

# Colorant Properties and Stability of *Amaranthus* Betacyanin Pigments

Yizhong Cai,<sup>†,‡</sup> Mei Sun,<sup>§</sup> and Harold Corke<sup>\*,†</sup>

Departments of Botany and Zoology, The University of Hong Kong, Pokfulam Road, Hong Kong, and Institute of Agricultural Modernization, Hubei Academy of Agricultural Sciences, Wuhan 430064, China

Color, spectral characteristics, and stability of betacyanin pigments of 21 genotypes from 7 *Amaranthus* species were evaluated. Wide variation in color characteristics and significant differences in pigment stability were found as much within as between *Amaranthus* species. *Amaranthus* betacyanins, like red beet pigments, were susceptible to temperature and also affected by pH, light, air, and water activity, with better pigment stability at lower temperatures ( $\leq 14$  °C) in the dark and in the absence of air. Storage stability at 25 °C of dried *Amaranthus* pigments ( $t_{1/2} = 23.3$  months) was longer than that of aqueous pigment extracts ( $t_{1/2} = 1.04$  months), indicating that the dried pigments were stable enough to be used as commercial colorants. The betacyanins from some better pigment genotypes (Cr015, Sheng07, Tr010, Cr072, Cr017, etc.) exhibited brighter red-violet color characteristics and were similar in stability to red radish anthocyanins under selected conditions. These characteristics of *Amaranthus* pigments give them considerable potential for development for use in the food industry, particularly for low-temperature uses.

**Keywords:** *Amaranthus*; betacyanins; food pigments; spectral characteristics; color stability

## INTRODUCTION

*Amaranthus* pigments are red-violet betacyanins, like the red beet pigments that have been extensively used in the food industry (Schnetzler and Breene, 1994). Since the 1980s, research on the characteristics and stability of the betacyanins of *Amaranthus tricolor* has been undertaken (Shen and Hwang, 1985; Huang and von Elbe, 1986; Chen, 1992). We identified 21 apparently superior pigment genotypes of 7 *Amaranthus* species, including *A. tricolor*, after screening 243 genotypes of 26 species from 38 countries and regions, and showed that grain amaranth species (e.g., *A. cruentus*) with high betacyanin content and biomass had potential as a new source of betacyanin pigments (Cai et al., 1998).

However, betacyanins, like other natural plant pigments (e.g., anthocyanins), are susceptible to color deterioration during processing and storage, with low stability, low tinctorial strength, and high cost, which limits their development and application (von Elbe et al., 1974, 1996; Driver and Francis, 1979; Baublis et al., 1994). The stability of betacyanins from red beet and *A. tricolor* was strongly affected by pH, temperature, oxygen, light, and water activity (Pasch and von Elbe, 1975; Saguy et al., 1978; von Elbe et al., 1981; Saguy et al., 1984; Huang and von Elbe, 1986; Huo and Guo, 1994).

*Amaranthus* pigments are easily obtainable at low cost because they can be removed by water extraction (Cai et al., 1998). However, to date, little has been reported about the color properties and stability of

betacyanins from diverse *Amaranthus* cultivated and wild species. Therefore, we conducted such studies to characterize pigments from better genotypes for the development of natural colorants.

## MATERIALS AND METHODS

**Materials.** We selected 21 better pigment genotypes from seven *Amaranthus* species (Table 1) and planted them in field experiments in 1995 and 1996 in Wuhan, China. The accessions were obtained from the USDA-ARS, Iowa State University, except for the *A. caudatus* and *A. hybridus*, which were from the Chinese National Grain Amaranth program (Chinese Academy of Agricultural Sciences, Beijing). The crude aqueous extract and dried samples (four replicates per genotype) of *Amaranthus* pigments were prepared and purified as described in Cai et al. (1998).

**Color and Spectral Analysis of *Amaranthus* Pigments.** The Hunter color parameters  $L^*$ ,  $a^*$ , and  $b^*$  (CIE 1976) were measured by a colorimeter (Chroma Meter CR-301, Minolta Co., Osaka, Japan) through square plastic optical cells (50 × 50 mm, 5 mm in depth), standardized with calibration plate sets CR-A47 and a white plate. UV-visible spectra of the pigment solutions were determined on a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY). The dried samples of *Amaranthus* pigments were dissolved in McIlvaine's buffer (pH 5.6) and standardized by dilution to an absorbance of 1.0 at  $\lambda_{max}$  (~535 nm) for analysis by tristimulus colorimetry so that all genotypes could be compared at similar  $L^*$  values. One selected sample (Cr072) was dissolved in McIlvaine's buffer (pH 2.2~8.0) and Clark-Lubs' buffer (pH 8.0–10.0) to determine color and spectral properties at different pH values (2.2–10.0). Absorbance spectra of the solution samples in 1.0 cm path length quartz cuvettes were recorded from 200 to 700 nm.

**Evaluation of Thermal and Storage Stability.** The crude aqueous betacyanin extracts ( $A_{538} \approx 6.465$ ) were tested at four temperatures (40, 60, 80, 100 °C) with exposure to light (with an intensity of 980 lx) and air at the natural pH (~6.0). The effects of light and air on stability were observed at 40 and 80 °C. The sample solutions were distributed in 50 mL

\* Author to whom correspondence should be addressed (fax 00852 2858 3477; e-mail harold@hku.hk).

<sup>†</sup> Department of Botany.

<sup>‡</sup> Institute of Agricultural Modernization.

<sup>§</sup> Department of Zoology.

**Table 1. Color and Spectral Characteristics of *Amaranthus* Pigments from 21 Genotypes of Seven Species<sup>a</sup>**

species	genotype	$\lambda_{\max}$ (pH 5.6)	$L^*$ ( $\pm$ SD)	$a^*$ ( $\pm$ SD)	$b^*$ ( $\pm$ SD)	$C$	$H^{\circ}$	$\Delta E^*_{ab}$ <sup>b</sup>	pigment retention <sup>c</sup>	
<i>A. cruentus</i> L.	Cr010	536	41.8 $\pm$ 0.13	20.7 $\pm$ 0.15	0.4 $\pm$ 0.07	20.7	1.1	1.56	62.8	
	Cr015	535	41.2 $\pm$ 0.17	23.0 $\pm$ 0.22	-2.4 $\pm$ 0.39	23.1	354.1 (-5.9)	4.23	73.4	
	Cr016	535	42.0 $\pm$ 0.14	20.6 $\pm$ 0.34	0.6 $\pm$ 0.20	20.6	1.7	1.63	58.4	
	Cr017	536	41.5 $\pm$ 0.09	22.7 $\pm$ 0.28	-3.9 $\pm$ 0.13	23.0	350.4 (-9.6)	5.56	69.5	
	Cr020	535	41.6 $\pm$ 0.14	22.3 $\pm$ 0.28	0.0 $\pm$ 0.25	22.3	0.1	1.75	62.7	
	Cr043	535	41.6 $\pm$ 0.16	22.6 $\pm$ 0.17	-1.3 $\pm$ 0.15	22.6	356.7 (-3.3)	3.07	59.4	
	Cr044	536	41.5 $\pm$ 0.10	22.3 $\pm$ 0.35	-4.7 $\pm$ 0.24	22.8	348.1 (-11.9)	6.32	67.9	
	Cr052	536	41.8 $\pm$ 0.17	21.5 $\pm$ 0.26	-0.4 $\pm$ 0.09	21.5	358.9 (-1.1)	2.06	58.8	
	Cr069	536	41.8 $\pm$ 0.01	22.0 $\pm$ 0.02	-0.4 $\pm$ 0.01	22.0	359.0 (-1.0)	2.07	64.2	
	Cr072	536	41.1 $\pm$ 0.13	24.3 $\pm$ 0.18	-5.0 $\pm$ 0.15	24.8	348.4 (-11.6)	7.14	69.6	
	Japan19	535	40.8 $\pm$ 0.23	22.2 $\pm$ 0.16	1.3 $\pm$ 0.26	22.2	3.3	0.83	60.7	
	V69	536	41.5 $\pm$ 0.11	21.9 $\pm$ 1.28	-3.2 $\pm$ 0.17	22.1	351.8 (-8.2)	4.79	63.9	
	mean $\pm$ SD		535.6	41.5 $\pm$ 0.35	22.2 $\pm$ 1.00	-1.6 $\pm$ 2.17	22.3	356.1 (-3.9)	3.23	64.3
	<i>A. caudatus</i> L.	Sheng07	535	41.0 $\pm$ 0.18	21.5 $\pm$ 0.24	0.9 $\pm$ 0.10	21.5	2.3	0.84	70.2
Sheng09		535	41.4 $\pm$ 0.21	19.5 $\pm$ 0.26	2.8 $\pm$ 0.09	19.7	8.1	2.41	62.6	
mean $\pm$ SD			535.0	41.2 $\pm$ -	20.5 $\pm$ -	1.8 $\pm$ -	20.6	5.2	1.17	66.4
<i>A. hypochondriacus</i> L.	Hy003	536	40.8 $\pm$ 0.00	22.1 $\pm$ 0.00	-2.4 $\pm$ 0.01	22.2	353.8 (-6.2)	4.07	59.6	
<i>A. hybridus</i> L.	Sheng12	535	40.4 $\pm$ 0.13	22.5 $\pm$ 0.16	0.6 $\pm$ 0.14	22.5	1.6	1.61	61.7	
<i>A. paniculatus</i> L.	Tibet Yellow	536	40.4 $\pm$ 0.10	22.5 $\pm$ 0.05	1.1 $\pm$ 0.20	22.5	2.9	1.42	61.9	
<i>A. lividus</i> L.	Lv001	529	40.5 $\pm$ 0.00	18.0 $\pm$ 0.02	4.9 $\pm$ 0.02	18.6	15.2	4.99	48.6	
<i>A. tricolor</i> L.	Tr010	535	41.1 $\pm$ 0.08	22.4 $\pm$ 0.32	1.1 $\pm$ 0.15	22.4	2.8		69.8	
	Tr015	535	41.4 $\pm$ 0.12	21.4 $\pm$ 0.13	1.5 $\pm$ 0.32	21.4	4.1		62.3	
	Tr017	535	41.6 $\pm$ 0.16	21.2 $\pm$ 0.32	2.2 $\pm$ 0.15	21.3	5.9		57.4	
	mean $\pm$ SD		535.0	41.4 $\pm$ 0.27	21.6 $\pm$ 0.63	1.6 $\pm$ 0.55	21.7	4.3	0.00	63.2
overall mean		535.1	41.3	21.8	-0.3	21.9	359.5 (-0.5)	3.12	63.1	
SD		1.49	0.43	1.31	2.53	1.30	6.72		5.63	

<sup>a</sup>  $L^*$ ,  $a^*$ ,  $b^*$  values were means of four samples at constant absorbance ( $A_{\lambda_{\max}} = 1.0$ ) for every genotype pigment. <sup>b</sup>  $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$  calculated by CIE  $L^*a^*b^*$  mean values of *A. tricolor* genotypes. <sup>c</sup> Aqueous solution, in dark, no air, 14 °C, 20 weeks.

glass test tubes. Absence of air was obtained by filling the tubes with pigment solution and sealing with screw caps. The samples without exposure to light were stored in tubes wrapped with aluminum foil. Pigment content and retention were determined at various time intervals. The thermal stability was expressed in terms of rate constant ( $k$ ) and half-life value ( $t_{1/2}$ ), calculated according to the modified methods of von Elbe et al. (1974), Saguy et al. (1978), and Shen and Hwang (1985), who applied the regression analysis of  $\ln$ (pigment retention %) versus storage time when plotted on a natural logarithmic scale.

Experimental samples for storage testing were kept in the dark in the absence of air in a refrigerator (4 °C) and at room temperature (~25 °C) for 1 year. The crude aqueous extract solutions and the dried powder samples were filled, respectively, in test tubes (50 mL) sealed with screw caps and in 5 mL bottles sealed with rubber stoppers. These tubes and bottles were sealed in aluminum foil bags. The degradation of pigments was investigated by triplicately measuring amaranthine concentration of each pigment solution in pH 5.6 at various time intervals. Dried pigment powder was accurately weighed and dissolved in pH 5.6 buffer to the required concentration. The storage stability was tested and expressed according to the same method as for the thermal stability tests.

**Stability Comparison Test.** Accurate amounts of dried pigment samples (0.03% w/v) from 21 *Amaranthus* genotypes were dissolved in the pH 5.6 McIlvaine's buffer and then filled in test tubes (50 mL) sealed with screw caps. Red radish anthocyanin pigments as control sample was obtained from Shanghai Food Additives Joint Co. (Shanghai, China). All of the experimental samples were stored in the dark and in the absence of air at 14 °C for 20 weeks. Pigment retention (percent) after 20 weeks of storage was determined to compare the stability of the pigments from different genotypes.

**Determination and Calculation of Experimental Parameters.** Because amaranthine and isoamaranthine, with the same  $\lambda_{\max}$  and similar structures, are the major components of *Amaranthus* betacyanins, the pigment content was measured and expressed as amaranthine milligrams per liter by a spectrophotometric method (Cai et al., 1998). Pigment

retention (percent) was calculated according to the following formula: (concentration of amaranthine at specified storage time)  $\times 10^2$  / (concentration of amaranthine at zero storage time).

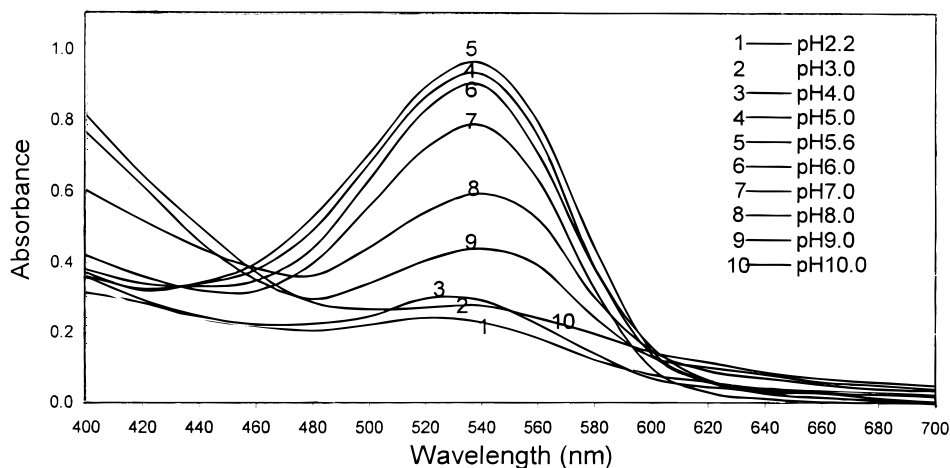
Color parameters were expressed as tristimulus parameters, that is,  $L^*$ ,  $a^*$ ,  $b^*$ ,  $C$ , and  $H^{\circ}$ . The  $C$  value indicates color purity, calculated as  $C = (a^{*2} + b^{*2})^{1/2}$ . Hue angle indicates sample color, calculated as  $H^{\circ} = \tan^{-1}(b^*/a^*)$ . For  $H^{\circ}$ , 0° or 360° = red, 90° = yellow, 180° = green, and 270° = blue (Sapers, 1994).  $\Delta E^*_{ab}$  denotes the total color difference between two samples, calculated as  $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$  (Francis, 1985; Sapers, 1994).

**Data Analysis.** All statistical computations were performed with STATISTICA for Windows (release 4.5) (Statsoft Inc., 1993).

## RESULTS AND DISCUSSION

**Color and Spectral Characteristics.** Betacyanins from the order Centrospermae, including the genus *Amaranthus*, were described as red-violet or purple-red pigments (Piattelli and Minale, 1964). Pigment solutions from 21 *Amaranthus* genotypes at constant absorbance ( $A_{\lambda_{\max}} = 1.0$ ) at pH 5.6 (Table 1) gave  $L$  values which varied within narrow limits (40.4–42.0), indicating that the standardized solutions were similar in lightness so that the color characters of the pigment samples could be appropriately compared using the other tristimulus parameters (Hong and Wrolstad, 1990).

Values of the hue angle ( $H^{\circ}$ ) ranged between 348.1° (-11.9°) and 15.2° for the 21 genotypes, visually showing that they varied in color from purplish-red to orange-red. Most of the genotypes from *A. cruentus* and *A. hypochondriacus*, for example, Cr044, Cr072, Cr017, V69, and Hy003, had lower  $H^{\circ}$ , indicating a more purple shade of red. The genotypes from *A. lividus*, *A. caudatus*, and *A. tricolor*, especially Lv001, Sheng09, and



**Figure 1.** Visible spectra of *Amaranthus* pigments (genotype Cr072) at different pH values after 24 h of storage at 25 °C.

Tr017, had higher  $H^*$ , indicating a more orange shade of red.  $C$  value, a measure of the purity of the color, varied between 18.6 and 24.8. Higher  $C$  values of the pigment samples, for example, Cr072, Cr017, and Cr015, indicated that these genotypes had a more vivid red color than the other genotypes with lower  $C$  values.

$\Delta E^*_{ab}$  represents the total color difference between two samples, in this case in comparison with the mean of three *A. tricolor* genotypes. From Table 1, it was generally found that *A. cruentus* ( $\Delta E_m = 3.23$ ), *A. lividus* (4.99), and *A. hypochondriacus* (4.07) were the most dissimilar in color to *A. tricolor*, whereas *A. caudatus* ( $\Delta E_m = 1.17$ ), *A. hybridus* (1.61), and *A. paniculatus* (1.42) were the closest in color to *A. tricolor*. For 12 *A. cruentus* genotypes,  $\Delta E^*_{ab}$  values ranged between 0.83 and 7.14, indicating substantial color differences among them.

The visible spectra absorption maxima ( $\lambda_{max}$ ) for the betacyanin pigments for most of the *Amaranthus* genotypes in pH 5.6 solution were between 535 and 536 nm (Table 1), quite similar to previously reported data for betacyanins (Piattelli and Minale, 1964; Huang and von Elbe, 1986). However,  $\lambda_{max}$  of *A. lividus* Lv001 was only 529 nm, probably due to the easy degradation of its betacyanin pigments.

The UV-visible spectral changes of *A. cruentus* Cr072 pigments in buffer between pH 2.2 and 10 were determined at 25 °C after 24 h of storage (Figure 1). In the pH range 5–7 the spectral curves were very close, their  $\lambda_{max}$  values were all 535 nm, and the color of the pigment solution was constant. Outside this pH range the spectral absorption shifted to a slightly shorter wavelength (526–532 nm) for pH 2.2–4 and a slightly longer wavelength (538–544 nm) for pH 8–10 at  $\lambda_{max}$ , with a marked decrease in the  $A_{\lambda_{max}}$ , and only for pH 8–10 with an increase in the absorbance values in the 600–700 and 400–450 nm regions. These alterations were accompanied by a marked change in color from purple to light red at pH 2.2–4 and from purple to dark purple to yellow at pH 8–10. Hence, the pigments from *A. cruentus* Cr072 had greatest stability at pH 5–7, at 25 °C, especially at pH 5.6. We also found that pigments from other *Amaranthus* genotypes had similar stability at pH 5–7. Huang and von Elbe (1986) reported that amaranthine from *A. tricolor* was more stable at pH 5–6 at 4 °C for 7 days.

**Thermal and Storage Stability.** Thermal stability of betacyanin pigments from *Amaranthus* species was

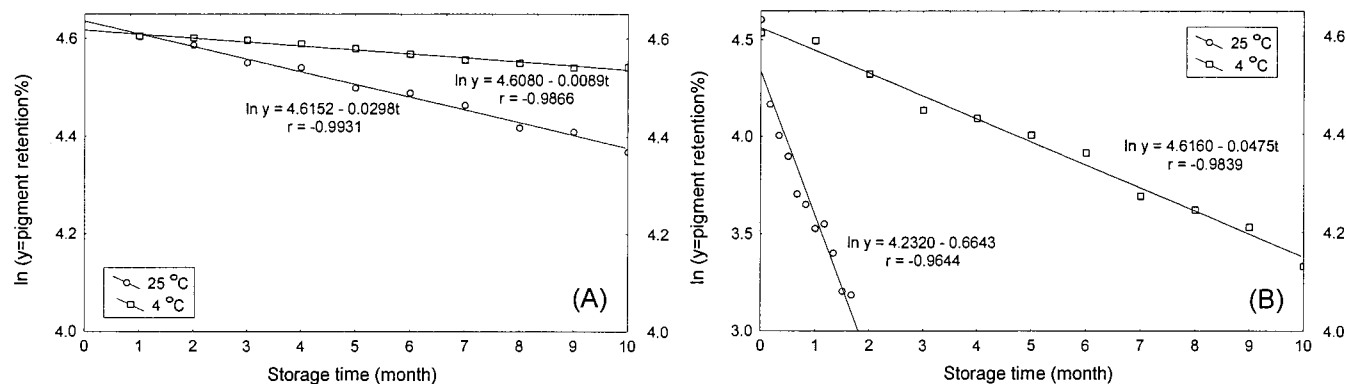
**Table 2. Thermal Stability of *A. cruentus* Cr044 Pigments**

experimental conditions	correlation coefficient ( $r^a$ )	rate constant $k$ ( $\text{min}^{-1}$ )	half-life time $t_{1/2}$ (min)
40 °C, light, air	-0.914	$(2.52 \pm 0.9) \times 10^{-4}$	2751
40 °C, light, no <sup>b</sup>	-0.937	$(1.97 \pm 0.6) \times 10^{-4}$	3519
40 °C, dark, air	-0.904	$(2.36 \pm 1.1) \times 10^{-4}$	2937
40 °C, dark, no	-0.942	$(1.31 \pm 0.4) \times 10^{-4}$	5291
60 °C, light, air	-0.961	$(3.14 \pm 0.5) \times 10^{-4}$	2208
80 °C, light, air	-0.979	$(5.56 \pm 0.6) \times 10^{-3}$	125
80 °C, light, no	-0.965	$(5.27 \pm 0.7) \times 10^{-3}$	132
80 °C, dark, air	-0.992	$(5.68 \pm 0.5) \times 10^{-3}$	122
80 °C, dark, no	-0.978	$(4.99 \pm 0.7) \times 10^{-3}$	139
100 °C, light, air	-0.983	$(3.71 \pm 0.4) \times 10^{-2}$	19

<sup>a</sup>  $\ln(\text{pigment retention \%}) = \text{const} + kt$ . <sup>b</sup> No = absence of air.

studied at four temperatures (40, 60, 80, and 100 °C), exposure/nonexposure to light, and in the presence or absence of air. When pigment retention (percent) versus heating time was plotted on a natural logarithmic scale, it followed first-order kinetics. The first-order reaction rate constant ( $k$ ) and half-life time ( $t_{1/2}$ ) values, indicating the degradation rate and stability of the pigments, respectively, were compared (Table 2), showing that *Amaranthus* betacyanins are susceptible to temperature in the light and air. When the temperature increased, the degradation of the pigments accelerated, with a  $k$  of  $2.52 \times 10^{-4} \text{ min}^{-1}$  and a  $t_{1/2}$  of 2751 min at 40 °C and a  $k$  of  $3.71 \times 10^{-2} \text{ min}^{-1}$  and  $t_{1/2}$  of 19 min at 100 °C in the light and air. However, at the same temperatures (40 and 80 °C) rate constants ( $k$ ) of the pigments were greater in the light and/or air than in darkness and/or absence of air, and the half-life time ( $t_{1/2}$ ) was shorter, indicating that light and air also affected the stability of the pigments. Moreover, the effects of light and air on the stability at low temperature were much greater than those at high temperature. To some extent, these results were similar to the previous studies by Huang and von Elbe (1986) and Reynoso et al. (1997), who reported the stability of betacyanins from red beet, cactacea fruit, and *A. tricolor*; but the  $k$  and  $t_{1/2}$  values in our study were better than in their results. This was likely partially due to our use of natural crude pigment extracts, which may be more stable because of protective effects conferred by other components (e.g., natural antioxidants and polyphenols) (von Elbe et al., 1974). There is also the likelihood of thermal stability differences between betacyanins from *A. cruentus* and other kinds of betacyanins.





**Figure 2.** Regression of  $\ln(\text{pigment retention } \%)$  versus storage time for (A) dried pigments and (B) aqueous extract from *A. cruentus* Cr072 at 25 and 4 °C.

**Table 3. Storage Stability of *A. cruentus* Cr044 Pigments in the Dark and in the Absence of Air at Different Temperatures**

pigment	temp (°C)	rate constant $k \times 10^{-3}$ (month <sup>-1</sup> )	half-life time $t_{1/2}$ (months)	pigment retention (after 10 months) (%)
aqueous extract	25	664	1.04	18.3 <sup>a</sup>
	4	47.5	14.6	62.3
dried sample	25	29.8	23.3	78.2
	4	8.9	77.9	93.4

<sup>a</sup> The value after 2 months.

Because of the susceptibility of *Amaranthus* betacyanins to the above factors, a storage experiment on aqueous and dry pigments from seven *Amaranthus* species was conducted in the dark, in the absence of air, and at 25 and 4 °C for 10 months. Regression of  $\ln(\text{pigment retention } \%)$  versus storage time was linear with negative slope when plotted on a natural logarithmic scale, indicating the degradation of the pigments also followed first-order kinetics (Figure 2). The dried *Amaranthus* pigments clearly had much higher storage stability than the aqueous pigments (Table 3). After 10 months of storage, the dried samples had very high pigment retention of 93.4% at 4 °C and 78.2% at 25 °C, whereas the aqueous extract had 62.3% at 4 °C and, notably, only 18.3% at 25 °C after only 2 months of storage. The dried samples had much a lower rate constant value ( $k$ ) and longer half-life period ( $t_{1/2} = 77.9$  months at 4 °C and 23.3 months at 25 °C) than the aqueous extract ( $t_{1/2} = 14.6$  months at 4 °C and 1.0 months at 25 °C).

Compared to previous studies by von Elbe et al. (1974) and Shen and Hwang (1985), who reported the half-life values for aqueous betanin ( $t_{1/2} = 19.2$  h at 25 °C) and aqueous amaranthine from *A. tricolor* ( $t_{1/2} = 18.5$  h at 25 °C and 29 days at 4 °C), our results were encouraging. In general, these were that (1) water activity is the most important factor for the storage stability of betacyanins in the dark, in the absence of air at any temperature; and (2) dried *Amaranthus* pigments are stable enough for use as commercial colorants. The great differences for stability of aqueous betacyanins between our experiment and previous studies may have resulted from different control of the storage conditions as well as differences in storage stability between *Amaranthus* betacyanin pigments from *A. cruentus* and other betacyanins.

**Stability Differences of Pigments from Different Genotypes.** In our studies, significant differences in pigment stability among genotypes were observed at

14–25 °C. At higher or very low temperature the stability differences among genotypes became smaller. The stability of the pigments at 80–100 °C was similarly low and below 4 °C similarly high for all genotypes. A stability comparison experiment was carried out in aqueous solution, in the dark, in the absence of air at 14 °C for 20 weeks. The pigment retention differences for betacyanin pigments from 21 genotypes (Table 1) showed that many genotypes retained good stability under the designed conditions, and there were significant differences among genotypes. Pigment retention varied between 48.6 and 73.4%, averaging 63.1%. The highest pigment retentions were shown by Cr015 (73.4%), Sheng07 (70.2%), Tr010 (69.8%), Cr072 (69.6%), and Cr017 (69.5%), which were near to control “red radish” (71.5%), identified as an acylated anthocyanin with better stability (Giusti and Wrolstad, 1996). Average pigment retention values of *A. caudatus* (66.4%), *A. cruentus* (64.3%), and *A. tricolor* (63.2%) were higher than those of *A. hybridus* (61.7%), *A. paniculatus* (61.9%), *A. hypochondriacus* (59.6%), and *A. lividus* (48.6%), indicating differences in pigment stability among species.

The probable causes of the stability differences in this study likely include the following: (1) Real stability differences between and within species might result from differences in molecular structure of the betacyanins from different genotypes. Many authors reported acylation of the anthocyanin molecules could improve the stability of anthocyanins in some plants, such as *Tradescantia pallida* (Shi et al., 1992), *Zebrina pendula*, and *Ipomoea tricolor* (Teh and Francis, 1988). Early studies also found acylated betacyanins in plants of the order Centrospermae, including *Amaranthus* species (Minale et al., 1966). Structure differences in acyl group substitutions of the betacyanins may cause the variation in the stability of the betacyanin pigments from different *Amaranthus* species or genotypes. (2) Differences in content of betacyanin pigments exist among genotypes. The extent of betacyanin degradation and regeneration depended not only on temperature and pH but also on the initial betacyanin concentration for red beet. As the initial betacyanin concentration increased, so did the color stability (von Elbe et al., 1981). Pigment retention (Table 1) was positively correlated ( $r = 0.55$ ,  $p < 0.05$ ) with betacyanin content of dried extracts from 21 genotypes. (3) Differences in harvest time or extraction time of pigments for each genotypes may result in differences in degradation and composition of the betacyanin pigments. (4) Differences in other constituents (polyphenols, antioxidants, etc.) among genotypes may

protect or leave open to attack betacyanin pigments. Further investigation of the basis of the stability differences between and within *Amaranthus* species is certainly warranted.

In summary, the results showed that the betacyanin pigments from most of the *Amaranthus* genotypes tested presented bright red-violet color characteristics and favorable stability in solution at low temperatures in the dark and in the absence of air over the pH range 5–7. The dried pigments were very stable at room temperature for long-term storage. Genotypic differences in the betacyanin stability of *Amaranthus* species were substantial. These results support the potential development of new natural colorants with stability comparable to or better than that of red radish anthocyanins.

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