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Identification of Betalains from Yellow Beet (*Beta vulgaris* L.) and Cactus Pear [*Opuntia ficus-indica* (L.) Mill.] by High-Performance Liquid Chromatography−Electrospray Ionization Mass Spectrometry

FLORIAN C. STINTZING, ANDREAS SCHIEBER, AND REINHOLD CARLE*

Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, Garbenstrasse 25, D-70599 Stuttgart, Germany

Betaxanthins, the yellow-orange water-soluble pigments from yellow beet (*Beta vulgaris* ssp. *vulgaris* cv. Bejo Zaden) and cactus pear (*Opuntia ficus-indica* cv. Gialla) have been investigated using an HPLC system compatible with mass spectrometry. Five novel betaxanthins were found and characterized as the immonium adducts of betalamic acid with serine, γ-aminobutyric acid, valine, isoleucine, and phenylalanine. To enable concentration of betalain samples, desalting was performed by solid-phase extraction. With this technique, betacyanins could be separated from the betaxanthins using the pH-dependent retention characteristics of red and yellow betalains. The betaxanthin fraction was taken for the preparation of betalamic acid as a precursor for semisynthetic standards. The HPLC method was applied to yellow beet and cactus pear, revealing a more complex betalain profile than described earlier, thus proving its suitability for screening of betaxanthin-containing plants as potential sources for natural food colors.

KEYWORDS: Betalains; betaxanthins; betacyanins; amino compounds; *Beta vulgaris*; yellow beet; *Opuntia ficus-indica*; cactus pear; solid-phase extraction; HPLC; mass spectrometry

INTRODUCTION

Natural colorants from plant sources are receiving increased interest from both food manufacturers and consumers. Whereas anthocyanins are well-known as water-soluble colorants, the betalains have not been as thoroughly investigated. This may be explained by the more widespread distribution of anthocyanins compared to betalains, the latter being restricted to 10 families of the plant order Caryophyllales and to the genus *Amanita* of the Basidiomycetes (*1*). Until now, red beet (*Beta vulgaris* L.) is the only betalain source exploited for use as a natural food-coloring stuff, yielding various shades of red and violet (*2*). There is an obvious need for water-soluble yellow pigments, and therefore sources of betaxanthins have become of interest (*3*). However, up to the present, little work has been performed on the analysis of betaxanthins for food-coloring purposes. Yellow beets (*Beta vulgaris* L. ssp. *vulgaris* cv. Golden Beet) have scarcely been investigated (*4, 5*), and only recently, *Opuntia ficus-indica* (L.) Mill. was found to be a potential source of both betacyanins and betaxanthins (*3*). Nineteen betaxanthin structures have hitherto been found in nature (*6*−*15*). Less common amino acids or amines related to the secondary metabolism of betalain-generating plants (*16*−*20*) may give rise to further structures that have not yet been described. Only very recently, betalamic acid conjugates of 3-methoxytyramine and tryptophan were elucidated in *Celosia* (*15*), thus indicating a more complex profile of natural betalamic acid derivatives than so far believed.

The analysis of betalains has mainly focused on the red-violet betacyanins (*21*), whereas betaxanthins were mostly considered for the elucidation of biochemical pathways (*22*, *23*). Moreover, the lack of commercially available standards has limited betalain analysis. Therefore, for betalain screening of edible plant material, an HPLC system compatible with mass spectrometric analysis was developed and used for the separation and identification of the complex betaxanthin mixtures found in cactus pear and yellow beet. Betaxanthin standards were synthesized by reaction of betalamic acid with amino compounds. In this study, betaxanthin mixtures were directly hydrolyzed for betalamic acid preparation. Solid-phase extraction (SPE) was used for the separation of betaxanthins from betacyanins, for their concentration, and for the purification of betalamic acid.

MATERIALS AND METHODS

Plant Material. Yellow beet (*Beta vulgaris* L. ssp. *vulgaris* cv. Bejo Zaden, Chenopodiaceae) was provided by Ernteband (Winnenden, Germany) and stored at 4 °C. Cactus pear [*Opuntia ficus-indica* (L.) Mill. cv. Gialla, Cactaceae] was purchased from Italy, stored at 4 °C, and processed within 1 week to prevent microbial spoilage.

* Author to whom correspondence should be addressed (telephone + 49-711-459-2314; fax + 49-711-459-4110; e-mail carle@uni-hohenheim.de).

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Pigment Extraction. For pigment analysis of the yellow flesh, 100 g of peeled beets was macerated in a blender and extracted by continuous stirring with 300 mL of 50% aqueous methanol containing 50 mM sodium ascorbate. The yellow solution was separated from the plant tissue on a Büchner funnel with a filter paper (Schleicher & Schuell, Dassel, Germany). To achieve complete discoloration of the plant material, the filter residue was rinsed twice with the extraction solution and finally with 100% methanol. The extract was concentrated in vacuo (30 °C), resuspended in 50 mL of purified water, and flushed with nitrogen before freezing at −80 °C. Cactus pear fruits were manually squeezed, and the filtered juice was flushed with nitrogen and stored at −80 °C.

Solvents and Reagents. Reagents and solvents were purchased from Merck (Darmstadt, Germany) and were of analytical or HPLC grade. Amino acids and amines were from Fluka (Buchs, Switzerland). The polymeric resin Amberlite XAD-16 HP (20−50 mesh) was purchased from Rohm & Haas (Darmstadt, Germany). C_{18} reversed phase cartridges (Chromabond, 1000 mg) were obtained from Macherey & Nagel (Düren, Germany).

Precipitation of Hydrocolloids and Proteins. Hydrocolloids and proteins were removed by adding 2 mL of 96% ethanol to 1 mL of sample as described previously (*24*). After 20 min, the mucilages were separated from the aqueous phase on a Büchner funnel with a filter paper (Schleicher & Schuell) and washed with an ethanol/water mixture (2:1, v/v) until the solid was colorless. Ethanol was removed under reduced pressure at 30 °C, and the residue was dissolved in 2 mL of acidified water (pH 3, see below).

Desalting and Fractionation of Betalain Mixtures on a Polymeric Resin. Acidified water was prepared by adding trifluoroacetic acid (TFA) to purified water until a pH of 2 or 3 was reached. Amberlite XAD-16 HP was slurried in purified water and kept overnight at 4 °C. Before use, the material was rinsed with purified water until the supernatant was clear. A glass column (400 × 15 mm i.d.) was filled with the slurry, and the sorbent material was rinsed with 1 L of purified water and then activated with 0.5 L of 2% aqueous sodium hydroxide solution. After neutralization by rinsing with purified water, the material was conditioned to pH 3 by washing with 1 L of acidified water. Aliquots of 10 mL of the sample were taken for precipitation of mucilages as described above. The sample was applied to the resin and subsequently desalted by rinsing with 0.3 L of acidified water (pH 3) at a flow rate of 10 mL/min. Then, the betaxanthins were eluted with 100% methanol while the betacyanins remained adsorbed until eluted with acidified methanol [95:5, methanol/acidified water (pH 2), v/v]. The yellow fraction was neutralized by addition of 3 M NH_4OH to prevent betaxanthins from acid hydrolysis, concentrated in vacuo at 30 °C, and resuspended in purified water for the recovery of betalamic acid.

When no fractionation was needed, all betalains were eluted in one step with acidified methanol [95:5, methanol/acidified water (pH 2), v/v] directly after desalting, then neutralized, and finally concentrated under reduced pressure at 30 °C as described above. After dissolving in purified water, the samples were directly analyzed or flushed with nitrogen prior to storage at −80 °C.

Desalting and Fractionation of Betalain Mixtures on a C_{18} Cartridge. The C_{18} cartridge was activated with 3 volumes of 100% methanol and then rinsed with 3 volumes of acidified water (pH 3). Aliquots of 1 mL of the sample were taken for precipitation of mucilages as described above, except that the colored material was dissolved in 1 mL of purified water. The sample was applied to the minicolumn and subsequently desalted by rinsing with 3 volumes of acidified water (pH 3). For fractionation, the betaxanthins were eluted with 100% methanol while the betacyanins remained adsorbed until eluted with acidified methanol [95:5, methanol/acidified water (pH 2), v/v]. The yellow fraction was neutralized by addition of 3 M NH_4OH to prevent betaxanthins from hydrolysis, then concentrated in vacuo at 30 °C, and resuspended in purified water. When no fractionation was required, all betalains were eluted in one step and handled as described above for the resin.

Preparation of Betalamic Acid. Juice from cactus pear was desalted, and betaxanthins were separated from betacyanins as described above. For betalamic acid preparation, betaxanthins were hydrolyzed at pH 11.3−11.5 through addition of 25% NH_4OH. The hydrolysis was followed spectrophotometrically using a Lambda 20 UV−vis spectrometer (Perkin-Elmer, Überlingen, Germany) at 424 nm (*25*). At the point of maximum absorption, the reaction was stopped by addition of 0.1 M HCl under ice cooling to reach pH 2. After Amberlite XAD-16 HP had been preconditioned with acidified water (pH 2), the bright yellow solution was applied and washed with 0.5 L of acidified water (pH 2) to remove amino compounds released through betaxanthin hydrolysis. Betalamic acid was then eluted with 100% methanol, and the eluate was immediately adjusted to pH 10 through addition of 3 M NH_4OH and then concentrated in vacuo at 30 °C. For stabilization of betalamic acid, the solution was again adjusted to pH 9 by addition of 3 M NH_4OH. The solution was directly taken for partial synthesis of betaxanthins or stored at −80 °C.

Partial Synthesis of Betaxanthins. Partial synthesis was modified according to a method described previously (*25*). Amino acid or amine was added to the alkaline solution of betalamic acid in a 10−20-fold molar excess. After vortexing, condensation was initiated by subsequent evaporation in vacuo (30 °C) to yield the respective betaxanthin resulting in a pH of 4−6. Before analysis, the semisynthetic standards were filtered (0.2 μm), or flushed with nitrogen, and stored at −80 °C.

Isolation of Betanin and Isobetanin. Betanin and isobetanin were obtained by semipreparative HPLC as described earlier (*20*).

High-Performance Liquid Chromatography (HPLC). The HPLC system (Merck) was equipped with an L-7200 autosampler, a D-7000 interface module, an L-7100 pump, an L-7350 column oven with Peltier cooling module, and an L-7450A diode array detector. Analyses were performed using an analytical scale (250 mm × 3 mm i.d.) LUNA C_{18} reversed phase column with a particle size of 5 μm (Phenomenex, Torrance, CA), fitted with a C_{18} ODS guard column (4 mm × 3.0 mm i.d.). HPLC conditions were as follows: Eluent A consisted of 0.2% TFA and 10% HCOOH (65:35, v/v), and eluent B was prepared by mixing 100% acetonitrile and 10% HCOOH (80:20, v/v). Complete separation of betalains was achieved within 75 min at 25 °C and at a flow rate of 1 mL/min. The first 15 min was performed isocratically with 100% A, followed by a linear gradient from 0 to 20% B in 60 min. Betalains were monitored at 405 nm for betalamic acid and at 470 and 538 nm for betaxanthins and betacyanins, respectively.

Electrospray Mass Spectrometry (MS). The Hewlett-Packard series 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) consisted of an ALS G1313A thermoautosampler, a G1311A binary gradient pump, a G1322A degasser, a ColComp G1316A column oven keeping a constant temperature of 25 °C, and a G1315A diode array detection system. Chromatographic conditions were as described above. The detector was a Platform II (Micro Mass, Manchester, U.K.) equipped with a cross-flow interface. The optimal tuning parameters for positive ion electrospray were found to be 3.50 kV for capillary and 40 eV for cone at a source temperature of 120 °C.

RESULTS AND DISCUSSION

Semipurification of Betalain Samples and Separation of Betacyanins and Betaxanthins. SPE with C_{18} sorbents (*26*, *27*) and polymeric resins (*28*−*30*) have been widely applied in anthocyanin and phenolic compound analysis but, so far, not routinely used for betalain separation. In this study, separation of yellow and red cactus pear pigments was achieved on both sorbents by using the pH-dependent retention characteristics of betaxanthins and betacyanins. Indicaxanthin, the major compound in cactus pear, displayed retention characteristics similar to those of betanin and was the only betaxanthin that could not be completely separated from the betacyanins. Although the C_{18} minicolumn proved to be applicable for desalting and fractionation of betalain samples, a polymeric resin was chosen for larger volumes and more highly concentrated samples. Using this method, desalting and fractionation were possible in one step. Whereas desalting of betanin solutions on a polymeric resin using acidified aqueous acetone as eluent has been described earlier (*31*), the separation of betacyanins and betaxanthins has

Table 1. Retention Times and HPLC-PDA and Mass Spectrometric Data of Betaxanthins, Betalamic Acid, Betanin, and Isobetanin and Their Occurrence in Yellow Beet and Cactus Pear

amino acid or amine moiety	trivial name	t_R standard[a] (min)	λ_{max} vis (nm)	*m/z* $[M + H]^{+}$ [b]	
				yellow beet	cactus pear
asparagine	vulgaxanthin III	4.1	470		
histidine	muscaaurin VII	4.2	474		
serine		4.9	470	299	299
taurine		5.0	463		
aspartic acid	miraxanthin II	6.2	470		
glutamine	vulgaxanthin I	6.9	470	340	340
hydroxyproline	portulacaxanthin I	7.1	477		
ornithine		7.3	460		
glycine	portulacaxanthin III	7.6	470		
histamine		8.3	467		
methionine sulfoxide	miraxanthin I	8.6	470		
arginine		8.8	470		
threonine		10.4	470		
glutamic acid	vulgaxanthin II	12.3	470		
β-alanine		14.9	460		
citrulline		15.0	470		
lysine		16.6	460		
alanine		23.4	467		
	betalamic acid	25.3	406		
γ-aminobutyric acid		29.9	460	297	297
proline	indicaxanthin	32.6	477	309	309
epinephrine		32.7	470		
Dopa	dopaxanthin	36.5	470		
norepinephrine		36.7	470		
cyclo-Dopa-glucoside	betanin	37.7	539		551
octopamine		40.1	470		
cyclo-Dopa-glucoside	isobetanin	40.8	539		551
dopamine	miraxanthin V	42.1	460	347	
5-hydroxytryptophan		42.8	470		
tyrosine	portulacaxanthin II	43.7	470		
methionine		45.4	470		
valine		47.4	470	311	311
serotonin		48.6	463		
norvaline		48.9	470		
tyramine	miraxanthin III	49.9	460		
isoleucine		59.9	470	325	325
leucine	vulgaxanthin IV	60.8	470	325	325
norleucine		62.4	470		
phenylalanine		63.5	470	359	359
tryptophan		65.2	470	398	
phenethylamine		68.9	464		
tryptamine		73.6	463		

[a] Retention times of semisynthesized betaxanthin standards. [b] *m/z* values indicate the occurrence of the respective betalain in the sample.

not yet been reported. In other studies betalain fractionation was performed on ion-exchange resins (*32*−*35*) or by stepwise extraction with acidified ethanol (*36*). However, an additional desalting procedure was still necessary (*37*, *38*). Using a series of volatile eluents containing formic acid with a pH between 0.9 and 2 for fractionation (*35*), additional desalting was dispensable. However, acid residues caused partial degradation of the pigments during evaporation (*39*). In this study, acidification was performed with TFA, possessing a higher acidity and a lower boiling point compared to formic acid. Whereas pH values of 2 and 3 were required for pigment retention, the eluates were adjusted to elevated pH. With respect to pigment stability, betacyanin fractions were kept at pH 5 (*40*), betaxanthins at pH 7, and betalamic acid solutions at pH 9 (*41*).

HPLC/ESI-MS Analysis. HPLC analysis of betaxanthins has been carried out by several groups (*23*, *25*, *42*). Thorough screening of cactus pear samples with a broad spectrum of betaxanthins showed that early-eluting betaxanthins could hitherto not be detected in an MS compatible system. When in this study 10% aqueous formic acid was used to retain all pigments, the most polar betaxanthins coeluted. The separation of early-eluting compounds could be improved through the addition of TFA. Optimum separation was achieved with mixtures of aqueous 10% formic acid and 0.2% aqueous TFA at a ratio of 35:65 (v/v). Fast atom bombardment mass spectrometry has been first exemplified for the structural elucidation of humilixanthin (*7*), and only lately was ion spray successfully adapted to the characterization of betaxanthins (*20*, *23*). When coupled with liquid chromatography, the latter technique allows for direct screening without prior isolation of individual betaxanthins. In accordance with previous studies (*15*, *20*, *23*), only the positive ionization mode proved to produce distinct molecular ions.

Partial Synthesis and HPLC of Betaxanthin Standards. **Table 1** shows retention times and spectral characteristics of 39 betaxanthin standards, together with betalamic acid, betanin, and isobetanin. Betanin and isobetanin were provided as described earlier (*20*), whereas betaxanthins were procured by partial synthesis as reported before (*25*). In this study, the precursor betalamic acid was obtained by alkaline hydrolysis of betaxanthin mixtures from cactus pear (**Figure 1**) and subsequent removal of released amino compounds by SPE. Vice versa, betalamic acid was condensed with amino compounds to yield the respective betaxanthins (**Figure 1**). A similar

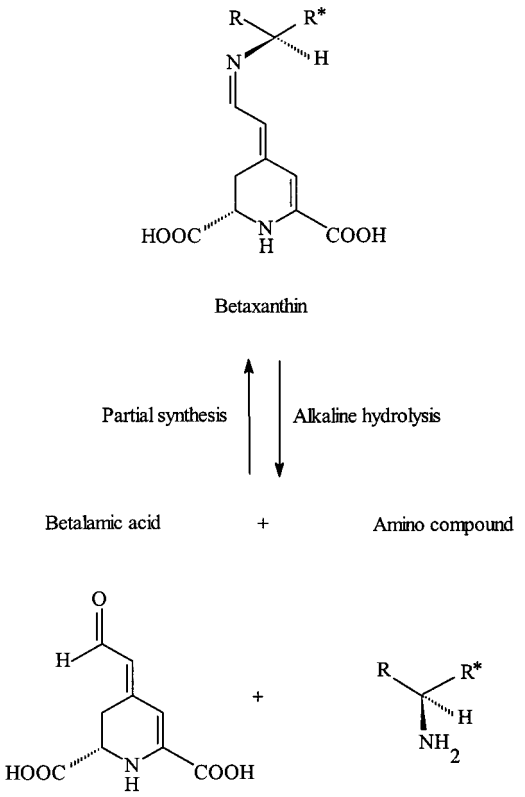

Figure 1. Alkaline hydrolysis and partial synthesis of betaxanthins. R* = COOH for amino acids and H for amines, respectively.

approach has previously been described (*14*) and presents a way to circumvent the semipreparative isolation of betanin as a source of betalamic acid (*23*, *25*). Compared to yellow beet, cactus pear proved to be a better source of betaxanthins due to the low content of phenolic compounds in *Opuntia* (unpublished results), thereby avoiding interferences with betalain analysis. Moreover, pressed cactus pear juices could be taken for sample preparation without extraction as necessary for yellow beet tissue.

The HPLC system presented was suitable for the separation of a great variety of betaxanthins. The first eluting standard was vulgaxanthin III with asparagine as amino acid moiety, whereas the least polar betaxanthin was the tryptamine derivative. In accordance with previous investigations (*23*, *25*), amine derivatives of betalamic acid eluted after their corresponding amino acid adjuncts due to the higher polarity of the latter. This elution order was demonstrated for histidine and histamine, aspartic acid and β-alanine, glutamic acid and γ-aminobutyric acid, Dopa and dopamine, 5-hydroxytryptophan and serotonin, tyrosine and tyramine, and phenylalanine and phenethylamine as well as for tryptophan and tryptamine, respectively. Epinephrine, valine, and leucine eluted prior to their respective 'nor'-amino adducts (**Table 1**).

HPLC-ESI-MS of Betaxanthin Samples. Through the combination of the HPLC system with electrospray mass spectrometry in the positive ionization mode, betaxanthins from yellow beet (*Beta vulgaris* L. ssp. *vulgaris* cv. Bejo Zaden) and cactus pear [*Opuntia ficus-indica* (L.) Mill. cv. Gialla] could be characterized (**Figures 2** and **3**). Although betaxanthins did not differ markedly in their absorbance maxima, their identification was possible by specific retention and mass characteristics. Both plant samples showed a great difference of the major betaxanthin, accounting for 85 and 75% of peak area at 470 nm, compared to the minor compounds ranging from 0.2 to 3.8%

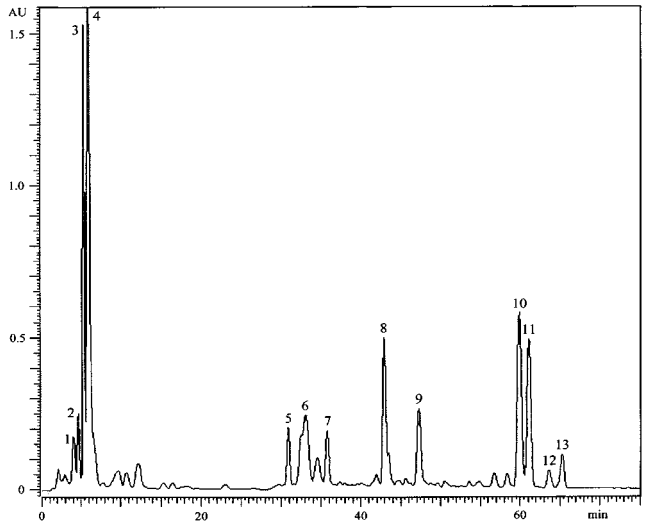

Figure 2. HPLC profile of betaxanthins (Bx) in yellow beet (470 nm). Peaks: 1, tentatively identified as muscaaurin VII, no mass signal could be obtained; 2, serine-Bx; 3, not identified, λ_{max} = 454 nm; 4, vulgaxanthin I; 5, γ-aminobutyric acid-Bx; 6, indicaxanthin; 7, not identified, λ_{max} = 474 nm; 8, miraxanthin V; 9, valine-Bx; 10, isoleucine-Bx; 11, leucine-Bx; 12, phenylalanine-Bx; 13, tryptophan-Bx.

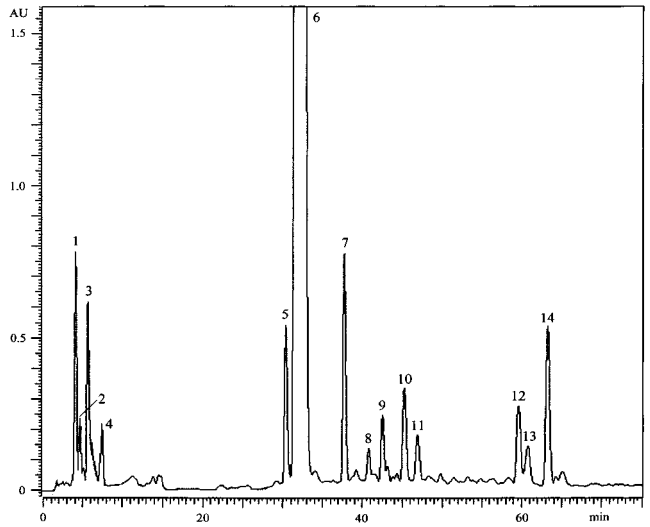

Figure 3. HPLC profile of betacyanins and betaxanthins (Bx) in cactus pear (470 nm). Peaks: 1, tentatively identified as muscaaurin VII, no mass signal could be obtained; 2, serine-Bx; 3, not identified, λ_{max} = 454 nm; 4, vulgaxanthin I; 5, γ-aminobutyric acid-Bx; 6, indicaxanthin; 7, betanin; 8, isobetanin; 9, not identified, λ_{max} = 474 nm; 10, not identified, λ_{max} = 474 nm; 11, valine-Bx; 12, isoleucine-Bx; 13, leucine-Bx; 14, phenylalanine-Bx.

and from 0.4 to 3.3% in yellow beet and cactus pear, respectively. Through removal of both pectic substances and sugars by SPE, a 40-fold concentration of betalain mixtures (40 g of yellow beet tissue or 40 mL of cactus pear juice/mL of sample) was possible with minor compounds reaching a sufficient quantity for MS detection. As a result, a broader profile of betaxanthins than previously reported could be identified in yellow beet and cactus pear. **Table 1** provides a list of synthesized betaxanthins and their occurrence in yellow beet or cactus pear.

Yellow Beet. As shown in **Table 1**, 10 betaxanthins were identified, with vulgaxanthin I being the major compound. The latter has been reported in yellow beetroot together with indicaxanthin (*4*, *5*, *14*, *42*). Due to their low concentration,

vulgaxanthin IV and miraxanthin V have hitherto been detected only in suspension cultures of red and yellow beets (*14*, *22*, *23*). Although the tryptophan adduct was very recently reported to impart the orange and yellow color to *Celosia argentea* inflorescences (*15*), it has never been found in beet before. To the best of our knowledge, adducts of betalamic acid with serine, γ-aminobutyric acid, valine, isoleucine, and phenylalanine have never been reported before as naturally occurring betaxanthins. Consistent with the literature, betacyanins were completely absent in extracts from the flesh of yellow beet (**Figure 2**).

Cactus Pear. Orange and yellow shades of cactus pear fruit are caused by different ratios of betanin and indicaxanthin (*3*, *8*, *43*). In this study, 10 betalains could be detected, with indicaxanthin as the major compound (**Table 1**). Indicaxanthin, vulgaxanthin I, betanin, and isobetanin have previously been reported (*3*, *8*, *43*), whereas vulgaxanthin II, miraxanthin II, and neobetanin, which were already described for different cactus pear cultivars (*3*), could not be identified in the cultivar investigated. Because the presence of neobetanin was also discussed as a result of harsh treatment during sample preparation (*44*), its absence in the present study can be taken as an indicator for a gentle workup procedure. In our investigation, vulgaxanthin IV was detected for the first time in cactus pear, whereas the conjugates of betalamic acid with serine, γ-aminobutyric acid, valine, isoleucine, and phenylalanine, respectively, were described as novel betaxanthins (**Figure 3**).

Through improved means of concentration and HPLC coupled with MS, this study demonstrated a greater variety of betaxanthins than so far known. Furthermore, a simple way for providing betaxanthin standards is presented which allows for thorough screening of plant matrices for their betalain spectrum. Because few data exist on betaxanthin stability and color properties (*4*, *5*), this is also a convenient way to provide betaxanthins for studying model solutions. The antiradical activity of betacyanins was attributed to free phenolic hydroxyl groups (*45*). Some betaxanthins such as dopaxanthin, miraxanthin V, or portulacaxanthin II display this active structural prerequisite, so betaxanthins may also be of interest not only as food colorants and for the supplementation of food with amino compounds (*46*) but also as functional compounds with potential antioxidative properties.

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LITERATURE CITED

(1) Waterman, P. G. Alkaloid chemosystematics. In *The Alkaloids—Chemistry and Pharmacology*; Cordell, G. A., Ed.; Academic Press: San Diego, CA, 1998; Vol. 50, pp 537−565.

(2) Stintzing, F. C.; Schieber, A.; Carle, R. Rote Beete als färbendes Lebensmittel−eine Bestandsaufnahme. *Obst Gemuese Kartoffelverarbeitung (Fruit Vegetable Potato Process.)* **2000**, *85*, 196−204.

(3) Stintzing, F. C.; Schieber, A.; Carle, R. Phytochemical and nutritional significance of cactus pear. *Eur. Food Res. Technol.* **2001**, *212*, 396−407.

(4) Savolainen, K.; Kuusi, T. The stability properties of golden beet and red beet pigments: Influence of pH, temperature, and some stabilizers. *Z. Lebensm.- Unters.- Forsch.* **1978**, *166*, 19−22.

(5) Singer, J. W.; von Elbe, J. H. Degradation rates of vulgaxanthin-I. *J. Food Sci.* **1980**, *45*, 489−491.

(6) Impellizzeri, G.; Piattelli, M.; Sciuto, S. A new betaxanthin from *Glottiphyllum longum*. *Phytochemistry* **1973**, *12*, 2293−2294.

(7) Strack, D.; Schmitt, D.; Reznik, H.; Boland, W.; Grotjahn, L.; Wray, V. Humilixanthin, a new betaxanthin from *Rivina humilis*. *Phytochemistry* **1987**, *26*, 2285−2287.

(8) Piattelli, M.; Minale, L.; Prota, G. Isolation, structure and absolute configuration of indicaxanthin. *Tetrahedron* **1964**, *20*, 2325−2329.

(9) Piattelli, M.; Minale, L.; Prota, G. Pigments of Centrospermae. III. Betaxanthins from *Beta vulgaris* L. *Phytochemistry* **1965**, *4*, 121−125.

(10) Piattelli, M.; Minale, L.; Nicolaus, R. A. Pigments of Centrospermae. V. Betaxanthins from *Mirabilis jalapa* L. *Phytochemistry* **1965**, *4*, 817−823.

(11) Döpp, H.; Maurer, S.; Sasaki, A. N.; Musso, H. Die Konstitution der Muscaaurine. *Liebigs Ann. Chem.* **1982**, *2*, 254−264.

(12) Piattelli, M. Betalains. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, U.K., 1976; pp 560−596, 831−842.

(13) Trezzini, G. F.; Zryd, J.-P. Two betalains from *Portulaca grandiflora*. *Phytochemistry* **1991**, *30*, 1897−1899.

(14) Hempel, J.; Böhm, H. Betaxanthin pattern of hairy roots from *Beta vulgaris* var. *lutea* and its alteration by feeding of amino acids. *Phytochemistry* **1997**, *44*, 847−852.

(15) Schliemann, W.; Cai, Y.; Degenkolb, T.; Schmidt, J.; Corke, H. Betalains of *Celosia argentea*. *Phytochemistry* **2001**, *58*, 159−165.

(16) Endress, R.; Jäger, A.; Kreis, W. Catecholamine biosynthesis dependent on the dark in betacyanin-forming *Portulaca* callus. *J. Plant Physiol.* **1984**, *115*, 291−295.

(17) Kuklin, A. I.; Conger, B. V. Catecholamines in plants. *J. Plant Growth Regul.* **1995**, *14*, 91−97.

(18) Smith, T. A. Phenethylamine and related compounds in plants. *Phytochemistry* **1977**, *16*, 9−18.

(19) Smith, T. A. Tryptamine and related compounds in plants. *Phytochemistry* **1977**, *16*, 171−175.

(20) Stintzing, F. C.; Schieber, A.; Carle, R. Amino acid composition and betaxanthin formation in fruits from *Opuntia ficus-indica*. *Planta Med.* **1999**, *65*, 632−635.

(21) Strack, D.; Wray, V. Recent advances in betalain analysis. In *Caryophyllales: Evolution and Systematics*; Behnke, H.-D., Mabry, T. J., Eds.; Springer-Verlag: Berlin, Germany, 1994; pp 263−277.

(22) Girod, P.-A.; Zryd, J.-P. Secondary metabolism in cultured red beet (*Beta vulgaris* L.) cells: Differential regulation of betaxanthin and betacyanin biosynthesis. *Plant Cell Tiss. Org. Culture* **1991**, *25*, 1−12.

(23) Schliemann, W.; Kobayashi, N.; Strack, D. The decisive step in betaxanthin biosynthesis is a spontaneous reaction. *Plant Physiol.* **1999**, *119*, 1217−1232.

(24) Stintzing, F. C.; Schieber, A.; Carle, R: Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose. *Food Chem.* **2002**, *77*, 121−126.

(25) Trezzini, G. F.; Zryd, J.-P. Characterization of some natural and semi-synthetic betaxanthins. *Phytochemistry* **1991**, *30*, 1901−1903.

(26) Ozmianski, J.; Ramos, T.; Bourzeix, M. Fractionation of phenolic compounds in red wine. *Am. J. Enol. Vitic.* **1988**, *39*, 259−262.

(27) Giusti, M. M.; Wrolstad, R. E. Characterization of red radish anthocyanins. *J. Food Sci.* **1996**, *61*, 322−326.

(28) Idaka, E.; Suzuki, K.; Yamakita, H.; Ogawa, T.; Kondo, T.; Goto, T. Structure of monoacylated anthocyanins isolated from red cabbage (*Brassica oleracea*). *Chem. Lett.* **1987**, *1*, 145−148.

(29) Di Stefano, R.; Guidoni, S. XAD 2 resin in separation of phenolics. *Vitis* **1990**, *29*, 71−78.

(30) Degenhardt, A.; Knapp, H.; Winterhalter, P. Separation and purification of anthocyanins by high-speed countercurrent chromatography and screening for antioxidant activity. *J. Agric. Food Chem.* **2000**, *48*, 338−343.

(31) Czapski, J. The effect of heating conditions on losses and regeneration of betacyanins. *Z. Lebensm.- Unters.- Forsch.* **1985**, *180*, 21−25.

(32) Colomas, J. Séparation des pigments bétalaiques par une méthode fondée sur la chromatographie sur gel de Séphadex appliquée à des plantules d'*Amaranthus caudatus* L. var. *pendula*. *Z. Pflanzenphys.* **1977**, *85*, 227−232.
(33) Colomas, J.; Barthe, P.; Bulard, C. Séparation et identification des bétalaines synthétisées par les tissus de tige de *Myrtillocactus geometrizans* cultivés *in vitro*. *Z. Pflanzenphys.* **1978**, *87*, 341−346.
(34) Wilkins, C. K. Technical note: Improved separations of beet pigments. *Int. J. Food Sci. Technol.* **1987**, *22*, 571−573.
(35) Bokern, M.; Heuer, S.; Wray, V.; Witte, L.; Macek, T.; Vanek, T.; Strack, D. Ferulic acid conjugates and betacyanins from cell cultures of *Beta vulgaris*. *Phytochemistry* **1991**, *30*, 3261−3265.
(36) Bilyk, A. Extractive fractionation of betalains. *J. Food Sci.* **1979**, *44*, 1249−1251.
(37) Döpp, H.; Musso, H. Isolierung und Chromophore der Farbstoffe aus *Amanita muscaria*. *Chem. Ber.* **1973**, *106*, 3473−3482.
(38) Strack, D.; Bokern, M.; Marxen, N.; Wray, V. Feruloylbetanin from petals of *Lampranthus* and feruloylamaranthin from cell suspension cultures of *Chenopodium rubrum*. *Phytochemistry* **1988**, *27*, 3529−3531.
(39) Schliemann, W.; Joy IV, R. W.; Komamine, A.; Metzger, J. W.; Nimtz, M.; Wray, V.; Strack, D. Betacyanins from plants and cell cultures of *Phytolacca americana*. *Phytochemistry* **1996**, *42*, 1039−1046.
(40) Von Elbe, J. H.; Maing, I.-Y.; Amundson, C. H. Color stability of betanin. *J. Food Sci.* **1974**, *39*, 334−337.
(41) Kimler, L.; Larson, R. A.; Messenger, L.; Moore, J. B.; Mabry, T. J. Betalamic acid, a new naturally occurring pigment. *Chem. Commun.* **1971**, *21*, 1329−1330.
(42) Strack, D.; Reznik, H. High-performance liquid chromatographic analysis of betaxanthins in Centrospermae (Caryophyllales). *Z. Pflanzenphys.* **1979**, *94*, 163−167.
(43) Fernández-López, J. A.; Almela, L. Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits. *J. Chromatogr. A* **2001**, *913*, 415−420.
(44) Wyler, H. Neobetanin: a new natural plant constituent? *Phytochemistry* **1986**, *25*, 2238.
(45) Escribano, J.; Pedreño, M. A.; García-Carmona, F.; Muñoz, R. Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. *Phytochem. Anal.* **1998**, *9*, 124−127.
(46) Leathers, R. R.; Davin, C.; Zryd, J.-P. Betalain producing cell cultures of *Beta vulgaris* L. var. *bikores monogerm* (red beet). *In Vitro Cell. Dev. Biol./Plant* **1992**, *28*, 39−45.

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