Studies on Betaxanthin Profiles of Vegetables and Fruits from the Chenopodiaceae and Cactaceae

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The present study provides an update on the betaxanthin (bx) compositions of red and yellow beetroots, yellow-coloured Swiss chard petioles, and yellow-orange cactus pear. Applying RP-HPLC coupled with positive ion electrospray mass spectrometry and by comparison with UV-vis and mass spectrometric characteristics as well as retention times of semi-synthesized reference compounds, 24 betaxanthins were identified in red and yellow beetroot hypocotyls. Twenty-five and thirteen betaxanthins were present in yellow Swiss chard petioles and the cactus pear cultivar 'Gialla', respectively. Ethanolamine-bx and threonine-bx were found to be novel betaxanthins in Chenopodiaceae representatives, which to the best of our knowledge have not been reported as genuine pigments so far. Furthermore, aspartic acid-bx (miraxanthin II), lysine-bx, and methionine-bx, hitherto found in other families, were identified in the Chenopodiaceae for the first time. Additionally, tyrosine-bx (portulacaxanthin II) and tryptophan-bx have not been earlier reported to occur in the Cactaceae. These findings provide valuable phytochemical information and may be useful for a better understanding of the functional properties of betaxanthins in plants.

Key words: Beta vulgaris, *Opuntia ficus-indica*, Betaxanthins

Introduction

Besides chlorophylls, carotenoids, and anthocyanins, betalains are the most common pigments in the plant kingdom. However, the latter comprising the red-purple betacyanins and the yellow-orange betaxanthins are restricted to fungi of some Basidiomycetes genera (Michelot and Melendez-Howell, 2003) and 13 families of the Caryophyllales (Clement and Mabry, 1996) including the Chenopodiaceae and Cactaceae. Besides their chemotaxonomic significance (Strack *et al.*, 2003), there is a renewed commercial interest in betalains for natural food colouring due to their exceptional stability from pH 3 to pH 7 (Stintzing and Carle, 2004). In addition, the betalains have been demonstrated to exhibit antioxidant properties being effective *in planta* (Sepúlveda-Jiménez *et al.*, 2005; Wang *et al.*, 2006) and *ex vivo* (Cai *et al.*, 2003; Stintzing *et al.*, 2005; Wettasinghe *et al.*, 2002). Therefore, the pigment composition of betalainic plants, especially edible sources such as yellow beetroot (Stintzing *et al.*, 2002), cactus pear fruits (Stintzing *et al.*, 2002, 2005) and coloured Swiss chard (Kugler *et al.*, 2004), is of recent scientific interest.

In earlier studies on the betalain patterns of Chenopodiaceae representatives, electrophoresis (Savolainen and Kuusi, 1978), high-performance liquid chromatography (HPLC) (Strack and Reznik, 1979; Vincent and Scholz, 1978), and thin-layer chromatography (Piattelli *et al.*, 1965a, b) have been applied. Today, HPLC coupled with mass spectrometry (MS) is the method of choice used for thorough characterization of pigment patterns in betalain-bearing plants (Strack *et al.*, 2003). Hitherto, several betaxanthins were reported to occur in yellow and red beetroots (Hempel and Böhm, 1997; Savolainen and Kuusi, 1978; Stintzing *et al.*, 2002; Vincent and Scholz, 1978), the latter being only scarcely investigated with respect to its yellow betalains. Only recently, an improved method applying a highly polar stationary phase allowed for the separation of the complex betaxanthin pattern in coloured Swiss chard stems as well as in inflorescences from *Bougainvillea* sp. and *Gomphrena globosa* L. (Kugler *et al.*, 2004, 2007). This approach appeared to be promising to reinvestigate the betaxanthin patterns of red and yellow beetroot hypocotyls, yellow Swiss chard petioles, as well as yellow-orange cactus pear.

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Material and Methods

Plant material

Seeds of yellow beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef. cv. 'Burpee's Golden', Chenopodiaceae; Lange *et al.*, 1999) were sown in May 2004 on the Experimental Station for Horticulture of Hohenheim University, and fully developed hypocotyls were harvested in July 2004. Red beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef., Chenopodiaceae; Lange *et al.*, 1999) was purchased in October 2004 on a local market. After washing and removing hair roots, yellow beetroots were cut by cross-section and only the bottom half with regular pigment distribution in the peel was used. Whereas yellow beet material was immediately comminuted by cryogenic grinding (see below), red beetroots were cut in slices, frozen in liquid nitrogen, sealed in bags under reduced pressure and finally stored at −80 °C until further sample preparation.

Yellow petioles from coloured Swiss chard [*Beta vulgaris* L. ssp. *cicla* (L.) Alef. cv. 'Bright Lights', Chenopodiaceae; Lange *et al.*, 1999] harvested at the end of September 2004, 13 weeks after sowing, were purchased from Pommerenke (Steinheim am Albuch, Germany). After washing, the leaves were removed and the petioles were frozen in liquid nitrogen and sealed in polyacrylamide-polyethylene bags under reduced pressure for storage at −80 °C until further processing.

Yellow-orange cactus pear fruits [*Opuntia ficus-indica* (L.) Mill. cv. 'Gialla', Cactaceae] from Sicily (Italy) were purchased in October 2004.

Solvents and reagents

Reagents and solvents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Amino acids and amines were from Fluka (Buchs, Switzerland). Purified water was used throughout.

Cryogenic grinding of beetroot hypocotyls and Swiss chard petioles

As described earlier (Kugler *et al.*, 2004), comminution of beetroot hypocotyl material and Swiss chard petioles was performed in a Waring blender model 38BL41 (Waring Products, Torrington, CT, USA). To preclude enzymatic action during grinding, liquid nitrogen was added. The resulting homogenous powder was kept at −80 °C until extraction.

Extraction of beetroot and Swiss chard powder

Betalains were extracted from 100 g of beetroot or 150 g of Swiss chard powder with pre-cooled 60% aqueous methanol (v/v) to inhibit residual enzyme activity (Kugler *et al.*, 2004). 50 mM sodium ascorbate were added to prevent oxidation. For extraction of Swiss chard powder, 600 mL of solvent were used, while 300 mL and 600 mL were applied for yellow and red beetroot samples, respectively. After stirring for 20 min at 4 °C, the plant material was separated from the extract by filtering through a glass frit under reduced pressure. For complete extraction, the residue was rinsed with 100 mL of extraction solvent and finally with 100 mL of methanol. Red beetroot residues were re-extracted with 200 mL of methanol saturated with sodium ascorbate for 10 min at 4 °C and then filtered through a glass frit. Extracts were then gently concentrated *in vacuo* at 30 °C, diluted to a volume of 50 mL with purified water, flushed with nitrogen and stored at −80 °C. For HPLC-MS analyses as well as for photometric quantifications, extracts were membrane-filtered (0.45 μm; Acrodisc Premium Filter, Pall, Ann Arbor, MI, USA).

Extraction of cactus fruits

Opuntia ficus-indica fruits were cut in halves, manually squeezed, and the resulting juice was filtered through a paper filter (604 A $^1/_2$; Schleicher & Schuell, Dassel, Germany). To 300 mL of pre-cooled 60% aqueous methanol (v/v) containing 50 mM sodium ascorbate about 100 g of juice were added to reach a final volume of 400 mL. The cactus fruit extract was concentrated *in vacuo* at 30 °C, diluted to 50 mL with purified water and treated as described for Swiss chard and beetroot extracts, respectively.

Identification of betaxanthins by HPLC-DAD-MS analysis

Betaxanthin analysis was performed on an Agilent HPLC series 1100 instrument (Agilent, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/1330A autosampler, a model G1316A column oven, and a model G1315A diode array detector.

The HPLC system was connected in series with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an electrospray ionization source operating in the positive 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 to 365 °C.

An analytical scale (250 × 4.6 mm i. d.) Atlantis dC_{18}-reversed phase column with a particle size of 5 μm (Waters, Wexford, Ireland), fitted with a C_{18}-ODS (4 × 3.0 mm i. d.) security guard column was used for pigment analysis, operating at a flow rate of 1 mL/min and a temperature of 30 °C.

The betaxanthin patterns were investigated with 1% formic acid in water (v/v, eluent A) and aqueous acetonitrile (80/20, v/v, eluent B) starting isocratically with 100% A for 2 min, followed by linear gradients from 0 to 20% B in 60 min and 20 to 100% B in 5 min before re-equilibration to starting conditions (Kugler *et al.*, 2004). The injection volume for all samples was 80 μL and monitoring was performed at 470 nm. Betaxanthins (bx) were identified by comparison with the UV-vis and mass spectrometric characteristics as well as retention times of semi-synthesized reference compounds obtained as described earlier (Kugler *et al.*, 2004). Dopamine-bx (miraxanthin V) and 3-methoxytyramine-bx were assigned by comparison with retention and absorption characteristics of the corresponding betaxanthins from an extract of yellow inflorescences from *Celosia argentea* var. *plumosa* (Burvenich) Voss (Schliemann *et al.*, 2001; Kugler *et al.*, 2004). All betaxanthin standards were checked for identity by LC-MS analyses.

Photometric quantification of betalains

Betalains were quantified photometrically as described previously (Kugler *et al.*, 2004): Pigment extracts were diluted to obtain absorption values of $0.8 \leq A \leq 1.2$ at their respective maxima. To consider co-absorbing non-betalainic substances, the obtained values were corrected by the absorption at 650 nm. Applying a mean molecular extinction coefficient [ε = 48,000 L/(mol cm)], betaxanthins were calculated as glutamine-bx (vulgaxanthin I; M = 339 g/mol) equivalents for extracts from beetroots and Swiss chard petioles, and as proline-bx (indicaxanthin; M = 308 g/mol) for cactus pear juice, respectively. Concentrations of individual betalains were calculated by multiplying the photometrically assessed value with the relative chromatogram area of the particular betaxanthin at 470 nm.

Results and Discussion

Applying a highly polar stationary phase, the present study aimed at providing a deeper insight into the complex betaxanthin composition of red and yellow beetroot hypocotyls, yellow Swiss chard petioles, as well as yellow-orange cactus pear fruits. Delayed retention of early eluting polar betaxanthins was achieved, thus ensuring an improved separation from co-eluting phenolic compounds especially abundant in Chenopodiaceae representatives (Kujala *et al.*, 2001; Pyo *et al.*, 2004).

Red beetroot

Up to now, red beetroot is the most common betalain source. Whilst the betacyanin and phenolic compositions in red beetroots have been thoroughly characterized (Kujala *et al.*, 2001, 2002; Vincent and Scholz, 1978), only little efforts have been made to scrutinize the betaxanthin patterns, although the latter are considered to act as background colours, thus determining the overall appearance (Stintzing *et al.*, 2007; Vincent and Scholz, 1978). In red beetroots, betaxanthins (bx) are visually overpowered by betacyanins which is reflected by betaxanthin-betacyanin ratios of below 1 (Stintzing *et al.*, 2007). To date, glutamine-bx (vulgaxanthin I, **5**), glutamic acid-bx (vulgaxanthin II, **11**), and proline-bx (indicaxanthin, **14**) have been assigned in red beetroots (Kujala *et al.*, 2001; Piattelli *et al.*, 1965a; Vincent and Scholz, 1978). Additionally, Dopa-bx (dopaxanthin, **15**), tyrosine-bx (portulacaxanthin II, **16**), and dopamine-bx (miraxanthin V, **17**) were earlier reported to occur in differently coloured cell lines derived from the red beet cultivar *Bikores monogerm* (Girod and Zryd, 1991). In the present study, 18 additional betaxanthins were found in an extract from red beetroot hypocotyls in the following order of retention: histidine-bx (muscaaurin VII, **1**), asparagine-bx (vulgaxanthin III, **2**), serine-bx (**3**), aspartic acid-bx (miraxanthin II, **6**), glycine-bx (portulacaxanthin III, **7**), ethanolamine-bx (**8**), lysine-bx (**9**), threonine-bx (**10**), alanine-bx (**12**), γ-aminobutyric acid-bx (**13**), methionine-bx (**18**), valine-bx (**19**), tyramine-bx (miraxanthin III, **20**), 3-methoxytyramine-bx (**21**), isoleucine-bx (**22**), leucine-bx (vulgaxanthin IV, **23**), phenylalanine-bx

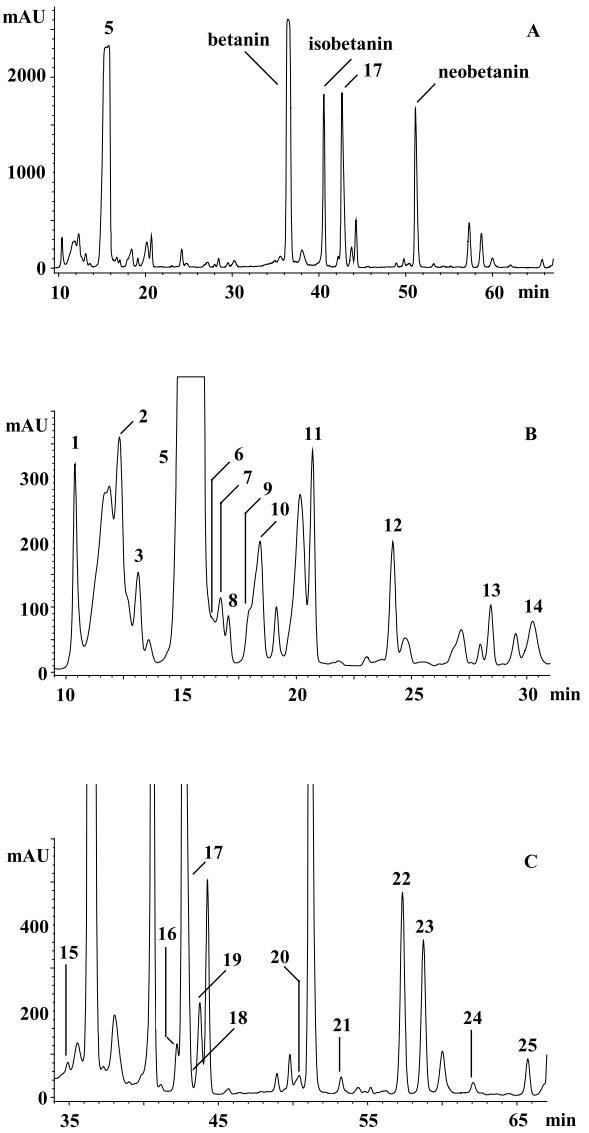

Fig. 1. HPLC fingerprint (470 nm) of betaxanthins in red beetroot hypocotyls. (A) Full scale; (B) 10–30 min of (A); (C), 35–65 min of (A); for peak assignment see Table I.

(**24**), and tryptophan-bx (**25**) (see Fig. 1 and Table I). From Fig. 1 it becomes evident that glutamine-bx (vulgaxanthin I, **5**) is clearly dominating in red beetroots, followed by dopamine-bx (miraxanthin V, **17**). All other betaxanthins were present in comparatively low quantities.

Whereas most of these betaxanthins have already been reported as genuine compounds of diverse representatives of the Cactaceae, Chenopodiaceae, Nyctaginaceae, and Amaranthaceae (Kugler *et al.*, 2004, 2007; Schliemann *et al.*, 2001; Stintzing *et al.*, 2002, 2005; Strack *et al.*, 2003) to the best of our knowledge, the adducts of ethanolamine and threonine with betalamic acid (see Fig. 2) have so far not been reported as genuine betaxanthins before. Recently, ethanolamine was found to be involved in the salt stress response of the mangrove species *Avicennia marina* (Forssk.) Vierh. (Suzuki *et al.*, 2003). This halophytic shrub also exhibits glycinebetaine which is assumed to serve as osmolyte to enhance resistance towards high salinity. Glycinebetaine is produced from choline which itself derives from ethanolamine. Another study on *Helianthus annuus* L. demonstrated that betaine biosynthesis is stimulated by addition of the precursor ethanolamine, thereby improving protection against salinity stress (Kogan *et al.*, 2000). Thus, ethanolamine, which may be formed by direct decarboxylation of serine catalyzed by serine decarboxylase or via decarboxylation of phosphatidyl-serine by a phosphatidyl-serine decarboxylase resulting in phosphatidyl-ethanolamine (Rontein *et al.*, 2003), may also be involved in stress response reactions of betalain-bearing plants. It may thus be suspected that the adduct of ethanolamine with betalamic acid may serve as a reservoir that modulates the cellular level of free ethanolamine in plant tissues by synthesis or cleavage of the respective betaxanthin thereby being indirectly involved in salt stress responses. Two possible routes may be assumed for ethanolamine-bx biosynthesis: Decarboxylation of serine-bx and/or condensation of serine-derived ethanolamine with betalamic acid. Hence, further studies should reveal the actual role of ethanolamine-bx in plant physiology and elucidate its biosynthesis in betalainic plant tissues.

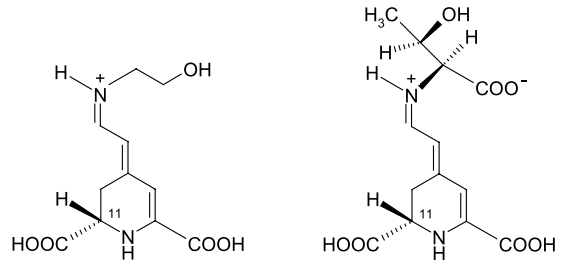

Fig. 2. Structures of ethanolamine-bx (left, **8**) and threonine-bx (right, **10**).

In addition to ethanolamine-bx and threonine-bx, lysine-bx (**9**), aspartic acid-bx (miraxanthin II,

Table I. Betaxanthins detected in red and yellow beetroots, yellow Swiss chard petioles as well as yellow-orange cactus pear[a].

No.[b]	Betaxanthins (bx) (trivial name)	t_R^c [min]	λ_{max}^c [nm]	*m/z* $[M+H]^+$	Red beetroot	Yellow beetroot	Yellow Swiss chard	Yellow-orange cactus pear
1	Histidine-bx (muscaaurin VII)	10.8	472	349	+	+	+	+
2	Asparagine-bx (vulgaxanthin III)	12.4	469	326	+	+	+	–
3	Serine-bx	13.4	468	299	+	+	+	+[d]
4	Histamine-bx	14.0	468	305	–	–	+	–
5	Glutamine-bx (vulgaxanthin I)	15.9	470	340	+	+	+	+
6	Aspartic acid-bx (miraxanthin II)	16.2	469	327	+[d]	+	+	–
7	Glycine-bx (portulacaxanthin III)	16.4	466	269	+	+	+	–
8	Ethanolamine-bx	16.7	460	255	+	+	+	–
9	Lysine-bx	17.7	458	340	+[e]	+	+[d]	–
10	Threonine-bx	18.8	469	313	+	+	+	–
11	Glutamic acid-bx (vulgaxanthin II)	21.0	469	341	+	+	+	+
12	Alanine-bx	24.8	466	283	+	+	+	–
13	γ-Aminobutyric acid-bx	28.7	460	297	+	+[e]	+[e]	+
14	Proline-bx (indicaxanthin)	30.9	479	309	+	+[e]	+[e]	+
15	Dopa-bx (dopaxanthin)	35.2	472	391	+[e]	+[e]	+[e]	–
16	Tyrosine-bx (portulacaxanthin II)	42.6	471	375	+	+	+[e]	+
17	Dopamine-bx (miraxanthin V)	42.8	459	347	+	+	+	–
18	Methionine-bx	44.1	470	343	+	+	+	+
19	Valine-bx	44.7	469	311	+	+	+	+
20	Tyramine-bx (miraxanthin III)	50.5	460	331	+[e]	+	+[e]	–
21	3-Methoxytyramine-bx	53.2	462	361	+	+	+	–
22	Isoleucine-bx	58.1	470	325	+	+	+	+
23	Leucine-bx (vulgaxanthin IV)	59.7	469	325	+	+	+	+[e]
24	Phenylalanine-bx	62.9	472	359	+	+	+	+
25	Tryptophan-bx	66.3	473	398	+	+	+	+

[a] + = present; – = not detected.
[b] Peak assignment refers to Fig. 1 and Fig. 3, respectively.
[c] Retention times and absorption maxima were obtained from reference betaxanthins.
[d] Mass signal was not unambiguous.
[e] Absorption maximum was not unambiguous.

6), and methionine-bx (**18**) were detected for the first time in the Chenopodiaceae. Only recently, lysine-bx (**9**) was reported as a genuine pigment of yellow-coloured *Bougainvillea* inflorescences (Nyctaginaceae) and red-coloured petals from *Gomphrena globosa* L. (Amaranthaceae), respectively (Kugler *et al.*, 2007). Since lysine disposes of two amino groups, the semi-synthetic reaction of L-lysine with betalamic acid resulted in two isobaric betaxanthins both yielding an *m/z* signal at 340, but differing absorption maxima at 469 nm (R_t = 12.1 min) and 458 nm (R_t = 17.7 min) (data not shown). Although the precise structure of the detected adduct of lysine with betalamic acid remains to be elucidated, only the second eluting matched compound **9** in the samples investigated. Up to now, aspartic acid-bx (miraxanthin II, **6**) has been reported in *Mirabilis jalapa* L. (Nyctaginaceae) (Piattelli *et al.*, 1965b) and in diverse flowers of the Cactaceae (Strack *et al.*, 1981), and only recently, methionine-bx was described for the first time to genuinely occur in fruits from *Opuntia ficus-indica* clones (Stintzing *et al.*, 2005).

Yellow beetroot

In contrast to red beetroots, more intensive research has been conducted to reveal the betaxanthin pattern in yellow beetroots in the past. An early investigation applying electrophoresis revealed glutamine-bx (vulgaxanthin I, **5**) and glutamic acid-bx (vulgaxanthin II, **11**) as the major betaxanthins besides several not more closely identified yellow pigments in *Beta vulgaris* var. *lutea* (Savolainen and Kuusi, 1978). Strack and Reznik (1979), who isolated betaxanthins from yellow beetroot extracts by high voltage electrophoresis prior to HPLC analysis, confirmed the presence of glutamine-bx (vulgaxanthin I, **5**) and glutamic acid-bx (vulgaxanthin II, **11**) and additionally detected proline-bx (indicaxanthin, **14**). Another study on yellow beetroot revealing the betaxanthin pattern of hairy roots from *Beta vulgaris* var. *lutea* (Hempel and Böhm, 1997) substantiated the occurrence of glutamine-bx (vulgaxanthin I, **5**), glutamic acid-bx (vulgaxanthin II, **11**), and proline-bx (indicaxanthin, **14**) and additionally revealed the presence of histidine-bx (muscaaurin VII, **1**), asparagine-bx (vulgaxanthin III, **2**), Dopa-bx (dopaxanthin, **15**), tyrosine-bx (portulacaxanthin II, **16**), and leucine-bx (vulgaxanthin IV, **23**). In a more recent investigation on the betaxanthin pattern of the yellow beetroot cultivar 'Bejo Zaden' (Stintzing *et al.*, 2002), the presence of seven further betaxanthins, namely serine-bx (**3**), γ-aminobutyric acid-bx (**13**), dopamine-bx (miraxanthin V, **17**), valine-bx (**19**), isoleucine-bx (**22**), phenylalanine-bx (**24**), and tryptophan-bx (**25**), has been found, although the earlier reported occurrence of asparagine-bx (vulgaxanthin III, **2**), histidine-bx (muscaaurin VII, **1**), glutamic acid-bx (vulgaxanthin II, **11**), Dopa-bx (dopaxanthin, **15**), and tyrosine-bx (portulacaxanthin II, **16**) could not be confirmed. In the present work, 24 betaxanthins were detected in an extract from powdered hypocotyls of the cultivar 'Burpee's Golden' (see Table I) and nine of them, namely aspartic acid-bx (miraxanthin II, **6**), glycine-bx (portulacaxanthin III, **7**), ethanolamine-bx (**8**), lysine-bx (**9**), threonine-bx (**10**), alanine-bx (**12**), methionine-bx (**18**), tyramine-bx (miraxanthin III, **20**), and 3-methoxytyramine-bx (**21**) were for the first time found to occur in yellow beetroot.

The most plausible reason for the more complex pattern in the present investigation is ascribed to the fact that whole beetroots including peel with a higher pigment proportion compared to flesh

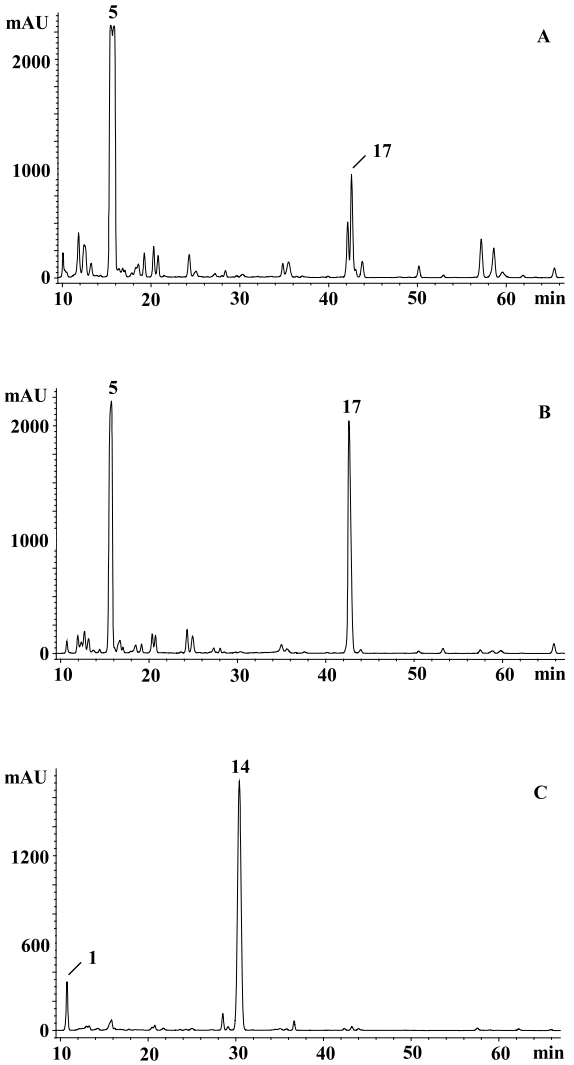

Fig. 3. HPLC fingerprints (470 nm) of betaxanthins in yellow beetroot hypocotyls (A), yellow Swiss chard petioles (B), and yellow-orange cactus pear (C) (for peak assignment see Table I).

(Stintzing *et al.*, 2007) were used amounting to 297.3 mg betaxanthins/kg fresh weight. Interestingly, red beetroots displayed a much higher betaxanthin content of 720.9 mg/kg fresh weight as compared to their yellow counterparts again demonstrating the underestimated importance of betaxanthins in red beetroots. Strikingly, an identical set of betaxanthins was found in both yellow and red beetroots (see Table I), thus underlining their close phylogenetic relationship. In both beet varieties, glutamine-bx (vulgaxanthin I, **5**) was found to

be the distinctly dominating betaxanthin amounting to 275.0 and 135.9 mg/kg fresh weight, followed by dopamine-bx (miraxanthin V, **17**) with 77.6 and 22.1 mg/kg fresh weight in red and yellow beetroot, respectively (see Fig. 1A, Fig. 3A).

Yellow Swiss chard petioles

Yellow Swiss chard petioles from the cultivar 'Bright Lights' were only recently subjected to a detailed investigation of their betaxanthin pattern and a broad pattern consisting of 19 structures was reported (Kugler *et al.*, 2004). In addition to these earlier findings, aspartic acid-bx (miraxanthin II, **6**), ethanolamine-bx (**8**), lysine-bx (**9**), threonine-bx (**10**), Dopa-bx (dopaxanthin, **15**), and methionine-bx (**18**) could be detected in yellow Swiss chard petioles (see Table I). Again, this might be attributed to the higher petiole betaxanthin contents investigated in the present study amounting to 107.4 mg/kg fresh weight compared to 49.7 mg/kg fresh weight (Kugler *et al.*, 2004). Furthermore, edaphic factors at the cultivation site and harvest time should generally be considered with respect to qualitatively and quantitatively differing betaxanthin patterns (Stintzing *et al.*, 2007). As could be observed earlier (Kugler *et al.*, 2004), glutamine-bx (vulgaxanthin I, **5**) and dopamine-bx (miraxanthin V, **17**) clearly dominated over all other betaxanthins amounting to 41.6 and 33.5 mg/kg fresh weight, respectively (see Fig. 3B).

Yellow-orange cactus pear fruits

Juice extract from the pulp of the cactus pear cultivar 'Gialla' revealed betaxanthin and betacyanin contents of 50.7 and 4.9 mg/kg juice, respectively. Furthermore, the presence of tyrosine-bx (portulacaxanthin II, **16**) and tryptophan-bx (**25**) both of which have not been detected in the Cactaceae until now were assigned in this study. Hitherto, tyrosine-bx (portulacaxanthin II, **16**) has been detected in the Portulacaceae (Trezzini and Zryd, 1991), the Chenopodiaceae (Kugler *et al.*, 2004; Stintzing *et al.*, 2002), the Aizoaceae (Gandía-Herrero *et al.*, 2005), the Amaranthaceae (Kugler *et al.*, 2007), and the Nyctaginaceae (Kugler *et al.*, 2007). Tryptophan-bx (**25**) was first detected in the Amaranthaceae (Schliemann *et al.*, 2001) and also found to be present in the Chenopodiaceae (Kugler *et al.*, 2004; Stintzing *et al.*, 2002) as well as the Nyctaginaceae (Kugler *et al.*, 2007). Whereas the latter two betaxanthins were present in comparatively low amounts, proline-bx (indicaxanthin, **14**) was found to be the predominant betaxanthin in yellow-orange cactus pear fruits amounting to 37.8 mg/kg juice (see Fig. 3C), thus confirming earlier findings (Stintzing *et al.*, 2002, 2005), followed by histidine-bx (muscaaurin VII, **1**) with 3.5 mg/kg juice.

In summary, the present study extends the knowledge about the betaxanthin profiles in betalainic vegetables and fruits. In each sample investigated, two betaxanthins were dominating and the Chenopodiaceae could be easily differentiated from the Cactaceae representatives due to the more complex betaxanthin pattern of the former. In addition, a set of betaxanthins exhibited both a particular amino acid and its corresponding amine. Hence, decarboxylase activity appears to be a common event in betaxanthin-bearing plants.

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