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Fruit Flesh Betacyanin Pigments in *Hylocereus* Cacti

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Determination of profiles and total contents of betacyanins in cactus fruits of *Hylocereus* species using chromatographic and spectrophotometric method is described. The investigated species were *H. polyrhizus*, *H. purpusii*, *H. costaricensis*, *H.* sp. *487* (all red-flesh species and hybrids made among them), and the white- or red-flesh species *H. undatus*. Hybrids included hybrid 1 (*H. undatus* white-flesh clone and *H.* sp. *487*), hybrid 35 (*H.* sp. *487* and *H. polyrhizus*), and the reciprocal hybrid hybrid 95 (*H. polyrhizus* and *H.* sp. *487*). Fruits of *H. polyrhizus* exhibited the highest relative concentration (expressed as percentage of the total HPLC peak area) of hylocerenin, a recently discovered pigment, and a high relative concentration of phyllocactin. Hylocerenin and isohylocerenin, present in fruits at relative concentrations of 11.7 and 5.8%, respectively, are probably responsible for the fluorescent color of the fruit pulp. *H. costaricensis* fruits have a much higher content of phyllocactin (63.9%), which is almost 4 times higher than the betanin content. These differences in pigment concentrations might explain the differences in red hues of the flesh of these fruits.

KEYWORDS: Vine cacti; dragon fruit; pitaya; pitahaya; *Hylocereus polyrhizus*; *H. undatus*; *H. costaricensis*; *H. purpusii*; Cactaceae; betacyanins; betalains; betanin; phyllocactin; hylocerenin

INTRODUCTION

Betacyanins are a class of water soluble pigments that provide the colors in a wide variety of flowers and fruits. The red-violet betacyanins and the yellow betaxanthins belong to the betalain pigments. Betanidin and isobetanidin (the corresponding C-15 diastereoisomer) are the simplest betacyanins (*1*, *2*).

Currently ∼30 stuctures of betacyanins are known and are well documented (*3*). Most of these are 5-*O*-glucosides, such as betanin, which is present in almost all plants containing betacyanins and is the major red-violet pigment in red beet root (*Beta vulgaris*), but 6-*O*-glucosides have also been detected. Further glycosylation of the 5-*O*-glucoside is very common and as is esterification with hydrocinnamic acids such as ferulic or *p*-coumaric acids (*3*) or malonic acid (*1*, *4*). Recent research reported the structural elucidation and discovery of a new betacyanin, named hylocerenin [betanidin 5-*O*-[6′-*O*-(3′′-hydroxy-3′′-methylglutaryl)-*β*-D-glucopyranoside]] in newly domesticated species of *Hylocereus* cacti (*5*).

Hylocereus polyrhizus (**Figure 1**) and its related species belong to the vine cacti from the subfamily Cactoideae of the tribe Cacteae (*6*). This tribe contains many species with edible fruits. Several species of climbing cacti of the genus *Hylocereus* have recently been developed as fruit crops (*7*−*9*). There are ∼16 species of *Hylocereus* in Central America and Mexico, and *H. undatus* is widely used as garden plant in the tropical world. Fruits of *H. undatus* need no more than ∼40 days to complete development. Other species of *Hylocereus*, such as *H. costaricensis* (**Figure 1b**) and *H. polyrhizus*, are also candidates for domestication because they produce large attractive fruits (*6*, *10*). Crosses between these species occur and can result in hybrids with good horticultural characteristics (*11*).

The fruits of *Hylocereus* species, known as red pitaya or pitahaya, which means "the scaly fruit", in Latin America, are medium-large berries bearing large green or red scales (*12*). The peel is usually red, and the pulp varies from red or purple colors of various hues to white. The pulp is delicate and juicy and contains numerous small soft seeds. The plants are grown in the open in tropical areas but must be protected from intense solar radiation and subfreezing temperatures when cultivated under subtropical conditions such those prevailing in Israel (*9*, *13*). In Israel, some of these cacti are already produced commercially, among them *Hylocereus polyrhizus*, which has a glowing deep red-purple fruit flesh (*14*). The pulp of *H. polyrhizus* is already used in Israel for the production of red-violet ice cream. These fruits have also the potential to be used in low-temperature dairy drinks and in light drinks with or without other fruit juices.

The objective of this study was to determine profiles and total contents of betacyanins in fruits of *Hylocereus* species using chromatographic and spectrophotometric methods.

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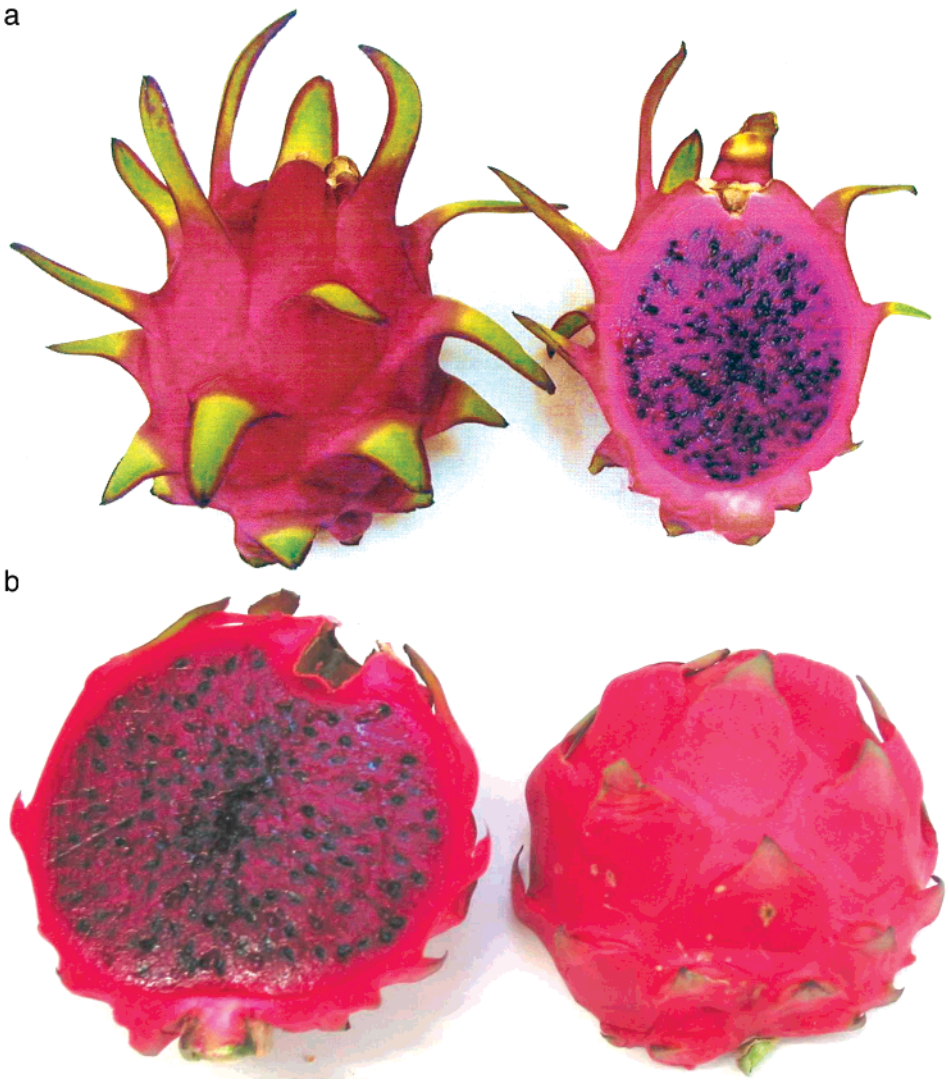

Figure 1. Photographs of (a) *H. polyrhizus* and (b) *H. costaricensis*.

MATERIALS AND METHODS

Plant Materials and Growing Conditions. The species selected for this study were *H. polyrhizus*, *H.* sp. *487*, *H. purpusii*, *H. costaricensis*, *H. undatus* (red- and white-flesh species), and various hybrids made among them. The hybrids were hybrid 1 (white flesh clone of *H. undatus* & sp. *487*), hybrid 35 (*H.* sp. *487* and *H. polyrhizus*), and hybrid 95 (*H. polyrhizus* and *H.* sp. *487*). The clones were originally introduced as cuttings to Israel from the Huntington Botanical Garden in California or from other cacti gardens from Israel and elsewhere (*14*). Hybrids were made in Beer-Sheva, Israel, by Ben-Gurion University personnel (*11*). The study was carried out at the Ben-Gurion University of the Negev campus in Beer-Sheva, during 1998−2000. The experiments were performed on 3−4-year-old-plants grown on a trellis in a greenhouse. The plants were irrigated once a week with 2 L of water per plant during the cold wet season (November−April) and twice a week with 2.5 L of water per plant during the hot season (May−October); water contained 70 ppm of N, 9 ppm of P, and 70 ppm of K. The average maximum/minimum greenhouse temperatures were 28/6 °C in the coldest month (January) and 35/18 °C in the hottest month (August). The maximum temperature was 45 °C during the summer, and the minimum temperature was 2 °C during the winter. All flowers were hand-cross-pollinated with other compatible clones and tagged (*10*). The fruits were harvested for analysis after reaching full ripening stage (between 30 and 40 days after anthesis). The edible pulp was taken for analysis.

Pigment Extraction. Typically 100 g of the peeled fruit, of watery consistency, was shaken with 200 mL of 80% aqeuous EtOH for a few minutes, followed by fast filtration and centrifugation at 18000 rpm and −4 °C for 20 min. After careful decantation, the extract was concentrated in vacuo at 25 °C to 3−4 mL and stored in a dark vessel at −19 °C. The roots from red beet (*Beta vulgaris*) was processed by using the same procedure. The supernatant was analyzed directly by UV−vis spectrophotometry. For HPLC analysis the extracts were purified.

UV—Vis. Total betacyanin content and UV−vis absorption spectra in the range of 200−700 nm were determined on a Jasco U-530 UV−vis spectrophotometer (Jasco Corp., Tokyo, Japan).

Extract Purification. The concentrated betacyanin extracts were purified in two steps prior to analytical HPLC by column chromatography. The first cleanup was performed on a Sephadex G-25 (Sigma, Steinheim, Germany) according to the method of given in ref *15*. The extract (0.5 mL) was purified on gel in a 40 cm × 1.2 cm i.d. glass column, pre-equilibrated with 1% acetic acid. The 1% acetic acid eluent flow rate was 1.0 mL/min. The red betacyanin fraction, eluting between 40 and 45 min, was collected and lyophilized. The residue was redissolved in water and purified on LH-20-100 (Sigma) (*15*) in a 40 cm × 1.0 cm i.d. glass column with water as eluent (flow rate = 0.9 mL/min). The red betacyanin fractions, eluting between 35 and 40 min, were collected and lyophilized.

HPLC Analysis. Analytical HPLC was carried out on an LC-10AT-VP chromatograph with a photodiode array SPD-M10A VP detector (Shimadzu Corp., Kyoto, Japan) equipped with a LiChroCART 250-4 (250 × 4 mm i.d.), LiChrospher 60 RP-select B (5 μm) column, or a LiChroCART 250-4 (250 × 4 mm i.d.), LiChrospher 100 RP-18 (5 μm) column protected by a guard column. The separation was performed isocratically using a mixture of 90% solvent A (0.5%

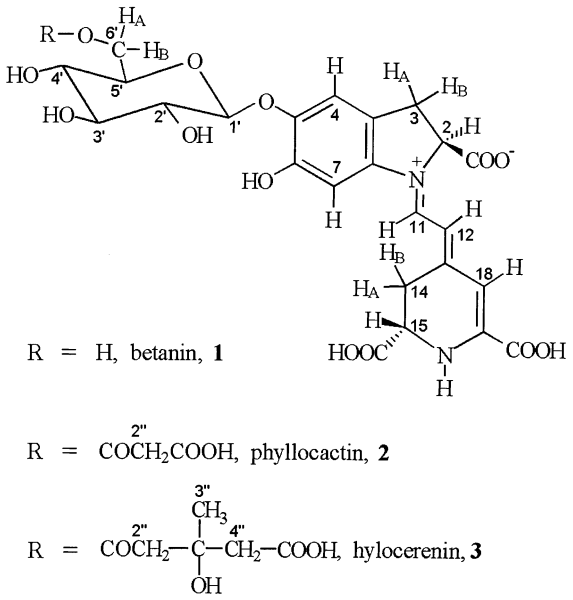

Figure 2. Chemical structures of analyzed betacyanins in the *Hylocereus* cacti. Compound numbers refer to **Figure 3** and **Table 1**.

aqueous TFA) with 10% solvent B (acetonitrile) for 35 min at a flow rate of 0.5 mL/min (injection volume = 10 μL; detection at 538 nm): **1**, t_R = 8 min; **2**, t_R = 18 min; **3**, t_R = 23 min. Slightly different elution patterns of betacyanins were observed depending on the column (compounds **2′** and **3** were eluted in different orders).

RESULTS AND DISCUSSION

The presence of the betacyanins (**1**−**3**) and their *15R*-isoforms (**1′**−**3′**) has been detected previously by HPLC of fruit pulp extracts from *H. polyrhizus*, and their structures were elucidated by electrospray MS-MS and ^{1}H NMR (**Figure 2**) (*5*). Fruit extracts of the other *Hylocereus* clones exhibited similar retention time profiles on HPLC chromatograms (**Figure 3**).

Parts A−C of **Figure 3** show the typical HPLC profiles of *H. polyrhizus*, *H.* sp. *487*, and *H. costaricensis* extracts obtained on the analytical LiChrospher 100 RP-18 column. Usually six pigments were observed. On an analytical column filled with LiChrospher 60 RP-select B stationary phase, compounds corresponding to peaks 2′ and 3 eluted in reversed order. Furthermore, the *15S*-forms are eluted in HPLC on ODS-type columns with acidic eluents earlier than the *15R*-forms (the isoforms) (*16*). Therefore, peaks 1−3 can be attributed to betanidin-based forms, whereas peaks 1′−3′ can be attributed to isobetanidin-based forms. Additionally in this work compounds **1**/**1′** were assigned as betanin and isobetanin by cochromatography with authentic betanin from red beet.

Distribution and quantification of the betacyanins in the *Hylocereus* species have not been described previously. The quantitative analysis results revealed that betanin and phyllocactin were the predominant betacyanins in all fruits of *Hylocereus* species (**Table 1**). The betanin concentration, expressed as percentage of the total HPLC peak area, ranged between 17.9% for *H. costaricensis* and 76.2% for hybrid 1 (*H. undatus* white clones and *H.* sp. *487*). The highest relative concentration of phyllocactin was found for *H. costaricensis* and was 63.9% of the total area. The *Hylocereus* fruits contained usually much lower amounts of betanin and phyllocactin isoforms.

The highest relative concentration of the newly discovered acylated betacyanin, hylocerenin, was observed in *H. polyrhizus* fruits (11.7%). The lowest amounts were found in fruits of *H.* sp. *487* (1.5%) and fruit 8 (hybrid of *H. undatus* white clones and *H.* sp. *487*) (1.3%). The isohylocerenin relative content was almost negligible in most of the fruits except for *H. polyrhizus* (5.8%).

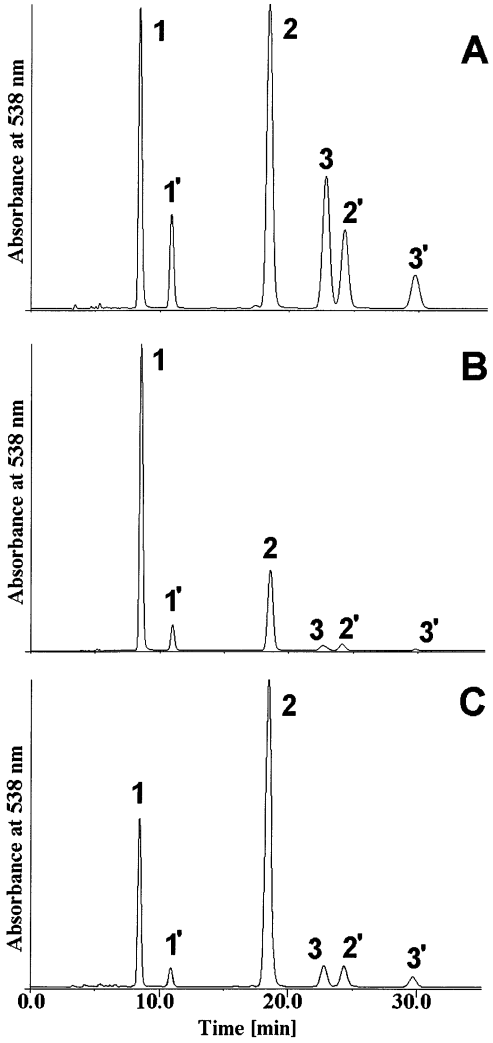

Figure 3. HPLC patterns of betacyanins from fruit pulp of *H. polyrhizus* (A), *H.* sp. *487* (B) and *H. costaricensis* (C). Peak numbers refer to **Figure 2**. The corresponding isoforms are depicted with numbers 1′, 2′, and 3′.

The total betacyanin contents were calculated (**Table 1**) in a similar way to that reported in ref *17*, with some modifications. The results were expressed as milligrams of betanin equivalents per gram of fresh fruit pulp, taking $E_{538}(1\%) = 1120$ for betanin (*18*) during spectrophotometric calculations. The concentration ranged between 0.39 and 0.23 mg/g, and the highest amount was found in fruits of *H. costaricensis*.

Different colors were observed in *Hylocereus* fruit pulps. Fruit pulp of *H. polyrhizus* is characterized by the glowing purple-red color in contrast to less glowing or nonglowing colors in the pulp of the other species. The difference was attributed to different pigments and their different concentration ratios in the pulp. The color of the fruit pulp of *H. polyrhizus* can be attributed to the presence of pigment **3** in the fruit.

Among the fruits investigated only fruits of *H. polyrhizus* exhibited much higher contents of hylocerenin, phyllocactin, and their isoforms. Hylocerenin and isohylocerenin, present in fruits at 11.7 and 5.8%, respectively, are probably responsible for the fluorescent color of the fruit pulp. *H. costaricensis* fruits have a much higher content of phyllocactin (63.9%), which is almost 4 times higher than the betanin content.

Table 1. Total Contents and Relative Concentrations (Expressed as Percentage of the Total HPLC Peak Area) of the Six Betacyanins Found in Analyzed *Hylocereus* Cacti

	relative concn (%) for peaks			
fruit	1 1′	2 2′	3 3′	total pigment content (mg/g)
H. polyrhizus	18.9 ± 1.3	36.1 ± 2.2	11.7 ± 1.1	0.28 ± 0.019
	7.2 ± 0.55	19.2 ± 1.5	5.8 ± 0.32	
H. sp. *487*	57.2 ± 4.2	34.2 ± 2.1	1.5 ± 0.11	0.30 ± 0.023
	3.4 ± 0.41	2.0 ± 0.18	0.2 ± 0.04	
H. purpusii	66.9 ± 4.1	21.3 ± 1.4	2.0 ± 0.18	0.23 ± 0.018
	7.2 ± 0.73	2.4 ± 0.17	0.1 ± 0.03	
H. costaricensis	17.9 ± 1.4	63.9 ± 4.1	6.4 ± 0.72	0.39 ± 0.041
	2.8 ± 0.32	7.4 ± 0.66	1.0 ± 0.15	
H. undatus	61.2 ± 4.3	28.0 ± 2.1	2.2 ± 0.17	0.29 ± 0.027
(red clone)	6.0 ± 0.51	1.9 ± 0.17	0.6 ± 0.07	
hybrid 1	76.2 ± 5.7	12.0 ± 1.0	1.3 ± 0.12	0.28 ± 0.024
(*H. u.* white & *487*)	9.6 ± 0.79	0.7 ± 0.09	0.2 ± 0.03	
hybrid 35	60.6 ± 4.2	19.5 ± 1.9	4.1 ± 0.34	0.33 ± 0.031
(*487* & *H. p.*)	13.6 ± 1.3	1.9 ± 0.17	0.2 ± 0.04	
hybrid 95	57.9 ± 3.8	19.7 ± 1.5	3.6 ± 0.44	0.30 ± 0.023
(*H. p.* & *487*)	11.3 ± 1.1	6.4 ± 0.53	1.0 ± 0.11	

[a] Values are means of three samples ± confidence interval at 95% level of confidence. Peak numbers refer to **Figure 2**.

It is interesting to note that the red flesh of *H. undatus* had a content of red pigments similar to that of the hybrid made of the white-flesh clone *H. undatus* with *H.* sp. *487*, but the ratio among the pigments was different. This dissimilarity might point to dissimilarity of pigment metabolism between these two genotypes. On the other hand, for the reciprocal hybrids (samples 7 and 8 in **Table 1**) both the content and the profile were similar, pointing to similar metabolisms.

The first results of betacyanin concentration analysis in fruits of diverse *Hylocereus* cacti make it clear that these cacti are the third richest betacyanin source for food-coloring agents after *Beta vulgaris* and *Amaranthus* species (*17*). Various contents of hylocerenin, phyllocactin, and their isoforms found in the cacti fruits may result in different properties of the coloring agents derived from these fruits.

This is the first report of its kind on these new fruit crops, and much more work should be carried out with various species, genera, and hybrids to get a better understanding of how profiles and hues are related, their mode of inheritance, and possible commercial uses of these pigments in the food industry.

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