ELSEVIER

Food Chemistry 77 (2002) 101–106

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose

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Received 15 May 2001; received in revised form 7 September 2001; accepted 7 September 2001

Abstract

The betalain pattern of *Hylocereus polyrhizus* (Weber) Britton & Rose is reported for the first time and a highly practicable technique is described for the simultaneous isolation of pigment and mucilage from the mesocarp by extraction with water. Whereas betaxanthins were totally absent, 10 betacyanins could be separated by HPLC-PDA. For eight betacyanins the respective molecular masses could be obtained by positive ion electrospray mass spectrometry. Five of them were unequivocally assigned to bougainvillein-r-I, betanin, isobetanin, phyllocactin, and iso-phyllocactin. The remaining betacyanins were tentatively identified as (6′-*O*-3-hydroxy-3-methyl-glutaryl)-betanin, its C_{15}-stereoisomer, and (6′-*O*-3-hydroxy-3–butyryl)-betanin, respectively. Using the β-glucosidase assay, acylated structures could be distinguished from non-acylated betacyanins. Contrary to white-fleshed *Hylocereus undatus* (Haworth) Britton & Rose with a slightly pink mesocarp, *H. polyrhizus* most likely possesses the same set of betalain forming enzymes in both pulp and mesocarp as the betacyanin pattern was found to be similar.

Keywords: Pitaya; *Hylocereus polyrhizus*; Betalain pigments; Natural food colorants

1. Introduction

Oblong shaped fruits of the genus *Hylocereus* (Berger) Britton & Rose originating from Latin America and known as red pitaya derive from climbing epiphytes belonging to the Cactaceae family. Due to Crassulacean Acid Metabolism (CAM), members of this family exhibit an extraordinarily high water-use efficiency with low water requirements (Raveh, Nerd, & Mizrahi, 1998; Winter & Smith, 1996). An additional ecological feature was very recently pointed out with CAM plants responding to high CO_2 atmospheric concentrations by increasing their biomass production (Drennan & Nobel, 2000). Compared to the more common cactus pear (*Opuntia* sp.), the usually red peel from *Hylocereus* possesses large scales instead of spines and its pulp only contains small digestible seeds (Raveh, Weiss, Nerd, & Mizrahi, 1993). Due to self-incompatibility, cross-pollination is required and hand-pollination is still necessary to obtain fruits (Lichtenzveig, Abbo, Nerd, Tel-Zur, & Mizrahi, 2000; Mizrahi & Nerd, 1999; Weiss, Nerd, & Mizrahi, 1994). Pulp colour in *Hylocereus* fruits varies from white to red and purple (Nerd, Gutman, & Mizrahi, 1999). Producing a deep purple-coloured flesh comparable to red beet (Stintzing, Schieber, & Carle, 2000b) or amaranth (Cai, Sun, & Corke, 1998), fruits from *Hylocereus polyrhizus* are highly appealing. Public concern about possible or proven harmful effects of artificial food colorants has increased the search for natural colour sources. Showing a stable appearance in the pH range from 3 to 7, betalains have a great potential in colouring a broad array of food. In this view, betacyanins from *H. polyrhizus* are most promising, not only as colouring agents but also in possessing antiradical potential (Escribano, Pedreño, García-Carmona, & Muñoz, 1998; Pedreño & Escribano, 2001).

Very recently, ripening and postharvest behaviour of *Hylocereus undatus* (Haworth) Britton & Rose and *H. polyrhizus* were studied to determine the optimum date of harvest in relation to colour development of pulp and peel (Nerd et al., 1999). However, no data were given about the pigment pattern of *H. polyrhizus*. Both the juice from the pulp and the aqueous extract of the deep-coloured mesocarp provide a hitherto little known

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0308-8146/02/$ - see front matter
PII: S0308-8146(01)00374-0

source of a natural food colorant. Peel and mesocarp could be exploited for the recovery of a natural thickening agent which has yet to be characterized. Mucilages in the pulp may display similar properties as recently reviewed for *Opuntia* sp. (Stintzing, Schieber, & Carle, 2001). Therefore, the aim of the present work was to develop a method for gaining both pigments and mucilages and thereby getting an insight in the betalain pattern of *H. polyrhizus* pulp and mesocarp.

2. Materials and methods

2.1. Plant material

Fruits were purchased from Israel and stored at −26 °C. After thawing at 4 °C, they were manually squeezed, and the filtered juice was flushed with nitrogen and kept at −80 °C. The mesocarp was removed from the pulp with a stainless steel knife and either stored frozen (−80 °C) or taken for analysis.

2.2. Solvents and reagents

Reagents and solvents were purchased from Merck (Darmstadt, Germany) and were of analytical or HPLC grade. C_{18}-Reversed phase cartridges Chromabond 1000 mg were from Macherey & Nagel (Düren, Germany). β-Glucosidase from sweet almonds was obtained from Serva (Heidelberg, Germany).

2.3. Extraction of mesocarp for pigment recovery

Ten millilitres of purified water were added per 1 g of plant tissue and macerated in a blender at low speed under ice-cooling. Extraction of the pigments was accomplished within a few minutes. Mucilagenous material was separated from the extract on a Buchner funnel to give a coloured solution. The residue was re-extracted with water and the filtrate concentrated in vacuo at 30 °C. Two millilitres of 96% EtOH were added to 1 ml of coloured solution for precipitation of the pectic substances. After separation of the pigment solution with a Buchner funnel, the filter residue was washed with EtOH/water (2/1, v/v) until all betacyanins were removed. The coloured solution was again concentrated under reduced pressure and the procedure repeated two times until no further precipitation by addition of EtOH was observed.

2.4. Precipitation of pectic substances and proteins from juice

For removal of pectic and other colloidal substances, 2 ml of 96% EtOH were added to 1 ml of sample. After 20 min, the mucilages were separated from the aqueous phase and washed with an EtOH/water mixture of 2/1 (v/v) until the solid was colourless. EtOH was removed under reduced pressure at 30 °C to restore the initial sample volume.

2.5. Purification and concentration of betacyanins

Desalting was carried out using C_{18}-reversed phase cartridges. Acidified water was prepared by adding TFA to purified water until a pH of 2 or 3 was reached, respectively. The sorbent material was activated with three volumes of MeOH, and then rinsed with three volumes of acidified water (pH 3). The unpurified sample was adjusted to pH 3 by addition of 0.1 N HCl and subsequently applied to the sorbent. The betacyanins non-ionically bound to the material were then desalted by eluting sugars and acids with three volumes of acidified water (pH 3). Elution of the betacyanins was accomplished by addition of acidified MeOH (95/5 MeOH/pH 2 water, v/v). The desalted sample was repetitively concentrated in vacuo at 30 °C and resuspended in water until a pH of 5–6 was reached. For HPLC analysis, the resulting pigments were rediluted with purified water.

2.6. β-Glucosidase assay

The β-glucosidase assay was modified according to Piattelli and Minale (1964). The acetate buffer (1 l) was prepared by dissolving 3.95 g of sodium acetate in purified water, before adding 3.0 g of glacial acetic acid as well as 20 ml of 1 N NaOH and was finally adjusted to pH 5 (Wilcox, Wyler, Mabry, & Dreiding, 1965). To a mixture of 750 μl of acetate buffer 50 μl of pressed juice were added. An aliquot of this blank solution was applied to the HPLC and the enzymatic hydrolysis started through the addition of 200 μl of β-glucosidase solution containing 83.5 units/ml when the first peak of the blank eluted on the HPLC-system. Every 60 min an aliquot of the reaction mixture was injected and the hydrolysis of the betacyanins was monitored over a total period of 12 h and compared to a blank solution.

2.7. HPLC analysis

2.7.1. System I

The HPLC-system (Hewlett-Packard Series 1100, Hewlett Packard, Waldbronn, Germany) was as follows: a thermoautosampler ALS G1313A, a binary gradient pump G1311A, a degasser G1322A, a column oven ColComp G1316A keeping a constant temperature of 25 °C, and a diode array detection system G1315A. Chromatographic analysis was performed using an analytical scale (250×4.6 mm i.d.) AQUA C_{18}-reversed phase column with a particle size of 5 μm (Phenomenex, Torrance, CA, USA), fitted with a security guard C_{18}

ODS (4×3.0 mm i.d.). The mobile phase A consisted of 2% (v/v) acetic acid in water, 0.5% acetic acid in water and acetonitrile (50/50, v/v) was used as B. The elution was carried out following a linear gradient from 10% B in A to 27% B in A in 30 min. Simultaneous monitoring was performed at 320 nm for hydroxycinnamoyl moieties, at 475 nm for betaxanthins and at 538 nm for betacyanins at a flow rate of 1 ml/min.

2.7.2. System II

The HPLC-system (Merck, Darmstadt, Germany) was equipped with an auto sampler L-7200, an interface module D-7000, a pump L-7100, a column-oven L-7350 with peltier cooling module, and a diode array detector L-7450A. Chromatographic analysis was performed using an analytical scale (250×3 mm i.d.) LUNA C_{18}-reversed phase column with a particle size of 5 μm (Phenomenex, Torrance, CA, USA), fitted with a security guard C_{18} ODS (4×3.0 mm i.d.). HPLC conditions were as follows: Eluent A consisted of 0.2% (v/v) formic acid in water, a mixture of ACN/water (80/20 v/v) was used as eluent B. Separation of all betalains was achieved in 30 min at 25 °C and a flow rate of 1 ml/min. Starting with 5% B in A at 0 min, a linear gradient was followed to 9% B in A at 33 min. Monitoring of betalains was performed at 320 nm for hydroxycinnamoyl moieties, at 475 nm for betaxanthins and at 538 nm for betacyanins.

2.8. LC-MS analysis

The HPLC-system (Hewlett-Packard Series 1100, Hewlett Packard, Waldbronn, Germany) was as follows: a thermoautosampler ALS G1313A, a binary gradient pump G1311A, a degasser G1322A, a column oven ColComp G1316A keeping a constant temperature of 25 °C, and a diode array detection system G1315A. Chromatographic analysis was performed using an analytical scale (250×3 mm i.d.) LUNA C_{18}-reversed phase column with a particle size of 5 μm (Phenomenex, Aschaffenburg, Germany), fitted with a security guard C_{18} ODS (4×3.0 mm i.d.). HPLC conditions were as described for system II. The MS-system was a Micromass Platform II equipped with a crossflow interface. The tuning parameters for positive ion electrospray (ES+) were 3.50 kV for capillary and 40 eV for cone at a source temperature of 120 °C.

3. Results and discussion

Up till now, the betalain pattern of *Hylocereus* sp. has not been reported. Although *H. undatus* has been subject to investigations (Barbeau, 1990; Rodríguez-Díaz, Ocampo Hurtado, & Casillas Gómez, 1995), no data exist about the pigments in *H. polyrhizus*. HPLC analyses of a purified juice and of aqueous extracts from the mesocarp revealed the presence of at least eight betacyanins and the absence of betaxanthins. Removal of both mucilage and sugars prior to pigment analysis turned out to be advantageous.

3.1. Extraction and purification of betacyanins from mesocarp tissue

For distinction of pigments in the pulp and mesocarp, a method reported by Rodríguez-Díaz et al. (1995) was optimized. As pigment recovery of mesocarp tissue with 0.5-cm cubes was low due to a limited surface, a different treatment was required. In addition, the temperature during extraction (40–50 °C) was deleterious to betacyanin integrity and would proliferate microbial growth. High shear stress during maceration or the extraction with aqueous MeOH as often described for phytochemical analyses (Strack, Steglich, & Wray, 1993) both resulted in gel formation and subsequent entrappment of the pigments. Aqueous extraction at temperatures below 10 °C after careful tissue maceration allowed total discoloration of the tissue. Further precipitation of the pectic-like substances and re-extraction yielded pigments devoid of mucilagenous material. Betacyanin fractions were subsequently desalted on a C_{18}-cartridge. The pigments from the mesocarp and from juice derived from the pulp had an identical HPLC pattern. The identity of individual compounds was confirmed by their spectral properties as well as by LC-MS studies.

3.2. β-Glucosidase assay

The β-glucosidase assay is a sensitive tool for differentiating acylated from non-acylated structures. As described by Minale, Piattelli, de Stefano, and Nicolaus (1966), steric hindrances may affect the action of emulsin: β-glucosidase did not cleave phyllocactin as the malonyl residue bound to the glucose unit hampered the enzymic hydrolysis. In this study, the assay yielded betanidin when compounds (1), (2) and (3) were hydrolyzed, indicating a glucose moiety attached to the *cyclo*-Dopa structure of betanidin. Due to the low stability of the aglyca, betanidin and isobetanidin readily degraded after being formed, the latter therefore being not detectable (data not shown). The remaining compounds, however, were not affected by enzymatic action indicating acylated structures of betanin.

3.3. HPLC-PDA and ion electrospray mass spectrometry

By applying two different HPLC-systems to the analysis of betacyanins from *H. polyrhizus*, similarity of the chromatographic patterns (System I, Fig. 1 and System

II, Fig. 2) was ascertained. However, two minor peaks (8 and 9; Fig. 2) could not be resolved in system I. This was due to coelution with compound (7) resulting in peak tailing and lower peak purity of (7). Consequently, system II was chosen for further investigation with all compounds showing a peak purity greater than 99%. The identity of these compounds was confirmed by HPLC (Fig. 2) coupled with electrospray mass spectrometry in the positive ionization mode yielding the following protonated molecular ions $[M+H]^+$: (1) *m/z* 713, (2) *m/z* 551, (3) *m/z* 551, (4) *m/z* 637, (5) *m/z* 637, (6) *m/z* 695, (7) *m/z* 637 and (10) *m/z* 695 (Table 1). All betacyanins produced a daughter ion at *m/z* 389, corresponding to [betanidin + H^+]. Based on its molecular mass, its sensitivity to β-glucosidase and its retention characteristics, the first eluting betacyanin (1) was identified as bougainvillein-r-I. Consistent with data on bougainvillein-v (Heuer, Richter, Metzger, Wray, Nimtz, & Strack, 1994), it eluted prior to betanin on the reversed phase system due to the presence of a di-glucose moiety. Hitherto, only Piattelli and Imperato (1970a) reported on bougainvillein-r-I in purple bracts of *Bougainvillea* 'Mrs. Butt' belonging to the red-violet 5-*O*-β-sophoroside derivatives of betanidin. In contrast to the violet-red 6-*O*-β-sophorosides of the bougainvillein-v type from *B. glabra* showing a bathochromic shift in their visible absorption (Heuer et al., 1994; Piattelli & Imperato, 1970b), the absorption characteristics of bougainvillein-r-I were similar to betanin consistent with the findings by Piattelli & Imperato (1970a). Results of co-chromatographic studies with authentic standards betanin and isobetanin from red beet were confirmed by mass spectrometric data for peaks (2) and (3). The relatively longer retention time and the lack of extended UV absorption compared to betanin, indicated betacyanin structures with aliphatic acyl groups: Betacyanins (4) and (7) corresponded to phyllocactin and its C_{15}-diastereoisomer. While the mass spectrometric data for phyllocactin were consistent with those of Kobayashi, Schmidt, Nimtz, Wray, and Schliemann (2000), the absorption maxima in the visible range slightly differed. The deviation of 3 nm may be ascribed to the different pH values of the mobile phases used. All remaining compounds were tentatively identified by their spectral properties and by their molecular masses. Resulting fragments at *m/z* 389 indicated betanidin type pigments. The occurrence of 'neo'-compounds which would have produced a daughter ion at *m/z* 387 corresponding to neobetanidin (Strack, Engel, & Wray, 1987) could therefore be excluded. With respect to the UV absorption, there is strong evidence for an acylation with other than cinnamic acid derivatives since the latter would cause an additional absorption band at 310–320 nm (Heuer, Wray, Metzger, & Strack, 1992). The same was

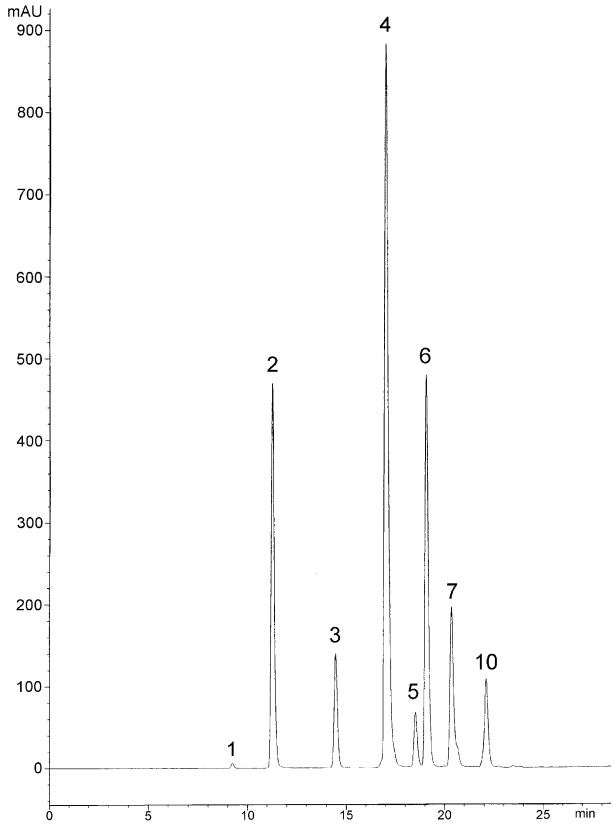

Fig. 1. HPLC separation of betacyanins from red-purple pitaya, *Hylocereus polyrhizus* (538 nm, system I; peak assignment is given in Table 1).

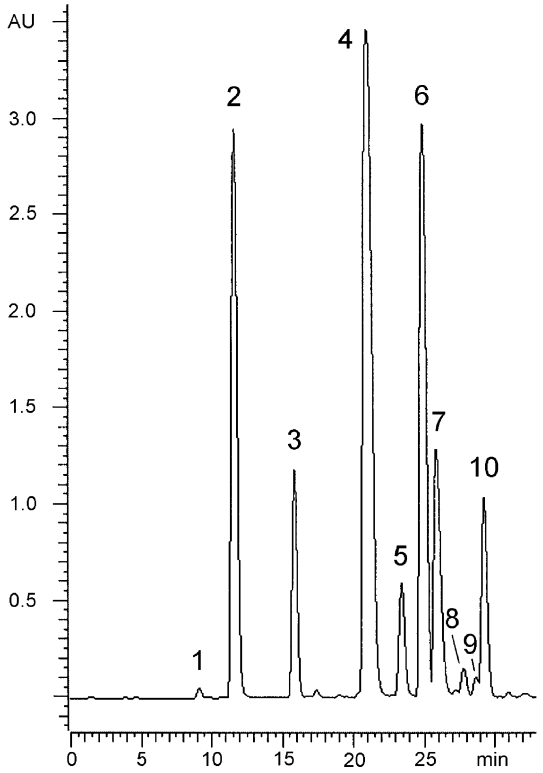

Fig. 2. HPLC separation of betacyanins from red-purple pitaya, *Hylocereus polyrhizus* (538 nm, system II; peak assignment is given in Table 1).

Table 1
Peak assignment for betacyanin pigments from pulp and mesocarp of *Hylocereus polyrhizus* (Weber) Britton & Rose

Peak number[a]	Definitive name (trivial name)	HPLC-System I		HPLC-System II		$[M+H]^+$
		R_t (min)	λ_{max} VIS (nm)	R_t (min)	λ_{max} VIS (nm)	
1	Betanidin-5-*O*-β-sophoroside (Bougainvillein r-I)	9.24	532	9.04	536	713
2	Betanidin-5-*O*-β-glucoside (Betanin)	11.28	536	11.43	536	551
3	Isobetanidin-5-*O*-β-glucoside (Isobetanin)	14.47	536	15.59	536	551
4	Betanidin-5-*O*-(6′-*O*-malonyl)-β-glucoside (Phyllocactin)	17.01	536	20.67	536	637
7[b]	Iso-betanidin-5-*O*-(6′-*O*-3-hydroxy-3-methyl-glutaryl)-β-glucoside	20.36	536	25.69	536	695
6[b]	Betanidin-5-*O*-(6′-*O*-3-hydroxy-3-methyl-glutaryl)-β-glucoside	19.07	536	24.67	536	695
10[b]	Betanidin-5-*O*-(6′-*O*-3-hydroxy-butyryl)-β-glucoside	22.10	536	29.12	536	637
8	Unknown betacyanin	–[c]	–[c]	27.70	536	–[c]
9	Unknown betacyanin	–[c]	–[c]	28.57	536	–[c]
5	Isobetanidin-5-*O*-(6′-*O*-malonyl)-β-glucoside (Isophyllocactin)	18.52	536	23.27	536	637

[a] As indicated in Figs. 1 and 2.
[b] Tentatively identified betanin-type structures.
[c] Not detectable.

true for compounds 8 and 9 (Fig. 2; HPLC-system II) for which no definite mass spectrometric data could be obtained. Evidence is given that compounds 6 and 10 are C_{15}-stereoisomers of betanin acylated with 3-hydroxy-methyl-glutaric acid. The identical acid was found in iresinin I and its C_{15}-epimer iresinin II which were reported for *Iresine herbstii* Hook. F. ex Lindl. (Cai, Sun, & Corke, 2001; Minale et al., 1966) as well as for *Amaranthus* species (Cai et al., 2001). Considering its molecular mass and its spectral properties, we assume compound 5 to be betanidin-5-*O*-glycoside acylated with 3-hydroxy-butyric acid.

Simultaneous occurrence of 5- and 6-*O*-glycosides of betanidin in the same plant tissue has been reported in flowers from *Gomphrena globosa* L. but was found to be very rare (Heuer et al., 1992). Recent investigations on *Phytolacca americana* L. (Schliemann et al., 1996) and on the *Amaranthaceae* (Cai et al., 2001) gave additional proof of this interesting phytochemical phenomenon. Other studies (Heuer et al., 1992; Piattelli & Imperato, 1970a) maintain that the attachment of sugar moieties to the C_6-position of betanidin induces a slight bathochromic shift in comparison to the corresponding C_5-adducts. However, a similar effect could not be observed for either of the betacyanins found (Table 1), thus indicating the presence of betanidin 5-*O*-glycosides. Both reversed phase systems parallely showed seven distinct peaks with uniform spectral data and a few minor ones, one of which was bougainvillein-r-I. Further studies are underway to fully elucidate pigments (5), (6), and (10) in *H. polyrhizus*.

4. Conclusion

This study on the pigment pattern of *H. polyrhizus* showed that seven major betacyanins with identical λ_{max} contribute to its deep-purple colour. Since betaxanthins tend to degrade more easily resulting in a shade of brown colour (Stintzing et al., 2000b), their absence is technologically advantageous. The betalain composition of mesocarp and pulp did not differ, thus indicating a similar set of enzymes in the two neighbouring tissues. Simple aqueous extraction of the mesocarp did not only yield the betalain pigments but also allowed their complete separation from the mucilagenous material at the same time. Considering an economical perspective, *H. polyrhizus* represents a potential commercial source of betalains, since by-products of fruit manufacture can also be exploited: whereas the pectic-like substance could be used in the food industry as a thickening agent or as a moisturizer in cosmetic products, the aqueous mesocarp extract as well as the juice from the pulp could serve as a colouring stuff for low acid food commodities. Additionally, betacyanins were very recently recognized as antiradical agents (Escribano et al., 1998; Pedreño & Escribano, 2001). Soluble fibres have been associated with blood sugar regulation in humans suffering from diabetes mellitus type II (Trejo-González et al., 1996). Other studies showed that mucilages may exert a positive influence on cholesterol metabolism (Luz-Fernández, Lin, Trejo, & McNamara, 1992; Luz-Fernández, Trejo, & McNamara, 1990, 1994) underlining the nutritional significance of *H. polyrhizus*. This again points out the high potential of hitherto little exploited plants as reviewed for the genus *Opuntia* of the Cactaceae family (Stintzing, Schieber, & Carle, 2000a; Stintzing et al., 2001).

Acknowledgements

The authors thank Dr. W. Armbruster, Institute of Food Chemistry, Hohenheim University, for perform-

ing LC-MS analyses. The authors are grateful to *fruit*-International Fruit Foundation, Heidelberg, Germany for financial support.

References

Barbeau, G. (1990). La pitahaya rouge, un nouveau fruit exotique. *Fruits*, *45*(2), 141–147.

Cai, Y., Sun, M., & Corke, H. (1998). Colorant properties and stability of Amaranthus pigments. *Journal of Agricultural and Food Chemistry*, *46*(11), 4491–4495.

Cai, Y., Sun, M., & Corke, H. (2001). Identification and distribution of simple and acylated betacyanins in the Amaranthaceae. *Journal of Agricultural and Food Chemistry*, *49*(4), 1971–1978.

Drennan, P. M., & Nobel, P. S. (2000). Responses of CAM species to increasing atmospheric CO_2 concentrations. *Plant, Cell and Environment*, *23*, 767–781.

Escribano, J., Pedreño, M. A., García-Carmona, F., & Muñoz, R. (1998). Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. *Phytochemical Analysis*, *9*, 124–127.

Heuer, S., Richter, S., Metzger, J. W., Wray, V., Nimtz, M., & Strack, D. (1994). Betacyanins from bracts of *Bougainvillea glabra*. *Phytochemistry*, *37*(3), 761–767.

Heuer, S., Wray, V., Metzger, J. W., & Strack, D. (1992). Betacyanins from flowers of Gomphrena globosa. *Phytochemistry*, *31*(5), 1801–1807.

Kobayashi, N., Schmidt, J., Nimtz, M., Wray, V., & Schliemann, W. (2000). Betalains from Christmas cactus. *Phytochemistry*, *54*(4), 419–426.

Lichtenzveig, J., Abbo, S., Nerd, A., Tel-Zur, N., & Mizrahi, Y. (2000). Cytology and mating systems in the climbing cacti *Hylocereus* and *Selenicereus*. *American Journal of Botany*, *87*(7), 1058–1065.

Luz-Fernandez, M., Lin, E. C. K., Trejo, A., & McNamara, D. J. (1992). Prickly pear (*Opuntia* sp.) pectin reverses low density lipoprotein receptor suppression induced by a hypercholesterolemic diet in guinea pigs. *Journal of Nutrition*, *122*, 2330–2340.

Luz-Fernandez, M., Lin, E. C. K., Trejo, A., & McNamara, D. J. (1994). Prickly pear (*Opuntia* sp.) pectin alters hepatic cholesterol metabolism without affecting cholesterol absorption in guinea pigs fed a hypercholesterolemic diet. *Journal of Nutrition*, *124*, 817–824.

Luz-Fernandez, M., Trejo, A., & McNamara, D. J. (1990). Pectin isolated from prickly pear (*Opuntia* sp.) modifies low density lipoprotein metabolism in cholesterol-fed guinea pigs. *Journal of Nutrition*, *120*, 1283–1290.

Minale, L., Piattelli, M., de Stefano, S., & Nicolaus, R. A. (1966). Pigments of Centrospermae. VI. Acylated Betacyanins. *Phytochemistry*, *5*, 1037–1052.

Mizrahi, Y., & Nerd, A. (1999). Climbing and columnar cacti: new arid land fruit crops. In J. Janick (Ed.), *Perspectives on new crops and new uses* (pp. 358–366). Alexandria, VA: ASHS Press.

Nerd, A., Gutman, F., & Mizrahi, Y. (1999). Ripening and postharvest behaviour of fruits of two *Hylocereus* species (Cactaceae). *Postharvest Biology and Technology*, *17*, 39–45.

Pedreño, M. A., & Escribano, J. (2001). Correlation between antiradical activity and stability of betanine from *Beta vulgaris* L. roots under different pH, temperature and light conditions. *Journal of the Science of Food and Agriculture*, *81*(7), 627–631.

Piattelli, M., & Imperato, F. (1970a). Betacyanins from *Bougainvillea*. *Phytochemistry*, *9*, 455–458.

Piattelli, M., & Imperato, F. (1970b). Pigments of *Bougainvillea* glabra. *Phytochemistry*, *9*, 2557–2560.

Piattelli, M., & Minale, L. (1964). Pigments of Centrospermae. I. Betacyanins from *Phyllocactus hybridus* Hort. and *Opuntia ficus-indica* Mill. *Phytochemistry*, *3*, 307–311.

Raveh, E., Nerd, A., & Mizrahi, Y. (1998). Responses of two hemiepiphytic fruit crop cacti to different degrees of shade. *Scientia Horticulturae*, *73*, 151–164.

Raveh, E., Weiss, J., Nerd, A., & Mizrahi, Y. (1993). Pitayas (Genus Hylocereus): a new fruit crop for the Negev Desert of Israel. In J. Janick, & J. E. Simon (Eds.), *New crops* (pp. 491–495). New York: Wiley.

Rodríguez-Díaz, A. J., Ocampo Hurtado, A. L., & Casillas Gómez, F. J. (1995). Extracción de un colorante natural a partir de la cáscara de la pitahaya (Hylocereus undatus). *Tecnología de Alimentos (México)*, *30*(6), 22–26.

Schliemann, W., Joy, R. W. IV, Komamine, A., Metzger, J. W., Nimtz, M., Wray, V., & Strack, D. (1996). Betacyanins from plants and cell cultures of *Phytolacca americana*. *Phytochemistry*, *42*(4), 1039–1046.

Stintzing, F. C., Schieber, A., & Carle, R. (2000a). Cactus pear—a promising component to functional food. *Obst-, Gemüse- und Kartoffelverarbeitung*, *85*(1), 40–47.

Stintzing, F. C., Schieber, A., & Carle, R. (2000b). Rote Bete als färbendes Lebensmittel—eine Bestandsaufnahme. *Obst-, Gemüse- und Kartoffelverarbeitung*, *85*(5/6), 196–204.

Stintzing, F. C., Schieber, A., & Carle, R. (2001). Phytochemical and nutritional significance of cactus pear. *European Food Research and Technology*, *212*(4), 396–407.

Strack, D., Engel, U., & Wray, V. (1987). Neobetanin: a new natural plant constituent. *Phytochemistry*, *26*(8), 2399–2400.

Strack, D., Steglich, W., & Wray, V. (1993). Betalains. In P. M. Dey, & J. B. Harborne (Eds.), *Methods in plant biochemistry 8, P.G. Waterman, alkaloids and sulphur compounds* (pp. 421–450). London: Academic Press.

Trejo-González, A., Gabriel-Ortiz, G., Puebla-Pérez, A. M., Huízar-Contreras, M. D., Munguía-Mazariegos, M. R., Mejía-Arreguín, S., & Calva, E. (1996). A purified extract from prickly pear cactus (*Opuntia fuliginosa*) controls experimentally induced diabetes in rats. *Journal of Ethnopharmacology*, *55*, 27–33.

Weiss, J., Nerd, A., & Mizrahi, Y. (1994). Flowering behavior and pollination requirements in climbing cacti with fruit crop potential. *HortScience*, *29*(12), 1487–1492.

Wilcox, M. E., Wyler, H., Mabry, T. J., & Dreiding, A. S. (1965). Die Struktur des Betanins-Über die Konstitution des Randenfarbstoffes Betanin. *Helvetica Chimica Acta*, *48*(1), 252–258.

Winter, K., & Smith, J. A. C. (1996). *Crassulacean acid metabolism. biochemistry, ecophysiology and evolution* (Ecological Studies 114). Berlin/Heidelberg/New York: Springer Verlag.