those of betaxanthins, whereas white cv. Bianca were virtually devoid of betalains (*10*, *12*). Indicaxanthin contents of yellow cactus pear amounting to 84 mg/kg of edible pulp (*10*) were virtually identical with that of cv. Orange in this study (80 mg/kg, **Table 3**). For blood-red *Opuntia* fruits harvested for maximum color development, high levels of 400 mg of betacyanins and 100 mg of betaxanthins per kilogram of edible portion have been reported (*38*), while 190 and 300 mg of betacyanins and betaxanthins per kilogram of edible pulp, respectively, were obtained for reddish purple cactus pear cultivars from Spain (*39*). These values are between those of cv. Purple or no. 1240 (*O. robusta*, **Table 3**). Other workers stated considerably higher values such as 670.0−800.1 mg/kg for *O. stricta*, 180.5−190.6 mg/kg for *O. undulata*, 140.3−150.2 mg/kg for an *O. ficus-indica* cultivar (*36*) and up to 1130.9 mg/kg for a purple *Opuntia* sp. (*37*). Besides fruit-specific variations, co-absorption of non-betalainic substances might have led to overestimation of pigment concentration (*12*). Second, betalain concentrations in peel may be higher or lower than those in pulp fractions, depending on the respective *Opuntia* sp. (*37*). Thus differences may be due to processing whole fruits including peels in earlier studies. It is worth mentioning that pigment quantification in unpeeled fruit does not allow prediction of betalain yield of the edible fruit part.

In general, the betalain contents in January fruit (**Table 2**) correlated well with those from November fruit (**Table 3**). The values of 0.4 and 0.1 mg/L for cv. Green came close to the zero values from November fruit. Within experimental error, the values for cv. Orange were virtually the same. The betaxanthin contents for cv. Red were within error for both November and January fruits, while the betacyanin contents were lower for the November fruits. The considerably higher betacyanin values of the January fruits from cv. Purple may be due to difficulties in selecting dark colored cactus pears of identical maturity.

Antioxidant Properties. *TEAC.* The TEAC values for cactus pear pulp and juice in **Table 2** are similar to those reported in an Italian survey of 34 vegetables, 30 fruits, 34 beverages, and 6 vegetable oils using three assays for antioxidant potential assessment (*40*). TEAC values ranged from 0.3 units for cucumber to 8.5 for spinach and from 0.6 for banana up to 20.2 for blackberry. Seventy-six percent of the fruits had a TEAC value less than 10, and prickly pear cactus produced a value of only 1.5 (*40*). In the present study, the TEAC values for cactus juice came close to those of red wine and green tea infusions (*41*), but cactus juice exhibited much lower TEAC values than previously assessed for blueberries or pomegranate juices (*35*, *41*).

ORAC. The total measured ORAC antioxidant values of 5.4−11.2 mmol/L for the cactus juice samples (**Table 2**) were in the same range as those reported for 12 fruit species yielding 1.0−15.4 mmol/L of juice (*42*). The values for cv. Green (5.4), cv. Orange (5.8), and cv. Red (6.3) were similar to white (4.4), yellow (5.3) and red (4.2) cactus pears from Sicily (*10*) but were much lower than 15.8−49.2 mmol/kg reported by ref *32*. It is worth mentioning that no ORAC activity could be detected in the lipophilic fraction.

Correlation between Compositional Data and Antioxidant Parameters. To gain a perspective of the relative antioxidant importance of the various compound classes, theoretical ORAC values were compared to experimentally determined values using literature conversion coefficients (**Table 4**; *16*, *43*, *44*). As earlier reported (*16*), a mean value of 4.00 ORAC units per micromole of phenolic compound was used. Similarly, a value of 0.52 ORAC per micromole was applied for ascorbic acid (*44*). Since no ORAC data for purified betaxanthins and betacyanins have been reported, auxiliary values were calculated from mean inhibition data for betacyanins and betaxanthins published in ref *43* and related to those of ascorbic acid (*44*). This afforded 1.54 ORAC per micromole for betacyanins and

Table 4. Semiquantitative Estimate of ORAC Values (Fluorescein-Based, units/μmol) from Total Phenolic, Total Betalain, and Ascorbic Acid Contents

cultivar	ORAC for total phenolics[a]	ORAC for betaxanthins[b]	ORAC for betacyanins[b]	ORAC for ascorbic acid[c]	total ORAC calcd	total ORAC measd
Green	5.69	0.00	0.00	0.15	5.84	5.45
Orange	5.81	0.43	0.02	0.21	6.47	5.83
Red	7.88	0.38	0.34	0.20	8.80	6.35
Purple	15.53	1.10	1.21	0.28	18.12	11.20

[a] Approximate mean ORAC based on a value of 4.00 for phenolics (*16*). [b] Approximate mean ORAC based on calculated values of 1.54 for betacyanins and 1.73 for betaxanthins (*43*, *44*). [c] Approximate mean ORAC based on a value of 0.52 for ascorbic acid (*44*).

Table 5. Correlation Matrix for Total Phenolics, Betaxanthins, Betacyanins, Ascorbic Acid, and Total Antioxidant Values of Cactus Pears Assessed by TEAC and Hydrophilic ORAC Assays (Fluorescein-Based)[a]

	total phenolics	betaxanthins	betacyanins	ascorbic acid	TEAC juice	TEAC pulp	ORAC juice	ORAC pulp
total phenolics	1							
betaxanthins	0.928	1						
betacyanins	0.998	0.926	1					
ascorbic acid	0.909	0.999	0.908	1				
TEAC juice	0.991	0.876	0.993	0.854	1			
TEAC pulp	0.999	0.941	0.998	0.924	0.987	1		
ORAC juice	0.995	0.943	0.988	0.924	0.974	0.994	1	
ORAC pulp	0.985	0.964	0.977	0.949	0.954	0.987	0.996	1

[a] For a single-tailed test and $n = 4$, levels of significance are as follows: $r = 0.974$, $P = 0.0005$; $r = 0.963$, $P = 0.001$; $r = 0.942$, $P = 0.0025$; $r = 0.882$, $P = 0.01$; $r = 0.811$, $P = 0.025$.

1.73 for betaxanthins. The estimated and measured ORAC values were in the same ranked order, but the predicted values were generally higher than the measured ones (**Table 4**). Total phenolics dominated the contribution to the total antioxidant indices: based on the phenolic contents themselves, a rather good approximation of the overall ORAC and TEAC values was obtained. Confirming earlier findings (*43*), the contribution supplied by the betalains was considerably greater than that provided by ascorbic acid. It is worth mentioning that even if the ascorbic acid values were to increase 10 fold, to be closer to other values in the literature (*10*, *13*), ascorbic acid would still be responsible for less than a third of the total calculated ORAC values.

To obtain an overall perspective of the antioxidant properties and the respective chemical constituents, pairwise correlations between all of these variables were performed (**Table 5**). The lowest correlation of 0.854 was significant at $P < 0.025$, and many of these values were significant at $P < 0.0005$. In contrast, values for both ORAC and TEAC of juice and pulp were best correlated yielding higher values for the former. Among the chemical constituents, highest correlation was found for total phenolics, then betacyanin and betaxanthin, and last ascorbic acid contents demonstrating that the major active principles in cactus pears are phenolics, followed by betacyanin and betaxanthin pigments, and last ascorbate. Cv. Green totally lacking betalains contained substantial quantities of antioxidants, further implicating the importance of colorless phenolics. The presence of phenolic hydroxyls in betacyanins may be responsible for higher correlations between total phenolics and betacyanins rather than between phenolics and betaxanthins. Although previous work by Galati et al. (*8*) pointed to higher antioxidant levels in cactus pear peels, there are no published data on phenolics and antioxidant measurements specifically addressing the nonedible fruit part.

Betalain Patterns of Cactus Pear Clones. Through improvement of a previous method (*45*), a broad range of betaxanthins and betacyanins could be separated and analyzed by HPLC−DAD−MS/MS. Altogether, five betaxanthins (**1**−**5**) and six betacyanin structures (**I, I′, II**−**V**) were detected in the different *Opuntia* fruits (**Table 6**; Figure 1). The main yellow betalain of all clones was proline-betaxanthin (indicaxanthin, **4**) which is the typical compound of cactus pears (*11*, *45*). The second most abundant betaxanthins were glutamine-betaxanthin (vulgaxanthin I, **2**) and the γ-aminobutyric acid-betaxanthin (**3**) in varying amounts (data not shown), both of which were very recently detected in juices of *O. ficus-indica* cv. Gialla (*45*). Previously only tentatively identified as a genuine compound of cactus pears (*45*), histidine-betaxanthin (muscaaurin VII, **1**) was unambiguously assigned in this study by its molecular mass, spectral properties, and finally co-injection with a semisynthetic reference compound. Low amounts of a further betaxanthin (R_t = 26.7 min, **5**) were detected in cv. Orange and clone no. 1320. Mass spectrometric analyses afforded a *m*/*z* value of $[M + H]^+ = 343$, corresponding to a methionine-betaxanthin structure. Through co-injection experiments, this assumption could be ascertained. Thus, for the first time, methionine-betaxanthin was shown to be a genuine betaxanthin of betalainic plants (*11*, *46*).

In each clone, except for cv. Orange and clone no. 1320, varying amounts of isobetanin (**I′**) accompanied the predominant betanin (**I**). Moreover, in those fruits having high betacyanin levels (nos. 1240 and 1281, cv. Purple), the betanin aglycone betanidin (**III**), indicative of endogenous β-glucosidase activity, was also detected. Closer inspection of the chromatograms (**Figure 1, part A2**) revealed a betacyanin structure that did not match the known retention times (*27*) but exhibited identical mass fragmentation characteristics as betanin ($[M + H]^+ = 551$ and 389; **Table 6**). Since this compound (R_t = 26.8 min, **II**) showed a bathochromic shift of 2 nm relative to betanin and eluted after the latter, the presence of the 6-*O*-glucoside of betanidin (gomphrenin I) was suspected (*47*−*49*). Confirmation was obtained by co-injection experiments with an aqueous extract from purple globe amaranth. Gomphrenin I (betanidin-6-*O*-glucoside) has so far not been described to occur in Cactaceae (*46*) and was hitherto thought to be restricted to *Basella rubra* L. fruits (Basellaceae; *50*) and purple inflorescences of *G. globosa* L. (Amaranthaceae; *47*−*49*). Furthermore, the simultaneous occurrence of 5- and 6-*O*-glycosides of betanidin in the same plant tissue is a rare event: in addition to

Table 6. LC–DAD and LC–MS/MS Data of Betacyanins and Betaxanthins (Bx) in Juices from Various *Opuntia ficus-indica* Clones[a]

peak number	name (trivial name)	R_t [min]	$\lambda_{max,vis}$ [nm]	*m/z* $[M + H]^+$	*m/z* HPLC-ESI(+)-MS/MS	clone no. 1240	clone cv. Purple	clone no. 1281	clone cv. Orange	clone no. 1320	clone no. 1379	clone cv. Red
					Betaxanthins							
1	histidine-Bx (muscaaurin VII)	11.0	477	349	305	+	tr	+	+	+	+	+
2	glutamine-Bx (vulgaxanthin I)	14.6	475	340	323	–	–	+	+	+	–	±
3	γ-aminobutyric acid-Bx	17.9	467	297	253	–	–	+	+	+	+	+
4	proline-Bx (indicaxanthin)	18.8	486	309	265	+	+	+	+	+	+	+
5	methionine-Bx	26.7	477	343	299	–	–	±	+	+	–	–
					Betacyanins							
I	betanidin-5-*O*-β-glucoside (betanin)	20.7	538	551	389	+	+	+	+	+	+	+
I′	isobetanidin-5-*O*-β-glucoside (isobetanin)	24.5	538	551	389	+	+	+	–	–	+	+
II	betanidin-6-*O*-β-glucoside (gomphrenin I)	26.8	540	551	389	+	+	+	–	–	tr	+
III	betanidin	28.0	540	389	345	+	+	+	–	±	–	+
IV	6′-*O*-malonylbetanin (phyllocactin)	29.0	538	637	593	–	tr	–	–	–	–	–
V	14,15-dehydrobetanin (neobetanin)	38.4	476	549	387	+	+	–	–	–	–	–

[a] + = present; ± = not unambiguously identified; – = not detectable; tr = present in trace amounts.

G. globosa L. (*47*, *48*) and *Phytolacca americana* L. (*51*) (Phytolaccaceae), *Opuntia* sp. is the third betalain source containing both 5- and 6-*O*-glycosyl-derivatives of betanidin.

Although phyllocactin (6-*O*-malonylbetanin, **IV**) is a typical compound of the Cactaceae (*11*, *52*−*54*), only trace amounts were found in cv. Purple (**Figure 1**). Also neobetanin (14,15-dehydrobetanin, **V**), which was earlier reported as an endogenous betalain of cactus pears (*55*), was only found in two clones, namely, no. 1240 and cv. Purple. Interestingly, the latter are those with highest betacyanin contents. Thus, neobetanin (14,15-dehydrobetanin) may result from betanin or isobetanin precursors by a dehydrogenase activity. To the best of our knowledge, neobetanin biosynthesis has not yet been considered (*46*). The chemical structures of all betacyanins and betaxanthins detected in the various *Opuntia* clones are depicted in **Figure 2**.

While indicaxanthin (**4**) was present in all *Opuntia* fruits, some compounds were found to be specific to the respective clones, such as phyllocactin (**IV**) in cv. Purple, neobetanin (**V**) in no. 1240 and cv. Purple, gomphrenin I (**II**) in no. 1240, cv. Purple, cv. Red, and no.1281, as well as methionine-betaxanthin (**5**) in cv. Orange and clone no. 1320 (**Table 6; Figure 1**). Further studies are therefore needed to clarify whether a specific clone can unequivocally be identified by its respective pigment pattern. It would also be worth investigating the influence of fruit maturity on the qualitative and quantitative betalain composition.

Correlation between Color and Betalains. While there are other papers reporting pigment concentrations or hue measurements of cactus pear juice (e.g., *12*, *36*, *38*), this is the first paper investigating both pigment concentrations and color measurements on cactus pears with a wide variety of shades. The absence or presence of indicaxanthin and the red betacyanin derivatives as well as their respective ratios basically govern the fruit color. The lime green fruits (no. 1288, cv. Green) are totally devoid of betalains. Fruits with high indicaxanthin concentrations (no. 1320) and a low betacyanin-to-betaxanthin ratio were yellow. Cactus pears from cv. Orange exhibit a higher ratio of red to yellow pigments than the yellow fruits. To the unaided eye, fruits of no. 1240 and cv. Purple appeared similarly dark purple. However, this was due to their high pigment concentrations and not to their respective pigment ratios (**Table 3; Table 7**). It must be noted that clone no. 1240 contained more than three times more yellow pigment than the most yellow clone no. 1320, but higher betacyanin concentration overpowered betaxanthin appearance.

In general, lightness, L^*, increased with higher relative betaxanthin content but decreased when betacyanins were predominant. Chroma, C^*, tended to increase with higher total betalain contents (no. 1379 vs no. 1240) but also depended on the relative ratios of betaxanthins and betacyanins (no. 1240 vs no. 1320). The hue angle, $h°$, ranged from 82.3° for the yellow no. 1320 to 347.2° for cv. Purple. Based on these findings, three groups could be distinguished: the yellow cactus pears of cv. Orange and no. 1320, the red clones no. 1281 and no. 1379, and finally the red-purple fruits of cv. Red, cv. Purple, and clone no. 1240. Lime-green juices from clone nos. 1288, 1458, and cv. Green were highly turbid, but colorless after filtration. Since measurements did not afford reproducible data, they were not included in **Table 7**.

While these general trends could be predicted from ratios of the respective absorbances at 480 and 538 nm (**Table 7**), the presence of the newly discovered gomphrenin I may open further possibilities in breeding trials: cactus fruits with higher ratios of 6-*O*-glycosylated structures would yield a more purplish

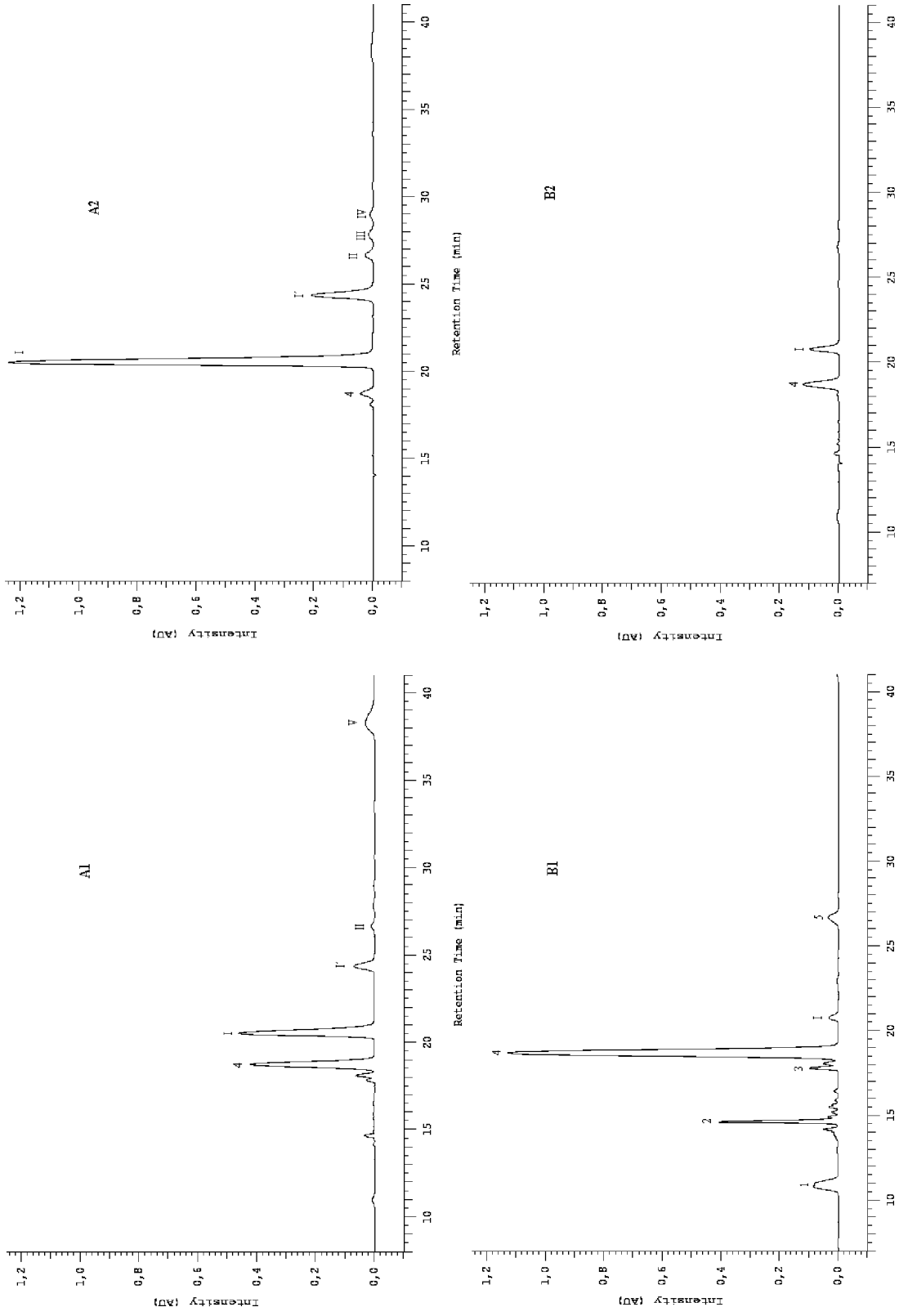

Figure 1. HPLC profile of betaxanthins and betacyanins in juices from a red-purple (cv. Purple, **A**) and a yellow (no. 1320, **B**) cactus pear clone monitored at 476 nm (**A1**, **B1**) and 538 nm (**A2**, **B2**), respectively. Peak assignment is given in **Table 6**.

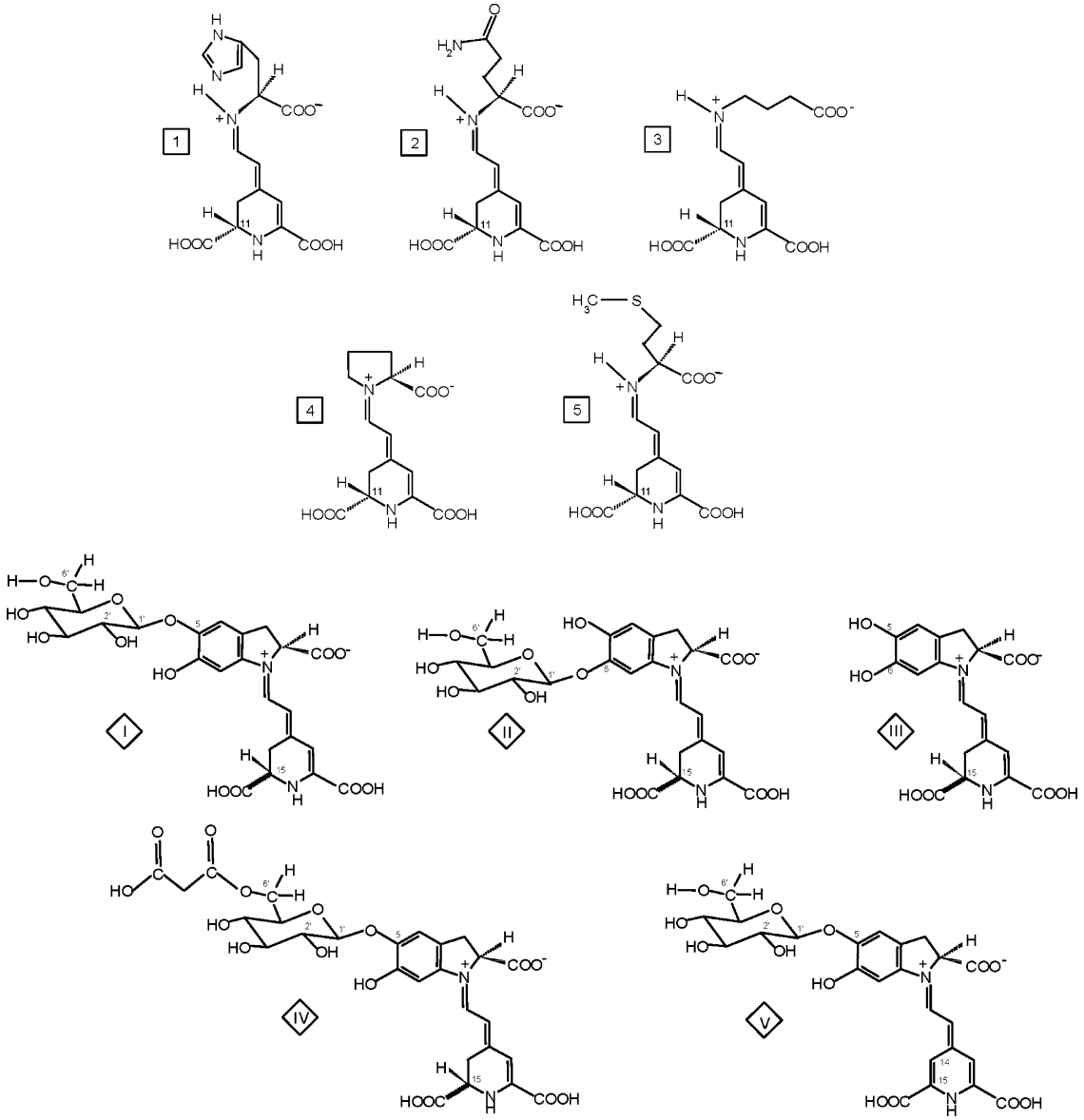

Figure 2. Structures of betaxanthins (**1**–**5**) and betacyanins (**I**–**V**) in various *Opuntia* sp. clones. Peak assignment is given in **Table 6**.

Table 7. Color Attributes for Fruit Juices from Various *Opuntia* Clones[a]

clone	*L**	*C**	*h*°	Bc/Bx[b]
no. 1240	64.5	53.2	6.1	1.06
cv. Purple	60.6	58.9	347.2	2.00
no. 1281	64.2	44.4	15.9	1.08
cv. Red	58.4	41.8	10.3	1.31
no. 1379	60.5	39.6	22.8	1.02
cv. Orange	78.6	45.7	80.2	0.13
no. 1320	83.8	52.3	82.3	0.09

[a] Color data were determined on the basis of similar tinctorial strengths of 0.95 ± 0.05. [b] Data are based on quantification as shown in **Table 3**. Bc = betacyanins; Bx = betaxanthins.

Table 8. Segregation of Colors in Progeny of Various *Opuntia* Crosses

	number of progeny			
cross	green fruit	orange fruit	red fruit	purple fruit
cv. Green × cv. Purple	3	0	0	23
cv. Green × cv. Red	21	0	40	0
cv. Green × cv. Orange	11	17	0	0
cv. Green × no. 1320	4	13	0	0

appearance than the corresponding 5-*O*-derivatives thereby broadening the array of color shades of cactus pears.

Color Heritage. Lime green fruits such as no. 1288, no. 1458, and cv. Green are evidently lacking the enzymes necessary for betalain synthesis. Thus, crosses between the lime green and the yellow-orange or red fruits could provide the genetic information for pigment production. However, in a freely outcrossing octaploid plant of unknown heterozygosity like *O. ficus-indica*, the results are difficult to predict. Data from a field hybridization program (**Table 8**) shed some light on the heritability of betalain pigments. In the cross of cv. Purple with cv. Green, 23 progenies had purple and only 3 produced green fruit. Although, there were no progenies with intermediate color such as red or pink, minor differences in the red-purple color intensity could be observed (data not shown). The progenies of the cv. Red × cv. Green displayed a similar pattern. In this case, however, almost a third of the progeny bore fruits without any pigment. Two different types of segregation patterns were found when cv. Green was crossed with two orange-yellow clones (cv. Orange or no. 1320). Thirty-nine percent of the progeny were devoid of pigments when cv. Orange was the

parent, whereas only 23% of the progeny were lacking betalains when no. 1320 served as parent. Since no. 1320 has about twice the betaxanthin concentration compared to cv. Orange, it might be suspected that the former possesses a higher number of color alleles in its octaploid chromosomes expressing in a greater percentage of colored progeny.

The present study substantiated the health-promoting properties of betalains that are the focal point of most recent studies (*56*). Betaxanthins and betacyanins (*10*, *14*), uncolored phenolics (*8*, *32*, *40*), mucilages, fibers, and other valuable nonvolatile constituents of *Opuntia* sp. (*11*, *13*) contribute to providing a complementary suite of nutraceuticals. Since cactus pears are low-input systems with a considerable genetic diversity, there is a bright future for this promising crop. However, due to the ability to freely hybridize with *Opuntias* of other ploidy levels (*19*, *20*), there are considerable taxonomic problems with the fruit producing flat stemmed cacti largely known as *O. ficus-indica*. Even though some of us (R.B., P.F.) have made germplasm collections throughout the ranges in Latin America and have worked with segregating progeny of full sib hybrids among various species, the genetic relationships are still most confusing. Even when considering all spiny and spineless, flat-stemmed platyopuntias to be *O. ficus-indica*, the species designation in the medical literature was incorrect (P.F., R.B., personal observation). These taxonomic problems are additionally confounded with ambiguity on which part of the fruit was analyzed (flower, whole fruit, pulp, peel). Therefore, scientists should improve characterization and standardization of the plant material and detail their sample work up. To prevent confusion, a free service for the taxonomic characterization of fruit bearing platyopuntias based on photographs of cladodes, spines, flowers, and fruits is provided (*57*).

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