HPLC Characterization of Betalains from Plants in the Amaranthaceae

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Abstract

HPLC characterization of reversed-phase (RP) high-performance liquid chromatography (HPLC) has been widely used in separation and identification of plant pigments. An effective RP-HPLC-based method is established to systematically isolate, identify, and quantitate the betalain pigments in the plants of 37 species of eight genera in the Amaranthaceae. A total of 16 betacyanins and three betaxanthins are characterized mainly using the RP-HPLC method and also with the aid of mass spectroscopy. The identified betacyanins include eight amaranthine-types, six gomphrenin-types, and two betanin-types. They are also divided into six simple (nonacylated) betacyanins and 10 acylated betacyanins. Acylated betacyanins are identified as betanidin 5-*O*-β-glucuronosylglucoside or betanidin 6-*O*-β-glucoside acylated with ferulic, *p*-coumaric, or 3-hydroxy-3-methylglutaric acids. Three betaxanthins were separated from *Celosia* species in the Amaranthaceae and identified to be immonium conjugates of betalamic acid with dopamine, 3-methoxytyramine, and (*S*)-tryptophan; the latter two are found to be new betaxanthins from plants.

Introduction

There has been an increasing trend towards replacement of synthetic pigments with natural plant pigments in the last 20 years because of the natural pigments' safety and health benefits. Reversed-phase (RP) high-performance liquid chromatography (HPLC) has been extensively employed in the separation and identification of plant pigments, such as betalains and anthocyanins (1,2). Vincent and Scholz first employed HPLC to analyze betalains from red beets (*Beta vulgaris*) (3). Betalains are water-soluble pigments, which can be divided into two major structural groups: red-violet betacyanins and yellow betaxanthins. So far it has been found that betalains in nature consist of approximately 50 red betacyanins and 20 yellow betaxanthins (4). Interestingly, red beets usually contain both red betacyanins (consisting of 75–95% betanin) and yellow betaxanthins (5–25%, mainly vulgaxanthin-I), in various ratios for different cultivars (4,5). Betalains from red beets, one of the most important natural food colorants, have been extensively used in the modern food industry for more than 20 years.

Betalains are distributed in only 10 families of the order Caryophyllales (6,7), including the family Amaranthaceae, which contains several important genera such as *Amaranthus*, *Celosia*, *Gomphrena*, and *Iresine* (8). Piattelli and Minale investigated various kinds of betacyanins from 16 species of six genera in the Amaranthaceae using chromatography on polyamide columns, paper chromatography, and paper electrophoresis (6). Huang and Hwang used HPLC to isolate and identify betacyanins (amaranthine/isoamaranthine) from vegetable amaranth (*Amaranthus tricolor*) (9). Heuer et al. identified betacyanins (gomphrenins/isogomphrenins) from flowers of *Gomphrena globosa* by RP-HPLC, mass spectrometry (MS), and NMR (10). However, earlier researchers could not systematically investigate the betalains in the Amaranthaceae by modern HPLC techniques.

Research on the betalain pigments from plants in the family Amaranthaceae have been carried out in our laboratory for over 6 years (11–15). Some plants in the Amaranthaceae (particularly *Amaranthus*) have attracted considerable attention as potential alternative sources of betalains. Some *Amaranthus* genotypes produce very high biomass and contain a high yield of betalain pigments. A main objective of this study was to separate, identify, and quantitate the many and varied types of betalain in the Amaranthaceae. It was the first systematic investigation of the betalains from 37 plant species of eight genera in the Amaranthaceae on a large scale by RP-HPLC. Sixteen betacyanins and three betaxanthins were characterized from the plants of this family, mainly using RP-HPLC and also with the aid of MS. These identified betacyanins in the Amaranthaceae included amaranthine-type, gomphrenin-type, and betanin-type (Table I), which were also divided into simple (free acylated) betacyanins and acylated betacyanins. Two of three betaxanthins isolated in the Amaranthaceae were found to be new natural compounds [i.e., 3-methoxytyramine-betaxanthin and (*S*)-tryptophan-betaxanthin].

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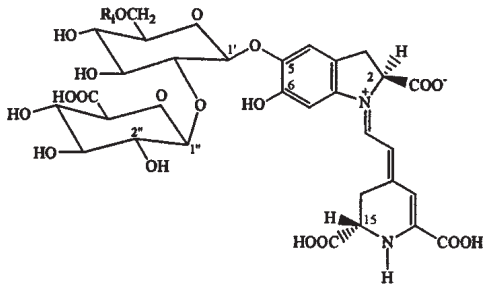
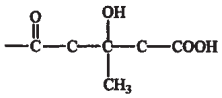
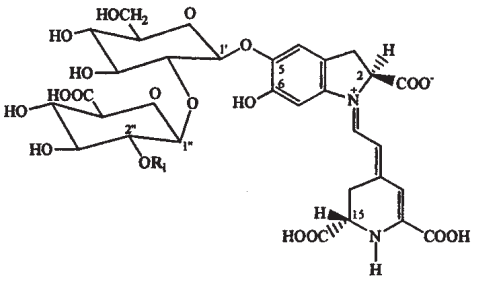
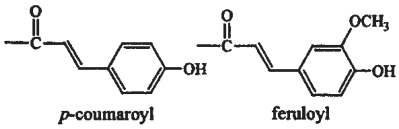
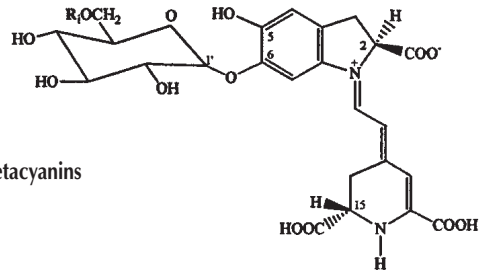
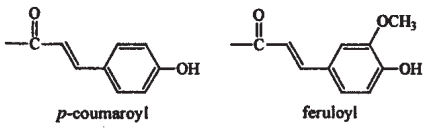
Table I. Chemical Structures and Identification of Betalains (Betacyanins and Betaxanthins) from Plants in the Amaranthaceae*

Number	Betalain name	Retention time (min)	HPLC–DAD UV–vis λ_{max} (nm)†	MS $[M+H]^+$	Representative plant source
	Amaranthine-type betacyanins	  **3-hydroxy-3-methylglutaryl (HMG)** **1: amaranthine (R_i = H)** **1': isoamaranthine (C_{15} epimer)** **3: iresinin I (R_i = HMG)** **3': isoiresinin I (C_{15} epimer)**			
1	Amaranthine	10.71	536	727	*Amaranthus cruentus*
1'	Isoamaranthine	12.01	536	727	*Amaranthus cruentus*
3	Iresinin I	17.49	298, 540	871	*Iresine herbstii*
3'	Isoiresinin I	18.37	298, 540	871	*Iresine herbstii*
	Amaranthine-type betacyanins	  **p-coumaroyl** **feruloyl** **4: celosianin I (R_i = *p*-coumaroyl)** **4': isocelosianin I (C_{15} epimer)** **5: celosianin II (R_i = feruloyl)** **5': isocelosianin II (C_{15} epimer)**			
4	Celosianin I	19.90	306, 546	872	*Celosia cristata*
4'	Isocelosianin I	20.56	306, 546	–	*Celosia cristata*
5	Celosianin II	21.32	312, 546	903	*Celosia cristata*
5'	Isocelosianin II	22.11	312, 546	–	*Celosia cristata*
	Gomphrenin-type betacyanins	  **p-coumaroyl** **feruloyl** **6: gomphrenin I (R_i = H) 6': Isogomphrenin I (C_{15} epimer)** **7: gomphrenin II (R_i = *p*-coumaroyl) 7': Isogomphrenin II (C_{15} epimer)** **8: gomphrenin III (R_i = feruloyl) 8': Isogomphrenin III (C_{15} epimer)**			
6	Gomphrenin I	32.83	540	551	*Gomphrena globosa*
6'	Isogomphrenin I	35.19	540	551	*Gomphrena globosa*
7	Gomphrenin II	58.34	310, 552	697	*Gomphrena globosa*
7'	Isogomphrenin II	61.58	310, 552	–	*Gomphrena globosa*
8	Gomphrenin III	59.10	322, 552	727	*Gomphrena globosa*
8'	Isogomphrenin III	63.59	324, 552	–	*Gomphrena globosa*

(Table continues on next page)

* Numbers correspond with the numbers in the text and the peak numbers in the figures. HPLC–DAD and MS data were obtained over the past several years.
† UV maximum wavelength (acyl moiety) was listed only for acylated betacyanins.

Experimental

Extraction and purification of betalain samples

Genotypes of 37 species from eight genera (mainly *Amaranthus*, *Celosia*, *Gomphrena*, and *Iresine*) in the Amaranthaceae from world collections, mainly provided by the United States Department of Agriculture–Agricultural Research Service (Iowa State University, Iowa City, Iowa), Chinese Academy of Agricultural Sciences (Beijing, China), and Hubei Academy of Agricultural Sciences (Wuhan, China), were used as materials for this study. All samples of fresh materials were harvested, frozen, and stored at –18°C until use.

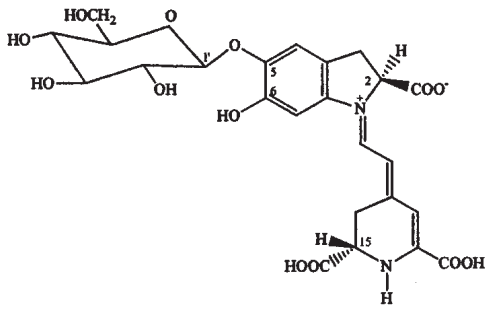
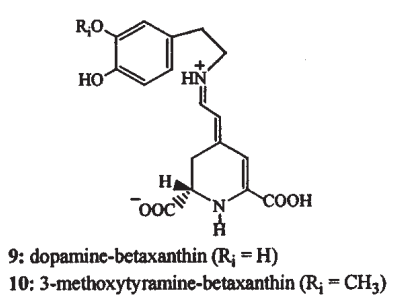
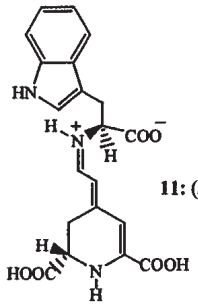
Water extraction was a simple, highly efficient, and low-cost method for *Amaranthus* betalain extraction, but led to difficulties in the separation of betalain and water-soluble protein components (16). Betalains were also easily extracted with 80% methanol and purified as described in previous reports (13–15). Methanol extraction significantly improved the separation of betalain and protein and reduced the interference of protein on betalain analysis. The methanolic extracts of betalains were filtered by a Millipore filter (Millipore, Bedford, MA) with a 0.2µM nylon membrane and centrifuged. The supernatant could be directly used for quantitative analysis by analytical HPLC and also used for further purification of betalains. The extracts were concentrated under vacuum at 22°C. The concentrate was transferred to a Sephadex LH-20-100 (Sigma, St. Louis, MO) column (100- × 2.5-cm i.d.) and separated by elution with ultrafiltered water adjusted to pH 5 to 6 with formic acid. The final purification of the betalains was carried out by preparative HPLC. The purified samples were freeze-dried and used for further analysis.

HPLC system and chromatographic conditions

Apparatus

The HPLC apparatus consisted of an HP 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany), equipped with a

Table I. Chemical Structures and Identification of Betalains (Betacyanins and Betaxanthins) from Plants in the Amaranthaceae* *(Continued)*

Number	Betalain name	Retention time (min)	HPLC–DAD UV–vis λ_{max} (nm) †	MS $[M+H]^+$	Representative plant source
	Betanin-type betacyanins	 **2: betanin** **2': isobetanin (C_{15} epimer)**			
2	Betanin	14.77	538	551	*Amaranthus powellii*
2'	Isobetanin	12.01	538	–	*Amaranthus powellii*
	Betaxanthins	 **9: dopamine-betaxanthin (R_i = H)** **10: 3-methoxytyramine-betaxanthin (R_i = CH_3)**  **11: (*S*)-tryptophan-betaxanthin**			
9	Dopamine-betaxanthin	17.1	216, 260, 474	347	*Celosia cristata/plumosa*
10	3-Methoxytyramine-betaxanthin	20.8	216, 312, 474	361	*Celosia cristata/plumosa*
11	(S)-Tryptophan-betaxanthin	22.0	218, 268, 478	398	*Celosia cristata/plumosa*

* Numbers correspond with the numbers in the text and the peak numbers in the figures. HPLC–DAD and MS data were obtained over the past several years..
† UV maximum wavelength (acyl moiety) was listed only for acylated betacyanins.

binary pump and a diode-array detector (DAD). Data processing was performed using Hewlett-Packard HPLC 2D ChemStation software (Palo Alto, CA). A Nucleosil 100-C_{18} column (250- × 4-mm i.d., 5 µm) with Nucleosil 5 C_{18} guard column (4- × 4-mm, 5 µm) and a Zorbax SB-C18 column (250- × 9.4-mm i.d., 5 µm) (Agilent Technologies, Palo Alto, CA) were used for analytical and preparative HPLC, respectively.

Elution conditions for analytical HPLC

Linear gradient elution of red-violet betacyanins was within 100 min from 0% to 100% solvent B (1.5% H_3PO_4, 20% acetic acid, 25% acetonitrile in H_2O) in solvent A (1.5% H_3PO_4 in H_2O) for *Gomphrena globosa* (Figure 1D), and within 30 min from 10% to 55% solvent B in solvent A for others (Figure 1A–C) at a flow rate of 1 mL/min with a 20-µL injection volume and detection at 540 nm. Linear gradient elution of yellow betaxanthins from *Celosia plumosa* (Figure 1E–F) was different, that is, within 30 min from 100% solvent A (55mM NaH_2PO_4, 2.5mM triethylamine, pH adjusted to 4.5 with H_3PO_4) and 0% solvent B (40% acetonitrile) to 70% A–30% B in 15 min; to 40% A–60% B in 5 min; to 20% A–80% B in 5 min followed by isocratic elution of 20% A–80% B for further 5 min at a flow rate of 1 mL/min with a 20-µL injection and detection at 480 and 538 nm. All UV–vis spectral data of the identified betacyanins and betaxanthins were recorded by HPLC–DAD.

Elution conditions for preparative HPLC

Solvent A was 1% aqueous formic acid, and solvent B was 80% aqueous MeOH. Different linear gradients were used: (*i*) amaranthines: 0–17 min, 10–45% B; 17–21 min, 45–100% B; (*ii*) iresinins: 0–25 min, 15–60% B; 25–35 min, 60–100% B; (*iii*) celosianins: 0–28 min, 20–70% B; 28–35 min, 70–100% B; (*iv*) betanins: 0–25 min, 10–55% B; (*v*) gomphrenins: 0–40 min, 20–100% B; and (*vi*) betaxanthins: 0–25 min, 20–70% B; 25–35 min, 70–100% B. The injection volume was 100 µL and a flow rate of 3.5 mL/min was used. The separations were monitored at 540 nm for betacyanins and at 474 nm for betaxanthins. The betalain samples obtained by preparative HPLC were used not only for qualitative analysis but also for antioxidant activity assay of the betalains (15).

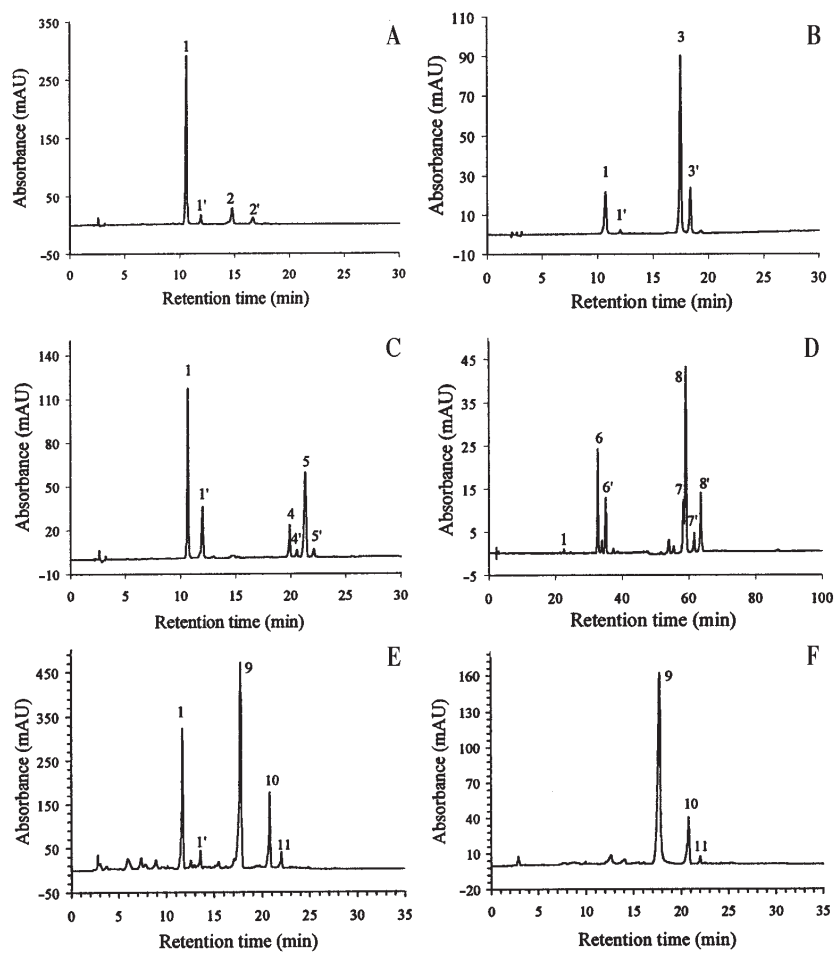


Figure 1. HPLC chromatograms of methanolic extracts from different species in the Amaranthaceae: seedlings of *Amaranthus powellii* (A); leaves of *Iresine herbstii* (B); inflorescences of *Celosia cristata* (violet) (C); inflorescences of *Gomphrena globosa* (D); orange-red inflorescences of *Celosia plumose* (E); and yellow inflorescences of *Celosia plumose* (F). Elution monitored at 540 nm (betacyanins, A–D) and 480 nm (betaxanthins, E and F). The numbers on the peaks refer to the betalains listed in Table I.

MS and LC–MS

Use of MS can further confirm the results of HPLC characterization. Betacyanin samples were analyzed by electrospray ionization (ESI) mass spectroscopy (ESI-MS and ESI-MS–MS) on a quadrupole ion-trap MS (Finnigan MAT LCQ) (San Jose, CA) at flow rate of 5 µL/min under ion spray voltage 4.5 kV, capillary temperature 200°C, capillary voltage 3–8 V, tube lens offset 20–40 V, and collision energy 25–40 eV.

Betaxanthin samples were analyzed by LC–MS as described by Schliemann et al. (17). Positive ion ESI mass spectra were recorded on a Finnigan MAT TSQ 7000 instrument (electrospray voltage 4.5 kV, capillary 220°C, sheath gas N_2) coupled to a Micro-Tech Ultra-Plus Micro LC system equipped with an ULTRASEP C_{18} column (100- × 1-mm i.d., 4 µm). The LC conditions inlcuded a gradient system starting from 10% B (0.2% aqueous acetic acid in acetonitrile) in 90% A (0.2% aqueous acetic acid) to 50% B in (A + B) within 10 min at a flow rate of 70 µL/min with a 2-µL injection volume. Positive LC–ESI–time-of-flight (TOF)-MS were recorded on an API QSTAR Pulsar instrument (PerkinElmer Sciex, Foster City, CA) (electrospray voltage 5.5 kV, sheath gas N_2) coupled to an Ultimate micro-HPLC system (LC Packings, Amsterdam, the Netherlands), equipped with a Fusica C_{18} column (150- × 0.3-mm i.d., 3 µm). The LC conditions included a gradient system starting from 100% A [0.05% aqueous

formic acid–acetonitrile (95:5)] to 100% B [formic acid–acetonitrile (0.05:99.95)] within 20 min at a flow rate of 4 mL/min with a 0.5-mL injection volume.

Identification of simple and acylated betacyanins

The betacyanins were systematically identified from 37 species of eight genera in the Amaranthaceae using RP-HPLC. Several selected typical HPLC profiles of methanolic extracts from different important plants in the Amaranthaceae display 16 distinct peaks altogether at 540 nm (Figure 1). Chemical structures and identification of 16 betacyanins and their stereoisomers (C-15 epimers) isolated in this family are summarized in Table I. The major peaks (**1** and **1'**) in Figure 1A–D were readily identified by co-chromatography with the reference extracts of amaranthine (betanidin 5-*O*-β-glucurconosylglucoside) and isoamaranthine (C-15 epimer) from *A. tricolor* leaves (9,13,16). The peaks (**2** and **2'**) were also easily identified by co-chromatography with the authentic samples of betanin (betanidin 5-*O*-β-glucoside) and isobetanin (C-15 epimer) from red beet pigments (No. 3600, Warner-Jenkinson, St. Louis, MO). Therefore, the results suggested that the peaks **1**, **1'**, **2**, and **2'** were simple betacyanins without any acyl groups.

According to the chromatographic profiles, UV–vis spectra, and ion spray mass spectra, the peaks **3** to **8'** (except the peaks **6** and **6'**) were identified as acylated betacyanins by comparison with literature data (1,7). The peaks (**3**, **4**, and **5**) separated from *Iresine herbstii* and *Celosia cristata* (violet) (Figure 1B and C) were assumed to be iresinin I, celosianin I, and celosianin II, respectively. They were the amaranthine-type (substituted at C-5 of betanidin) betacyanins with acyl groups (3-hydroxy-3-methylglutaryl, *p*-coumaroyl, and feruloyl) (Table I). The presence of their isobetacyanins (peaks **3'**, **4'**, and **5'**) was easily confirmed by treatment of the extracts with an aqueous citric acid solution as described by Piattelli and Minale (6). The six peaks (**6**, **6'**, **7**, **7'**, **8**, and **8'**) isolated from *Gomphrena globosa* (Figure 1D) completely matched those detected and identified at similar HPLC conditions by Heuer et al. (10).

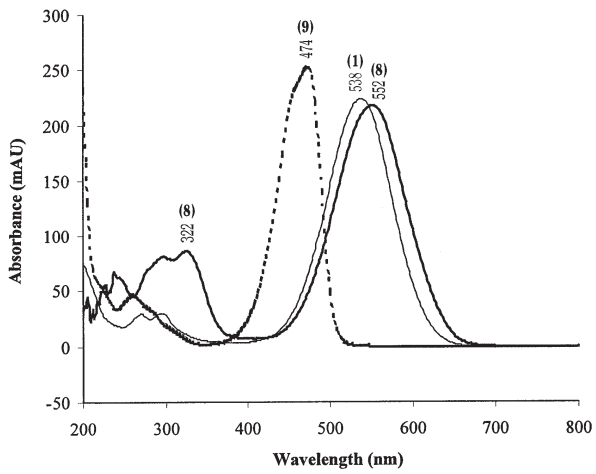


Figure 2. UV–vis spectra of nonacylated betacyanin (**1**, amaranthine), acylated betacyanin (**8**, gomphrenin III), and yellow betaxanthins (**9**, dopamine-betaxanthin) by HPLC–DAD.

They were gomphrenin-type betacyanins substituted at C-6 of betanidin (Table I). The results indicated that the peak **6** was gomphrenin I (betanidin 6-*O*-β-glucoside, nonacylated betacyanins) and the peaks **7** and **8** were their acylated forms with *p*-coumaroyl and feruloyl, (i.e., gomphrenin II and gomphrenin III). Their isomeric forms were isogomphrenin I (**6'**), isogomphrenin II (**7'**), and isogomphrenin III (**8'**). These acylated betacyanins could also be indirectly identified by alkaline hydrolysis (1,13,18).

The chromatographic behavior of the betacyanins provided effective identification (13). From Table I and Figure 1, HPLC elution order (retention time) revealed that:

(*i*) The retention of gomphrenin-type betacyanins (**6**, **6'**, **7**, **7'**, **8**, and **8'**) (6-*O*-glucosides) obviously exceeded that of amaranthine- (**1**, **1'**, **3**, **3'**, **4**, **4'**, **5**, and **5'**) or betanin-type (**2** and **2'**) betacyanins (5-*O*-glucuronosylglucosides or 5-*O*-glucosides) because of their structural difference at C-5 and C-6. In order to improve the separation of gomphrenin-type betacyanins with relatively weak polarity, the elution time of *Gomphrena globosa* extracts was extended from 30 to 100 min in this study (Figure 1D). (*ii*) Acylated betacyanins (**3**-**5'**, **7**, and **8'**) had a longer retention time than nonacylated betacyanins (**1**–**4'**, **6**, and **6'**) because acyl groups increased the retention of pigments. (*iii*) The retention of betacyanins decreased with the increase of glycosyl substitution, thus amaranthine-type betacyanins with two sugar units attached had a shorter retention time than the betacyanins with only one sugar unit attached (betanin- or gomphrenin-type). (*iv*) Because the configuration of C-15 isomeric forms for betacyanins allowed greater interaction with the stationary phase, isobetacyanins were retained slightly longer than their parents. The results agreed well with those predicted by RP chromatography principles (7).

UV–vis spectra data by HPLC–DAD further supported the identification. The maximal absorption (λ_{max}) values in the visible region were 540–552 nm for acylated betacyanins (**3, 3', 4, 4', 5, 5', 7, 7', 8**, and **8'**) but 536–540 nm for nonacylated betacyanins (**1**, **1'**, **2**, **2'**, **6**, and **6'**) (Table I). The acylated betacyanins exhibited a marked bathochromic shift, like anthocyanins acylated with aromatic acids (7,13). Additionally, the acylated betacyanins displayed an additional absorption in the UV region of approximately 300–330 nm, where the absorption of nonacylated betacyanins was weak. For example, gomphrenin III (**8**) (acylated betacyanin) displayed λ_{max} 552 nm in the visible region and a strong additional absorption (322 nm) in the UV region, though amaranthine (**1**) (nonacylated betacyanin) exhibited λ_{max} 538 nm in the visible region and a very weak additional absorption in the UV region (Figure 2). The findings were similar to earlier studies (6,7). In addition, a more reliable and direct confirmation of the betacyanin molecular composition came from the electrospray mass spectra that gave the expected protonated molecular ions and various fragment ions. Table I shows that the molecular weights of the betacyanins measured by ESI-MS in this study agreed quite well with the calculated masses for monoisotopic protonated molecular ions.

Identification of betaxanthins

RP-HPLC analysis of methanolic extracts from various geno-

types of *Celosia plumosa* (*Celosia argentea* var. *plumosa*) and *Celosia cristata* (*Celosia argentea* var. *cristata*) showed that three betaxanthins (**9**, **10**, and **11**) were identified from differently pigmented inflorescences (yellow, red, or orange-red) of *C. plumosa* and *C. cristata* (Table I and Figures 1E and 1F) in the family Amaranthaceae. Also, already known betacyanins (**1**, amaranthine and **1'**, isoamaranthine) were found in the red or orange-red inflorescences of *C. plumosa* (Figure 1E) and *C. cristata*. A large amount of dopamine and a minor constituent (betalamic acid) were also detected at 280 and 405 nm in the tested *Celosia* species, respectively (not shown in Figure 1) (14,17). The UV–vis spectral analysis of the betaxanthins by HPLC–DAD (Figure 2) showed maximal absorption (λ_{max}) at 216, 260, and 474 nm for the peak **9**; at 216, 312, and 474 nm for the peak **10**; and at 218, 268, and 478 nm for the peak **11** (**10** and **11** was not shown in Figure 2), respectively. The UV–vis spectra of the peaks **9**, **10**, and **11** are typical for betaxanthins (7), but clearly different from amaranthine (**1**, λ_{max} 538 nm).

The peaks **1** and **1'** in Figure 1E (orange-red *C. plumosa*) were easily identified as amaranthine and isoamaranthine by co-chromatography (HPLC) with authentic standards from *A. tricolor* and *A. cruentus* (13,16). Figures 1E and 1F show that the peaks **9** and **10** were the major components in yellow- and orange-/orange-red *Celosia* species. From the retention time and the characteristic HPLC–DAD data, the peak **9** (retention time 17.1 min; λ_{max} 216, 260, and 474 nm) was suggested to be dopamine–betaxanthin (miraxanthin V), previously found as the major betaxanthin in hairy root cultures of yellow beets (19). Co-chromatography (HPLC) with synthetically prepared authentic standard and LC–MS giving the protonated molecular ion $[M+H]^+$ at *m/z* 347 confirmed the suggestion. The CID mass spectrum of *m/z* 347 was shown to be identical in its fragmentation pattern with previously published data (19).

The UV–vis spectral data of the peak **10** (216, 312, 474 nm) were similar to the peak **9**, but it eluted 3.7 min later than the peak **9** (Figure 1E and F). The betaxanthins derived from 4-methoxy-3-hydroxy-phenylethylamine and 3-methoxy-4-hydroxy-phenylethylamine (3-methoxy-tyramine) were synthetically prepared and used as reference compounds in co-injection experiments. Both compounds were identical with the peak **10** with respect to UV–vis data. However, 4-methoxy-3-hydroxy-phenylethylamine-betaxanthin eluted late and was clearly different from the peak **10**, whereas 3-methoxy-4-hydroxy-phenylethylamine-betaxanthin (3-methoxy-tyramine-betaxanthin) showed complete coelution with the endogenous betaxanthin (the peak **10**). LC–ESI–TOF-MS analysis further confirmed the structural elucidation of the peak **10** as 3-methoxy-tyramine-betaxanthin (*m/z* 361), a new betaxanthin from plants (17).

The peak **11** was a minor constituent in yellow and orange-red *Celosia* species (Figure 1E and F). The peak **11** was suggested to be weak polar or unpolar betaxanthins, according to its longer retention time (22.0 min) and UV–vis spectral characteristics (218, 268, and 478 nm) (Table I). One of the most unpolar betaxanthins synthesized, (*S*)-tryptophan-betaxanthin, was a reference compound (19). The corresponding HPLC and MS data of the peak **11** perfectly matched with the synthetical standard of (*S*)-tryptophan-betaxanthin (*m/z* 398). It was also found that (*S*)-tryptophan-betaxanthin was a new betaxanthin from plants (17).

Quantitative analysis of betalains

Quantitative analysis of betalains was undertaken with HPLC and spectrophotometric methods. The results showed that the dominant betacyanin composition in the *Amaranthus* plant materials was amaranthine (average 81% of total peak area). Among 21 *Amaranthus* genotypes, total betacyanins ranged from 46 to 199 mg/100 g of fresh plant material and from 15.4 to 46.9 mg/g of dry extracts (16). Notably, *Amaranthus* cultivated species contained much more betacyanins than wild species. It was also found that acylated betacyanins were distributed among 11 species of six genera, with the highest proportion occurring in *Iresine herbstii* (iresinins, 79.6% of total peak area), *Gomphrena globosa* (gomphrenin II and III and their C-15 epimers, 68.4%), and *Celosia cristata* (celosianins, 39.8%) (13). The tested yellow and orange-red genotypes of *Celosia* species contained a large amount of betalains (mainly betaxanthins), ranging from 92.1 to 621.5 nmol/g of fresh weight (17). Pure yellow genotype of *Celosia plumosa* possessed a high level of yellow betaxanthins (95.7% of total peak area).

Conclusion

Over the past several years, an effective RP-HPLC-based method was developed to successfully separate, identify, and quantitate the betalains in the plants of 37 species of eight genera in the Amaranthaceae on a large scale. The HPLC method was employed with the aid of MS for the successful characterization of 16 red-violet betacyanins and three unidentified yellow betaxanthins from the plants of this family. The chromatographic system developed here may be used to isolate and identify the betalains from other plants in the order Caryophyllales.

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