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Characterization and Quantification of Betacyanin Pigments from Diverse *Amaranthus* Species

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The betacyanin pigments from 21 genotypes of 7 *Amaranthus* species were separated by gel filtration chromatography and HPLC. On the basis of their IR and UV−visible spectra, enzymatic hydrolysis, and chromatographic profiles, the pigments were identified as homogeneous betacyanins, which consisted on average of 80.9% amaranthine and 19.2% isoamaranthine. Dried crude betacyanin extracts contained 23.2−31.7% protein, and the purified sample retained 12.8% protein. The betacyanins were difficult to separate from protein. Total betacyanins in the *Amaranthus* species ranged from 46.1 to 199 mg/100 g of fresh plant material and from 15.4 to 46.9 mg/g of dry extracts. The mean extraction rate of the eight best genotypes was 2.18%. *Amaranthus* cultivated species contained much more betacyanin than wild species and had much higher biomass, indicating that certain cultivated genotypes had greater potential for commercial development as natural colorant sources. Dried extracts from *Amaranthus* species may form natural nutritive pigments for the food industry.

Keywords: *Amaranthus; betacyanins; pigments; amaranthine; isoamaranthine; colorants*

INTRODUCTION

The *Amaranthus* genus contains species and genotypes that can be used as high-quality multipurpose high-potential crops for modern agriculture, for food, feed, and forage uses, and also as an ornamental plant or pigment source plant because of its rich colorful leaves, inflorescences, stems, and seeds (Paredes-López, 1994).

Amaranthus pigments are red-violet and water-soluble betacyanins. Betacyanins occur in plants from about 10 families of the order Centrospermae such as the genus *Beta* (red beet) and *Amaranthus*. The presence of betacyanins in plants is mutually exclusive of the occurrence of anthocyanins (Stafford, 1994). The most studied betacyanins are those of red beet (*Beta vulgaris*), which have been extensively commercialized as a food colorant (von Elbe and Schwartz, 1996). Much less is known about *Amaranthus* betacyanins.

Early studies on *Amaranthus* betacyanins in the 1960s and 1970s considered their botanical classification, application, and biosynthetic pathways [e.g., Piattelli and Minale (1964a), Piattelli et al. (1969), and Koehler (1972a,b)]. The betacyanins in *Amaranthus tricolor* were identified as amaranthin (the 5-*O*-[2-*O*-(*β*-D-glycopyranosyluronic acid)-*β*-D-glucopyranoside] of betanidine) and isoamaranthin (C-15 epimer) (Piattelli et al., 1964, 1969). Mabry and Dreiding (1968) designated the pigments "amaranthine" rather than "amaranthin" so as to avoid confusing it with amaranthin (lectin). Also, the word "amarantin" has been used for a globulin protein from *Amaranthus* (Chen and Paredes-

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Table 1. Pigment Distribution and Harvest Time for 21 Genotypes of *Amaranthus*

species	genotypes	red-colored parts[a]	suitable harvest period for betacyanins[b] (days) seedlings	leaves	inflorescence	fresh wt[c] (kg/ha)
A. cruentus L.	12	Sl*, Lv*, In*, St, Sd, or Tp	32 ± 6	42 ± 8	81 ± 10	56390 ± 7225 (Lv, In)
A. caudatus L.	2	Sl, Lv, In*, Sd			74 ± 5	42520 (In)
A. tricolor L.	3	Sl*, Lv*, Sd, or Tp	27 ± 3	33 ± 4		20520 (Lv)
A. hypochondriacus L.	1	Sl*, In*, Sd	30 ± 3		76 ± 4	29160 (In)
A. hybridus L.	1	Sl, In*, Sd			80 ± 4	39285 (In)
A. lividus L.	1	Sl*, Lv*, Sd	28 ± 3	32 ± 4		17370 (Sl)
A. paniculatus L.	1	Sl*, Lv, In*, St, Sd	30 ± 3		71 ± 5	44550 (In)

[a] Sl, seedlings; Lv, leaves; In, inflorescences; St, stem skin; Sd, seeds; Tp, total plant; *, particularly high pigments in the parts. [b] Days after sowing. [c] Estimated as Sl, Lv, or In weight per plant × planting density.

López, 1997). Amaranthine has the same basic structure—betanidin (aglycon) as the betacyanins from red beet. Recent biochemical investigations include the characterization of the betanidin glucosyltransferases that catalyze the formation of betanidin (Vogt et al., 1997) and studies on the mode of induction of betacyanins in cell cultures (Rudat and Göring, 1995).

Huang and von Elbe (1986) extracted amaranthine from leaves of *A. tricolor* (a vegetable amaranth) and described its stability. Shen and Huang (1985), Chen (1992), Huo and Guo (1994), and Yue and Sun (1993) also studied the red pigment in *A. tricolor*. Natural pigments from *A. tricolor* can be legally used as food ingredients in China (Hygienic Standards for Food Additives in China, GB2760-89) (Fan, 1993). Historically, the pigments from *Amaranthus* plants have been used to color foods, beverages, and bread products in numerous New World locations: the southwestern United States, Mexico, Bolivia, Ecuador, and Argentina (Lehmann, 1990).

More than 60 species of the genus *Amaranthus* are known to grow throughout the world, including cultivated and wild species (Schnetzler and Breene, 1994). The pigment composition, content, properties, and stability in a wide diversity of species of the genus *Amaranthus*, except *A. tricolor*, have not yet been reported. During our research on *Amaranthus* genetic resources, we observed that the red color was variously distributed in inflorescences (spikes), leaves, stems, and branches, or even in the whole plant in some genotypes of some grain amaranth species (e.g., *A. cruentus*). Grain amaranths have high biomass production potential and regenerative ability; that is, several biomass harvests per sowing are feasible (Yue and Sun, 1993). Hence, grain amaranths represent an underexploited potential source of betacyanin type pigments. Knowledge of genotypic differences in pigment composition, content, and stability is essential in the selection of *Amaranthus* varieties for the commercial production of food colorants.

The objective of this study was to elucidate the identification, composition, and content of the betacyanin pigments from 21 *Amaranthus* genotypes of 7 species.

MATERIALS AND METHODS

Materials. Forty-two genotypes with red inflorescences and/or red leaves and/or red stems were screened from 243 genotypes of 26 *Amaranthus* species from 38 countries and regions, provided by the USDA-ARS, Iowa State University, and evaluated in Wuhan, China, in 1994 (Wu et al., 1998). These pigment genotypes and several local genotypes were grown again for characterization and extraction at the same site in 1995 and 1996. Field evaluation results showed that 21 genotypes in 7 species appeared better in color and agronomic traits and were selected for further analysis (Table 1 and Table 5).

Preparation of Crude Extract or Dried Pigments. Samples (300−400 g) of fresh plant materials (seedling, leaves, inflorescences, or branches and stem skin) were taken at the appropriate growth stage (Table 1). The raw materials were chilled and cut into small pieces, which were blanched in 3−4 volumes of water at 80 °C for 5 min and filtered through multiple layers of gauze. The extraction was repeated until no further color was removed. The combined aqueous extracts were quickly cooled by use of refrigerated water and centrifuged to remove some impurities. Most of the supernatant was concentrated and dried under vacuum at 40 °C. The dried pigment samples and the remainder of supernatant were frozen and stored at 4 or −18 °C, respectively, until further use.

Purification of *Amaranthus* Pigments. *Amaranthus* pigments were purified following the method of Huang and von Elbe (1986) with modifications. The dried pigment samples were dissolved in deionized water. The pigment solutions were adjusted to pH 2.8 with 2 N HCl and centrifuged at 10 000 rpm for 20 min. The clear supernatant was placed on Sephadex G-25 and G-50 columns (50 × 3 cm) and eluted with deionized water. The major red-violet pigment band was collected and freeze-dried. The freeze-dried samples were dissolved in water and filtered through a 0.45-μm Millipore filter. The filter was passed onto another Sephadex G-25 column, and the freeze-dried pigment was obtained again. Basically purified pigments were prepared according to the method of Schwartz and von Elbe (1980) and were used for quantitative and qualitative analysis.

High-Performance Liquid Chromatography (HPLC). The HPLC system consisted of a Waters 990 photodiode array detector (PDA), two Waters 510 pumps (Waters Chromatography, Milford, MA), and an Ultrasphere C_{18} column (5 μm, 250 × 4.6 mm; Beckman), placed in a thermostated control system (Waters), operated at 30 °C throughout separations. The mobile phase consisted of solvent A, 0.005 mol/L potassium phosphate (KH_2PO_4), and solvent B, 5% acetonitrile (CH_3CN)/0.005 mol/L KH_2PO_4. The following elution profiles were used: 0−3 min, 100% A; 3−6 min, 50% B; 6−15 min, 100% B; 15−20 min, 100% A. The flow rate was 1.0 mL/min. The samples were injected with a Rheodyne 7725i valve with a 20-μL loop. Peaks were determined at the wavelength of 538 nm. All computations were performed using a Waters Millennium 2010 data system.

FTIR Spectroscopy and UV−Vis Spectral Analysis. Fourier transform infrared (FTIR) spectra were recorded by a Bio-Rad Tracer interface using the direct deposition technique in combination with the FTS-45 FTIR spectrometer (Bio-Rad Co., Hercules, CA). UV−vis spectra were measured on a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY) and a Waters 990 photodiode array detector.

Gel Filtration Chromatography. Gel filtration chromatography was performed on Sephadex series (G-25, G-50, G-100) columns (Sigma Co., St. Louis, MO), pre-equilibrated with deionized water. A fraction collector (Gilson Medical Electronics, France) was used to collect 4.0-mL fractions that were subsequently analyzed for *Amaranthus* betacyanins and protein. The distribution of the two components in eluates

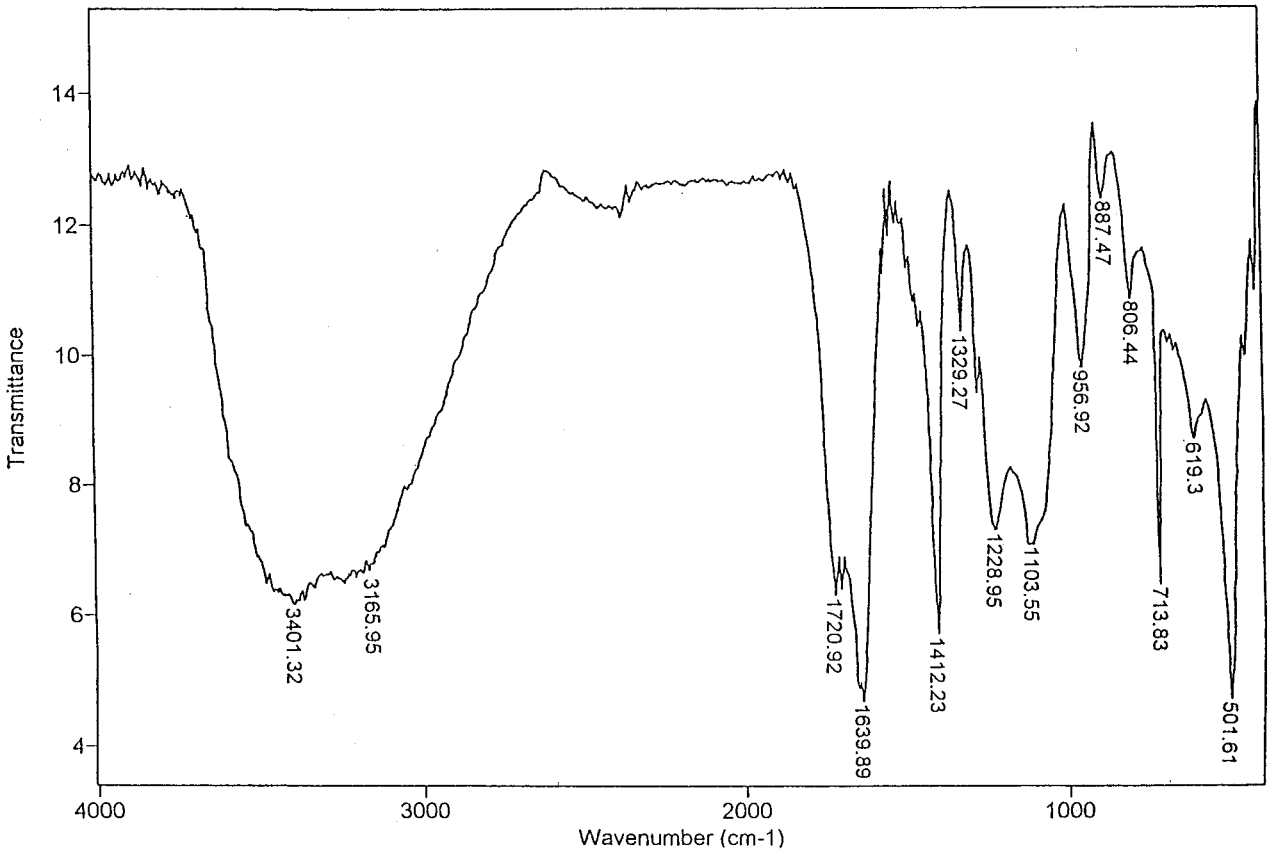

Figure 1. Tracer-FTIR solid-phase spectrum of *Amaranthus* betacyanins.

from the chromatographic column was assayed as follows: *Amaranthus* betacyanin content by spectrophotometric method and protein concentration by Lowry method (Bollag and Edelstein, 1993).

Enzymatic Hydrolysis of *Amaranthus* Pigments. Enzymatic hydrolysis of the purified *Amaranthus* betacyanins, according to the modified method of Piattelli et al. (1964a) and Schwartz et al. (1980), was conducted in β-glucuronidase (from *Helix pomatia*, Sigma) solution at pH 5.0 and 37 °C for 40 min. The samples with and without β-glucuronidase treatment were assayed by HPLC to compare the change of peaks and to identify the betacyanin composition.

Quantitative Determination of *Amaranthus* Pigments. *(1) Amaranthus* pigment content for crude aqueous extracts (AC_a) and dried extracts (AC_d), determined by spectrophotometric method, was calculated and expressed as amaranthine by the following formulas:

$$AC_a = A_{536}(MW)V_a(DF) \times 10^2/\epsilon L W_a$$

(mg/100 g of fresh weight)

$$AC_d = A_{536}(MW)V_d(DF)/\epsilon L W_d$$

(mg/g of dried extracts weight)

For amaranthine, ϵ (molar absorptivity) = 5.66×10^4 cm^{-1} mol^{-1} L (Piattelli et al., 1969) and MW = 726.6•$^{-}$. A_{536} is the absorbance at 536 nm (λ_{max}); L (path length) = 1.0 cm; DF is the dilution factor; V_a is the total extract volume (mL); V_d is the dried pigment solution volume (mL); W_a is the fresh weight of extracting materials (g); W_d is the dried pigment weight (g).

(2) Individual *Amaranthus* betacyanin (amaranthine and isoamaranthine) content for dried pigment samples was determined by HPLC method (as described above) and was expressed as percentage of peak area.

(3) Extraction rate of dried pigments (%) = weight of dried pigments $\times 10^2$/total fresh weight of parts extracted.

Determination of Protein and Amino Acid. Protein of dried pigments was estimated by the improved Kjeldahl method—copper catalyst modification (AACC, 1995). Amino acid of dried pigments was measured in duplicate by a Hitachi 835-50 AA automatic analyzer (Hitachi Co., Japan).

Table 2. FTIR Spectral Data for *Amaranthus* Betacyanins

wavenumber (cm^{-1})	intensity	assignment[a]
1720.92	weak	C=O str
1639.89	strong	C=N str
1412.23	very strong	C−H def
1329.27	medium	C−H def
1228.95	strong	C−O str
1103.55	strong	C−O str
956.72	strong	ben-r C−H def
887.47	medium	ben-r C−H def
806.44	medium	ben-r C−H def
713.83	very strong	ben-r C−H def

[a] str, stretch; def, deformation; ben-r, benzene ring.

RESULTS AND DISCUSSION

Identification of *Amaranthus* Pigments. The red-violet pigments, from 21 genotypes in 7 *Amaranthus* species, were isolated by gel filtration chromatography on Sephadex columns and by HPLC. The dried pigments were identified as homogeneous betacyanins by their IR spectra, UV−vis spectra, and HPLC analysis.

The wavelengths of absorption bands of the solid-phase FTIR spectrum (Figure 1) and some selected assignments to the type vibration (Table 2) of *Amaranthus* pigments from *A. cruentus* were determined. The IR spectrum behavior showed the pigments to be betacyanins, similar to those of *A. tricolor* (Huang and Hwang, 1981) and red beet (Maing, 1972). The FTIR spectral data in Table 2 further demonstrated that *Amaranthus* pigments had typical functional groups

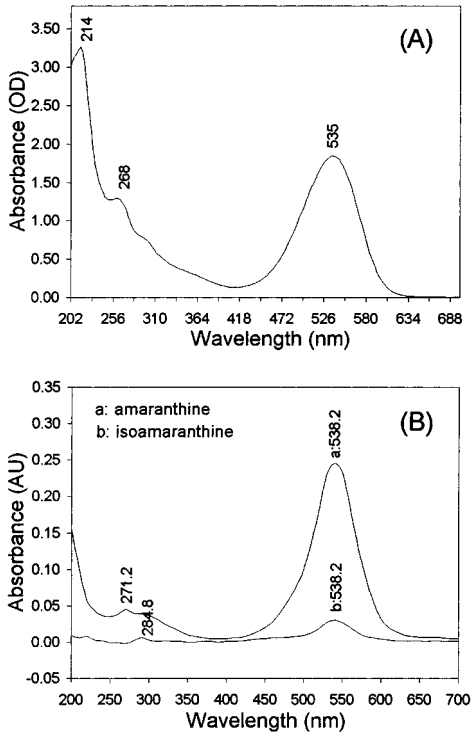

Figure 2. UV−vis spectrum of *Amaranthus* pigments (A) isolated by gel filtration chromatography of dried crude preparations and measured by spectrophotometer and (B) separated and measured by HPLC (with impurities removed).

(1720−714 cm^{-1}) of betacyanins. However, the vibration in the range from wavenumber 3400 to 3100 cm^{-1} and from 619.30 to 501.61 cm^{-1} might be caused by $-NH_2$, $-OH$, $-H_2O$, and C−S functional groups from the protein or amino acid components and trace water in the pigments (see next section).

Figure 2A displays the UV−vis spectra of a crude preparation of *Amaranthus* pigments determined spectrophotometrically. The pigments absorb light as strongly on a dry weight basis as betacyanins from red beet (von Elbe et al., 1974) and garambullo (Reynoso et al., 1997), with only one maximum absorbance peak (535−538 nm) at the range of visible light, and also have major absorbance peaks at 260−280 nm, especially below 235 nm. Figure 2B shows the UV−vis spectra of a purer preparation of two *Amaranthus* pigments separated and determined by PDA detector with the HPLC system, which are very similar to that in Figure 2A at the wavelengths for the two pigments. At lower wavelengths the spectra differ, reflecting the changes on partial purification.

A typical HPLC separation of *Amaranthus* pigments fraction after gel filtration is shown at 538 nm in line A in Figure 3. Two betacyanins of *Amaranthus* pigments separated by PDA detector of HPLC are amaranthine (peak a) and isoamaranthine (peak b), respectively. Amaranthine is eluted first (t_R = 10.6 min), followed by isoamaranthine (t_R = 12.2 min). Huang and Hwang (1981) separated only amaranthine from *A. tricolor* by use of a variable-wavelength detector.

Enzymatic hydrolysis of *Amaranthus* betacyanins could confirm our identification. The partially purified solution of the betacyanins obtained by Sephadex G-25 and G-50 columns was subjected to enzymatic hydrolysis with β-glucuronidase. The specificity of almond emulsion containing β-glucuronidase to the betacyanin glycons was reported by Piattelli and Minale (1964a). Figure 3 shows the comparison of HPLC chromatograms of *Amaranthus* betacyanins before and after enzyme treatment. After β-glucuronidase treatment, amaranthine (peak a) and its epimer (peak b) in part converted to betacyanin aglycons, that is, betanidin (peak c) and isobetanidin (peak d). Peaks a and b, the more polar betacyanin glycons, are eluted first, followed by peaks c and d, the less polar aglycons. This RP-HPLC eluted profile agrees well with that predicted by reversed-phase chromatography principle. The configuration of the C-15 epimers, isoamaranthine (peak b) and isobetanidin (peak d), allows greater interaction with the stationary phase and therefore has a greater retention value relative to their parent. This is similar to early results reported by Schwartz and von Elbe (1980), who identified the order of elution of betacyanins from red beet as betanin, isobetanin, betanidin, and isobetanidin, respectively.

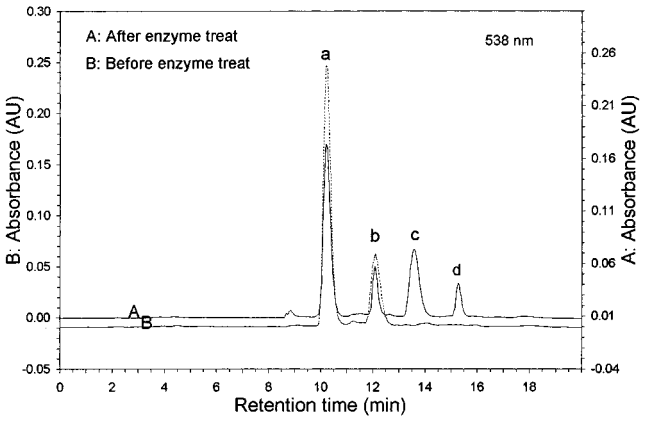

Figure 3. HPLC chromatograms of *Amaranthus* betacyanins before and after treatment with β-glucuronidase: (a) amaranthine; (b) isoamaranthine; (c) betanidin; (d) isobetanidin.

Composition of *Amaranthus* Pigments. As identified above, amaranthine and isoamaranthine are the two betacyanin pigments in *Amaranthus* and also the only betacyanin type in *Amaranthus* plants. Table 3 lists the amaranthine and isoamaranthine levels of the *Amaranthus* betacyanins. Amaranthine and isoamaranthine of the three genotypes (Tr010, Tr013, Tr017) from *A. tricolor* represented ca. 89 and 11% of the total peak area. This was in accordance with previous findings that mature leaves of *A. tricolor* contained amaranthine and isoamaranthine in a molar ratio of about 9:1 separated by an automatic analyzer on polyamide column (Piattelli et al., 1964, 1969). However, the molar ratio of amaranthine and isoamaranthine from most of the genotypes in other species was lower. The average amaranthine and isoamaranthine contents, expressed as percentage of peak area, were 81 and 19% of the total area in aqueous extracts, which were higher than the 67 and 33% in dried extracts. The reason was probably that the drying process and storage factors (e.g. drying temperature, oxygen level) resulted in isomerization of amaranthine to isoamaranthine. Our observations indicated that the conversion of the parent amaranthine to its epimer could occur easily. Previous researchers had reported these isomerizations of amaranthine (Piattelli and Minale, 1964b) and betanin (Vincent and Scholz, 1978; Schwartz and von Elbe, 1980), when a solution of betacyanin in 5% citric acid

Table 3. Pigment Composition (Percent) of *Amaranthus* Betacyanins in Aqueous and Dried Extracts[a]

	aqueous extracts		dried extracts	
genotype	amaranthine	isoamaranthine	amaranthine	isoamaranthine
Cr010	76.5	23.5	60.2	39.8
Cr015	81.0	19.0	71.3	28.7
Cr016			59.4	40.6
Cr017	88.5	11.5	83.4	16.7
Cr020	84.3	15.8	63.8	36.2
Cr043	77.2	22.8	71.1	28.9
Cr044	88.9	11.1	70.7	29.3
Cr052			68.7	31.3
Cr069			70.7	29.3
Cr072	85.6	14.4	73.6	26.4
Japan19	76.6	23.4	57.8	42.2
V69	84.9	15.1	67.5	32.5
Tr010	90.3	9.7	75.7	24.3
Tr015	88.8	11.2	70.3	29.7
Tr017	87.1	13.0	68.8	31.2
Sheng07	81.1	18.9	73.5	26.5
Sheng09			67.0	33.0
Sheng12	74.4	25.6	65.4	34.7
Hy003	71.1	28.9	60.5	39.5
Lv001	60.3	39.7	52.6	47.4
Tibet Yellow	78.0	22.0	56.9	43.1
mean	80.9	19.2	67.1	32.9

[a] Individual pigment content was expressed as percentage of peak area determined by HPLC. Samples were stored at 4 °C in the absence of oxygen for 10 months before analysis.

was allowed to stand at room temperature for 36 h or when it was stored in the absence of oxygen.

An unexpected finding was that the *Amaranthus* betacyanins had a special relationship with protein. Both crude dried pigment extracts and purified samples contained abundant water-soluble protein components, which seemingly bound to the betacyanins. When the UV−visible spectral determination of *Amaranthus* pigments was conducted, it was observed that dried extracts and the purified samples by gel filtration had abnormally strong absorbance at the range of UV light (260−280 nm and <235 nm; Figure 2A), indicating that the extracts or samples might contain protein. To confirm that the absorbance was from proteins, not phenolics, additional tests were conducted. Protein estimation (by Kjeldahl method) showed that the average protein content of dried pigment extracts was 28%, ranging from 23 to 32% among 21 *Amaranthus* genotypes (Table 5). *Amaranthus* betacyanin content was positively correlated ($r = 0.81$, $p < 0.05$) with protein content among 21 *Amaranthus* genotypes, indirectly suggesting that *Amaranthus* betacyanins are associated with protein components. Amino acid analysis (Table 4) further confirmed that the material was protein. The high nitrogen content of vegetative tissues of *Amaranthus* has been noted in relation to its use in animal feed (Corke et al., 1997), certainly providing enough nitrogen for protein synthesis. Previous studies on the relation of betacyanin (amaranthine) synthesis with kinetin, inhibitors, and photocontrol in *A. tricolor* and *A. caudatus* found that a large part of pigments were produced dependent on protein and nucleic acid synthesis, as expected from the biosynthetic pathway (Piattelli et al., 1970; Koehler, 1972a,b).

During purification of the pigments, it was found that the betacyanins could not readily be completely isolated from the protein. $(NH_4)_2SO_4$ salting-out or HCl treatment and centrifugal separation removed only part of the protein. The treated pigment samples were then

Table 4. Amino Acid Content of Protein from *A. cruentus* Pigments[a]

	content (%)	
amino acid	dried crude sample	Sephadex G-25 and G-50 separated sample
glutamine	2.53	1.01
asparagine	2.01	0.63
leucine	1.27	0.57
glycine	1.09	0.49
alanine	1.06	0.68
valine	1.05	0.51
lysine	1.02	0.40
isoleucine	0.92	0.36
phenylalanine	0.89	0.49
arginine	0.82	0.01
proline	0.74	0.01
serine	0.73	0.14
threonine	0.65	0.15
tyrosine	0.50	0.14
histidine	0.32	0.06
methionine	0.14	0.18
cysteine	0.09	0.19
total amino acid	15.83	6.02
crude protein	30.27	12.76

[a] Genotype Cr044.

gradually isolated by Sephadex G-25 and G-50 columns to remove the remnant protein and nonpigment substances. However, the purified pigment samples still contained 12.8% protein (Table 4). Piattelli et al. (1964) identified amaranthine as having a molecular weight of 726.6. If so, the smaller individual molecules of amaranthine should be separated easily from the much larger protein molecules and should be eluted after protein molecules on Sephadex G-25. However, gel filtration chromatographic elution profiles (Figure 4) showed *Amaranthus* betacyanin content peak and protein concentration peak almost coincide, clearly indicating that *Amaranthus* betacyanins together with protein are eluted at the same time on Sephadex G-25, G-50, and G-100 columns. This showed that *Amaranthus* betacyanins are strongly bound to protein. Amaranthine and isoamaranthine molecules perhaps combine with protein molecules by noncovalent bonding or other means. According to the protein calibration curve, the molecular weight (MW) of the pigment−protein was estimated at ∼20 000−35 000. Yue and Sun (1993) reported the MW of the pigment−protein from *A. tricolor* ranged from 10 000 to 50 000 determined by SDS−polyacrylamide gel electrophoresis. Table 4 gives the amino acid composition of *Amaranthus* pigment−protein before and after separation by gel filtration chromatography. For our purposes, effective pigments with presumed nutritional advantages were formed by leaving the pigment−protein complex more or less intact. We expect that practical usage of pigments would follow a simple extraction procedure, so our evaluation of the pigments would not require total separation from protein.

The pigments purified by gel filtration chromatography were separated again by HPLC (Figure 3). The HPLC-isolated amaranthine and isoamaranthine components exhibited the UV−visible spectra as shown in Figure 2B. The UV spectral peaks (260−268 and <235 nm) of the two pigments looked like the typical UV absorbance spectra of protein or amino acid. Piattelli (1976) considered that the absorbance peak at ∼280 nm was a spectral characteristic of the betacyanins.

Quantification and Distribution of *Amaranthus*

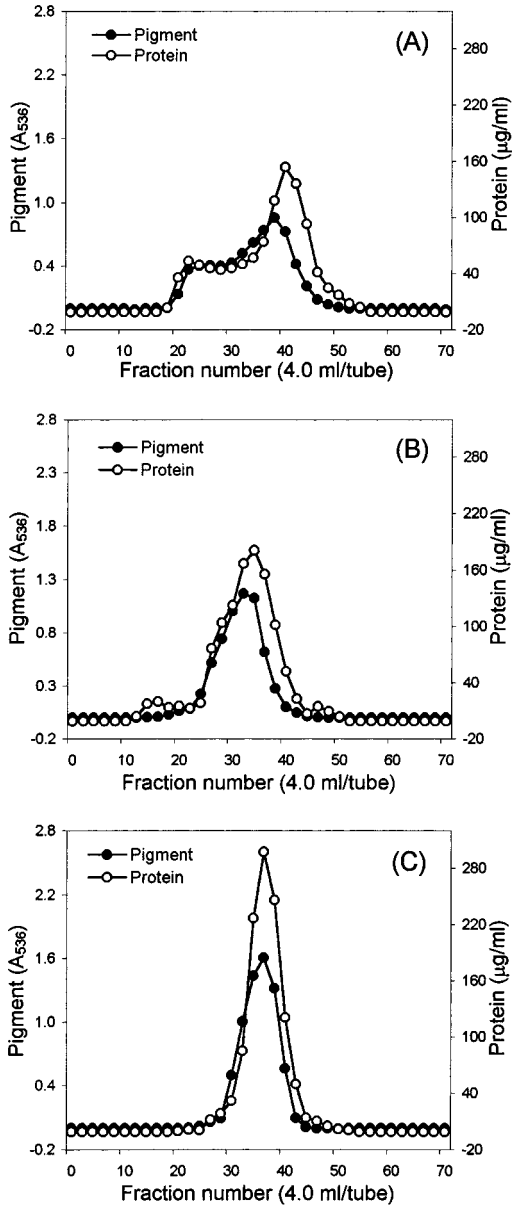

Figure 4. Gel filtration chromatographic elution profiles of *Amaranthus* pigments on columns of Sephadex G-25 (A), G-50 (B), and G-100 (C).

Pigments. The pigment distribution in plant parts of seven *Amaranthus* species is shown in Table 1. Two vegetable amaranth species (*A. tricolor and A. lividus*) contained more betacyanins in young leaves, while the five grain amaranth species had more betacyanins in very large inflorescences or young leaves and seedlings. Notably, two cultivated genotypes, Cr072 and Tr010, were entirely red in color.

Quantitative analysis of the 21 better pigment genotypes is given in Table 5. The other 20 genotypes screened were substantially lower in pigment content and are not included in this paper. Spectrophotometric determination revealed that the pigment content of crude aqueous extracts varied between 42.3 and 198.9 mg of amaranthine/100 g of fresh weight (FW) for the 21 genotypes. The pigment content of dried extracts ranged from 17 to 47 mg of amaranthine/g of dried extract weight (DW). The highest pigment content was possessed by three *A. cruentus* genotypes, that is, Cr072 (199 mg/100 g of FW and 47 mg/g of DW), Cr044 (171 mg/100 g of FW and 41 mg/g of DW), and V69 (145 mg/100 g of FW and 34 mg/g of DW). Among eight genotypes of pigment content over 100 mg/100 g, five were *A. cruentus*, a grain amaranth species that is the most widespread species in agricultural practice in many countries; the other genotypes belonged to *A. tricolor* (Tr010), *A. caudatus* (Sheng07), and *A. paniculatus* (Tibet yellow). Table 5 also showed cultivated species contained much more betacyanins than wild species (*A. lividus*).

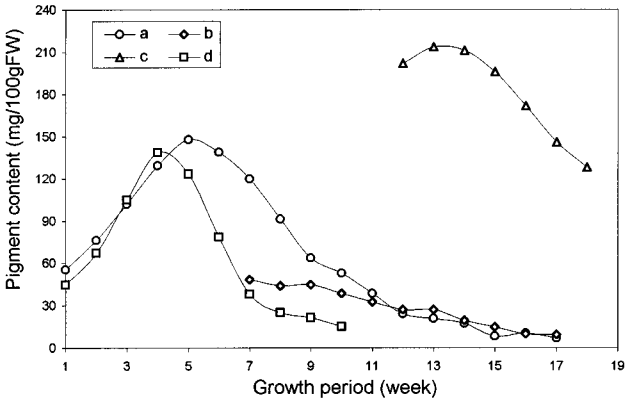

Figure 5. Change of *Amaranthus* pigment content at growth period: (a) Cr072 leaves; (b) Cr072 stem skin; (c) Cr072 inflorescences; (d) Tr010 leaves.

Not only did large genotypic differences in betacyanin content exist among *Amaranthus* species, distributed differently in plant parts, but also the pigment contents changed significantly during different growth stages (Figure 5). For vegetable amaranth genotypes (Tr010), pigment content of leaves was over 100 mg/100 g for 3 weeks and reached the highest level at 139 ± 4.3 mg/100 g. The result was similar to that of a previous study (Shen and Hwang, 1985). However, for grain amaranth genotypes (Cr072), the highest pigment contents occurred at 12−14 weeks after sowing (early-medium flowering period) in inflorescences (~213 mg/100 g), at 4−6 weeks after sowing in leaves (148 mg/100 g), and at ~7 weeks after sowing in stem skin (48 mg/100 g).

Interestingly, cultivated grain-type Cr072 contained much more betacyanin in inflorescences and leaves than did ornamental-type Tr010 in leaves, and its pigment content was >100 mg/100 g for 12 weeks in inflorescences and leaves combined (Figure 5). Moreover, after grain harvest the inflorescences still had high betacyanin content (>120 mg/100 g). This suggested that cultivated grain amaranth genotypes can give high grain yield and natural pigments at the same time. The average extraction of dried pigments in 21 *Amaranthus* genotypes was 1.68% (Table 5), whereas the average for the eight genotypes of higher pigment contents was 2.18%. The extraction was highly positively correlated ($r = 0.95$, $p < 0.05$) with the pigment content of crude extracts. The pigment content of dried extracts also had a high positive correlation ($r = 0.89$, $p < 0.05$) with the pigment content of crude extracts. Grain amaranth genotypes had much higher biomass weight and grain yield than vegetable amaranth genotypes (Table 1; Wu et al., 1998). Thus, the grain amaranth genotypes have greater commercial potential to be exploited as food colorants than vegetable amaranth genotypes which were reported before (Huang and von Elbe, 1986). Besides, the best *Amaranthus* genotypes contained much more betacyanins, compared to the results for red

Table 5. Quantitative Analysis of *Amaranthus* Pigments from 21 Genotypes

			pigments from fresh parts		pigments from dried extracts		
species	genotype	origin	plant part	betacyanins[a] (mg/100 g)	extraction rate[b] (%)	betacyanins[c] (mg/g)	protein (%)
A. cruentus	Cr010	USA	inflores	54 ± 3.4	1.18	20	25.7
	Cr015	Mexico	inflores	134 ± 5.6	2.17	29	29.9
	Cr016	Zaire	inflores	53 ± 3.3	1.21	17	24.6
	Cr017	unknown	inflores	71 ± 3.8	1.43	24	26.5
	Cr020	Mexico	inflores	77 ± 3.0	1.48	27	30.6
	Cr043	Mexico	inflores	45 ± 2.8	1.20	19	25.2
	Cr044	Mexico	inflores	171 ± 4.7	2.35	41	30.3
	Cr052	Peru	inflores	42 ± 3.3	1.15	26	29.3
	Cr069	Guatemala	inflores	44 ± 2.9	1.20	16	25.1
	Cr072	India	inflores	199 ± 6.9	2.57	47	30.5
	Japan19	Japan	inflores	113 ± 3.2	1.87	27	29.9
	V69	China	inflores	145 ± 8.6	1.91	34	31.7
A. tricolor	Tr010	China	leaves	143 ± 4.5	2.36	33	30.8
	Tr015	India	leaves	74 ± 3.2	1.65	24	27.4
	Tr017	China	leaves	51 ± 3.9	1.34	26	28.7
A. caudatus	Sheng07	China	inflores	144 ± 5.4	2.28	28	30.0
	Sheng09	China	inflores	57 ± 2.4	1.49	24	27.1
A. hybridus	Sheng12	China	inflores	82 ± 3.8	1.66	17	25.8
A. hypochondriacus	Hy003	Mexico	inflores	47 ± 1.9	1.23	19	23.2
A. lividus	Lv001	India	seedlings	46 ± 3.5	1.57	19	24.2
A. paniculatus	Tibet Yellow	China	inflores	127 ± 4.5	2.12	30	31.2
mean				91.4 ± 4.0	1.69	26	28.0
$LSD_{0.05}$[d]				6.6		1.0	0.8

[a] Calculated as mg of amaranthine/100 g of fresh material. [b] Percentage of weight of dried pigments/total fresh weight of parts extracted. [c] Calculated as mg of amaranthine/g of dried extracts. [d] Least significant difference for comparison of genotype means.

beet and cactus fruit reported by Sapers and Hornstein (1979) and Reynoso et al. (1997).

In conclusion, the betacyanin pigments from 21 *Amaranthus* genotypes were identified as homogeneous betacyanins that consisted of amaranthine and isoamaranthine. Extensive variability in betacyanin content was seen among and within species. Grain amaranth species with much higher biomass and more betacyanins over a longer growth period had greater commercial potential for the development of natural pigments than vegetable amaranth species. Some superior genotypes, such as Cr072, Cr044, V69, Tibet Yellow, and Sheng07, were screened for use in the future commercial production of food colorants.

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