Chemical Stability and Colorant Properties of Betaxanthin Pigments from *Celosia argentea*

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The chemical stability and colorant properties of three betaxanthins recently identified from *Celosia argentea* varieties were evaluated. Lyophilized betaxanthin powders from yellow inflorescences of *Celosia* exhibited bright yellow color and high color purity with strong hygroscopicity. The aqueous solutions containing these betaxanthins were bright yellow in the pH range 2.2−7.0, and they were most stable at pH 5.5. The betaxanthins in a model system (buffer) were susceptible to heat, and found to be as unstable as red betacyanins (betanin and amaranthine) at high temperatures (>40 °C), but more stable at 40 °C with the exclusion of light and air. The three betaxanthins had slightly higher pigment retention than amaranthine/isoamaranthine in crude extracts at 22 °C, as verified by HPLC analysis. Lyophilized betaxanthins had much better storage stability (mean 95.0% pigment retention) than corresponding aqueous solutions (14.8%) at 22 °C after 20 weeks. Refrigeration (4 °C) significantly increased pigment retention of aqueous betaxanthins to 75.5%.

Keywords: *Celosia; betaxanthins; betalains; yellow pigments; colorant properties; stability*

INTRODUCTION

Considerable interest exists in the development of new natural colorants for use in the food industry (*1*). Water-soluble natural yellow pigments as food colorants are scarce because of their limited sources and poor stability. Betalains, the red-violet betacyanins and the yellow betaxanthins, are characteristic water-soluble pigments occurring in plants of most families of the plant order Caryophyllales (with the exception of Caryophyllaceae and Moluginaceae) and in some higher fungi (*2, 3*). Betaxanthins are the conjugation products of betalamic acid with different amino acids or amines. They may be used as a means of introducing essential dietary amino acids into foodstuffs, giving rise to "essential dietary colorants" (*4*). There is much less knowledge and research on yellow betaxanthins than on red-violet betacyanins (5, 6). Betaxanthins were not reported previously in plants of the family Amaranthaceae (*7*).

The plant genus *Celosia*, consisting of about 60 species in the family Amaranthaceae (Caryophyllales), is native to subtropical and temperate zones of Africa, South America, and South East Asia. *Celosia argentea* var. *cristata* (L.) Kuntze, (*Celosia cristata* L.) (common cockscomb), and *Celosia argentea* var. *plumosa* (Burvenich) Voss, (*Celosia plumosa* Burvenich) (feathered amaranth), are widespread ornamental plants. Seedlings, young leaves, and inflorescences are used as vegetables in China and other countries (*8*), and dried leaves, inflorescences, and seeds are used in traditional Chinese medicine (*9*). Lee et al. (*10, 11*) evaluated colorant properties and stability of betacyanins from red cockscomb flowers (*Celosia cristata*). Their results demonstrated that the red pigments had potential value as a food colorant under selected conditions.

The stability of betalains is generally influenced by pH, temperature, oxygen, light, and water activity (*5, 12*). The majority of work to date has focused on the effects of these various factors on red betacyanins. Less research has been conducted on the effects of these factors on yellow betaxanthins. Savolainen and Kuusi (*13*), Saguy (*14*), and Singer and von Elbe (*15*) reported thermostability and degradation rates of vulgaxanthin-I, a yellow betaxanthin isolated from yellow (golden) beets. The yellow vulgaxanthin-I was not as stable as the red betanin.

Since 1996, research has been performed in our laboratory on the development and application of betalains (especially *Amaranthus* betacyanins) from plants of the Amaranthaceae (*16*−*18*). Some yellow/orange-red leaf or inflorescence genotypes have been screened from the U.S. Department of Agriculture (USDA) and Chinese national and provincial collections. Very recently the betaxanthins from *Celosia argentea* varieties were isolated and characterized as immonium conjugates of betalamic acid with dopamine (miraxanthin V or dopamine−betaxanthin, B_1), 3-methoxytyramine (3-methoxytyramine−betaxanthin, B_2) and (*S*)-tryptophan [(*S*)-tryptophan−betaxanthin, B_3] (Figure 1), besides the known betalamic acid and amaranthine/isoamaranthine (A_1/A_2) (*19*). This was the first report that the three betaxanthins (B_1−B_3) occur in the Amaranthaceae; furthermore, the latter two (B_2 and B_3) are new natural compounds. The objective of this study, therefore, was to determine the colorant properties and stability of *Celosia* betaxanthins as a potential new source of water-soluble yellow colorants.

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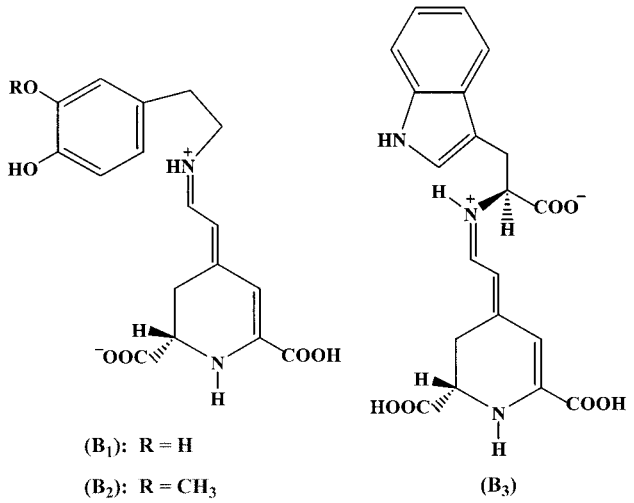

Figure 1. Structures of miraxanthin V (dopamine−betaxanthin) (B_1), 3-methoxytyramine−betaxanthin (B_2), and (S)-tryptophan−betaxanthin (B_3).

MATERIALS AND METHODS

Materials. Four yellow-colored or orange-red-colored inflorescence genotypes of *Celosia argentea* var. *plumosa* and var. *cristata* were selected from the genetic resource collections of the USDA Agricultural Research Service (ARS) at Iowa State University, and the Hubei Academy of Agricultural Sciences, Wuhan, China. All genotypes were planted in Wuhan in 1999 and 2000.

Extraction, Purification, and Drying of Pigments. Samples of fresh inflorescences were taken at full-bloom period, frozen at −18 °C, ground and homogenized in a frozen mortar, and extracted with 80% aqueous MeOH until no further color was removed. The combined extracts were filtered by a Millipore filter (Millipore Corp., Bedford, MA) with a 0.2-μM nylon membrane under vacuum at 22 °C, and centrifuged at 20000*g* for 15 min at 4 °C. The supernatant was concentrated under vacuum at 22 °C to yield crude pigment extracts. The extracts were transferred onto a Sephadex LH-20 column (100 × 2.5 cm i.d.) (Sigma Co., St. Louis, MO) and separated by elution with water adjusted to pH 5−6 with formic acid. Three visible yellow betaxanthin fractions were collected. They were then lyophilized in a Heto FD3 freeze-dryer (Heto-Holten A/S, Denmark) and yielded yellow powders of partially purified pigments. Crude extracts and yellow powders were stored at −18 °C and 4 °C, respectively.

HPLC. HPLC analysis was carried out by means of a Hewlett-Packard 1100 Series HPLC System equipped with a HP 1100 photodiode-array detector and a data processor (Hewlett-Packard HPLC^{2D} ChemStation Software). The chromatographic column was a 5-μm Nucleosil 100-C18 column (250 × 4 mm i.d.) with a 5-μm Nucleosil 5 C18 guard column (4 × 4 mm) (Agilent Technologies, Palo Alto, CA). Stepwise linear gradient elution was performed within 30 min as described previously (*20*) with minor modification: from 100% solvent A (55 mM NaH_2PO_4, 2.5 mM triethylamine, pH adjusted to 4.5 with H_3PO_4) and 0% solvent B (40% acetonitrile) to 70% A and 30% B in 15 min; to 40% A and 60% B in 5 min; to 20% A and 80% B in 5 min; followed by isocratic elution of 20% A and 80% B for a further 5 min at a flow rate of 1 mL/min, with a 20-μL injection with multiwavelength detection (280, 424, 480, and 538 nm).

Measurements of Physicochemical Parameters. UV/Vis spectra were obtained by HPLC−photodiode array detection (HPLC−DAD, Hewlett-Packard) with the chromatographic conditions described above. Color characteristics were measured using a Chroma Meter CR-301 colorimeter (Minolta Co., Osaka, Japan). Pigment powder color was determined through the surface of plastic bags filled with experimental samples, and pigment solution color was determined through the bottom of cylindrical optical cells (30 mm i.d., 35 mm in height). Color traits were expressed as tristimulus parameters [L^*, a^*, b^*, C (chroma), $H°$(hue angle)] as described in our previous publication (*16*). All measurements were conducted in triplicate and averaged.

For the effect of different pH on the pigments, spectra were measured with a spectrophotometer (Spectronic Genesys 5, Milton Roy, NY) in duplicate. Absorbance spectra of the samples in 1.0-cm path length quartz cuvettes were recorded from 200 to 700 nm. Partially purified dried pigment powders were dissolved in different McIlvaine's buffer (pH 2.2∼7.0) and Clark-Lubs' buffer (pH 9.2) to prepare pigment solutions ($A_{\lambda max} \approx 0.8$). The solution samples were kept at 22 °C in the dark for 24 h prior to determining their spectral properties and color parameters in triplicate.

For hygroscopicity, 2 g of yellow pigment powder samples from *C. plumosa* inflorescences were placed into Petri dishes at 22 °C in an airtight plastic container (39 × 23 × 18 cm, L × W × H) filled with a saturated solution of Na_2SO_4 (81% RH) for one week. Maltodextrin 15 DE (20%) was added into the purified aqueous betaxanthin solution prior to lyophilization. Comparison of hygroscopicity between the betaxanthin powders without and with maltodextrin was conducted. Hygroscopicity (hygroscopic moisture) was measured in duplicate and expressed as g of moisture per 100 g dry solids (g/100 g) (*18*).

Evaluation of Thermal and Storage Stability. Crude pigment extracts and betaxanthin solutions from an orange-red genotype of *C. plumosa* were tested at three temperatures (40, 60, and 80 °C), and results on thermal and storage stability were compared to those of standards, i.e., amaranthine and betanin from *A. tricolor* pigment extracts and red beet powders (no. 3600, Warner-Jenkinson Co., Inc., St. Louis, MO), respectively. Betaxanthins, amaranthine, and betanin were partially purified by Sephadex LH-20 column chromatography. The sample solutions (pigment concentration ≈ 400 mg/L, pH 5.3−5.5) were distributed into 50-mL glass tubes sealed with screw caps and placed in a water bath for the required heat treatment. The tubes were wrapped with aluminum foil to avoid exposure to light. Pigment content and retention were determined in triplicate for each sample 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 our previous method (*16*) using regression analysis of *ln* (pigment retention %) versus heating time.

For the storage experiment, partially purified betaxanthin solutions and dried powders from the yellow genotype of *C. plumosa* were filled into 70-mL plastic cups sealed with screw caps or in 2.5-mL vials with caps sealed in a plastic bag, respectively. These samples were kept in the dark in a refrigerator (4 °C) and at room temperature (∼22 °C) for 20 weeks. Betaxanthin storage stability, expressed as pigment retention (%), was investigated by photometric measurement, in triplicate, of the pigment content of each sample before and after storage. Dried pigment powders were accurately weighed and dissolved in pH 5.5 buffer to determine pigment concentration. However, the crude pigment extracts from yellow and orange-red genotypes of *C. plumosa* and *C. cristata* were placed in 70-mL plastic cups sealed with screw caps and stored only at 22 °C in the dark to observe the change of betalain HPLC profiles during 5 week storage.

Determination of Betalain Content and Pigment Retention. Betalain content was measured at 474 nm for betaxanthins and 538 nm for betacyanins using a Spectronic Genesys 5 spectrophotometer (Milton Roy, NY). Duplicate measurements were used for each sample. Betalain content was expressed as mg of betaxanthin or betacyanin per liter and calculated according to Cai and Corke (*7*) and Cai et al. (*17*). The mean molar absorptivity (ϵ) value for betaxanthins is 4.80×10^4 (*21*), for amaranthine is 5.66×10^4 (*22*), and for betanin is 6.16×10^4 (*23*). Pigment retention (percent) was calculated as follows: (betalain content at X storage time) × 10^2/(betalain content at zero storage time).

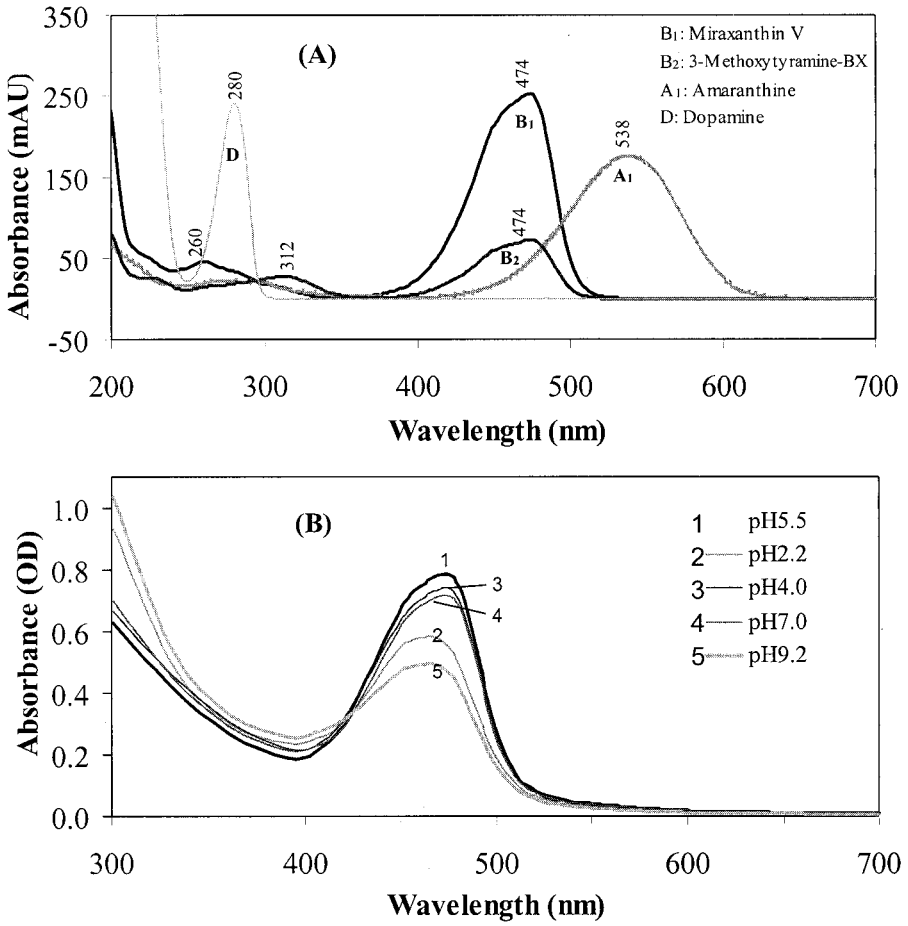

Figure 2. UV/Vis spectra of (A) *Celosia* betalains isolated and measured by HPLC−DAD, and (B) *Celosia* betaxanthins isolated by Sephadex LH-20 column chromatography and measured spectrophotometrically at different pH values after 24 h of storage at 22 °C.

RESULTS AND DISCUSSION

Physicochemical Characteristics and pH Effect. Three betaxanthins, i.e., miraxanthin V (B_1), 3-methoxytyramine−betaxanthin (B_2), and (*S*)-tryptophan−betaxanthin (B_3), were recently identified from *C. plumosa* and *C. cristata* (*19*). The UV/Vis spectral analysis of the betaxanthins by HPLC−DAD (Figure 2A) resulted in absorption maxima (λ_{max}) at 260 and 474 nm for B_1, at 312 and 474 nm for B_2, and at 268 and 478 nm for B_3 (not shown in Figure 2A), respectively. The UV/Vis spectra of B_1, B_2, and B_3 are typical for betaxanthins, but clearly different from that of amaranthine (A_1, λ_{max} 538 nm). Figure 2B shows the effect of pH 2.2−9.2 on spectra of yellow betaxanthins at 22 °C after 24 h of storage. The results indicated that betaxanthin solutions had high stability in the pH range 4.0−7.0 (the pH range of most foods). The optimal pH for maximum stability of the betaxanthins was 5.5. To some extent, the results were similar to those for betacyanins (amaranthine and betanin) reported by Cai et al. (*16*) and von Elbe et al. (*24*).

Lyophilized betaxanthin powders of *Celosia* inflorescences were water-soluble with high tinctorial strength and strong hygroscopicity. Purified betaxanthin powders absorbed moisture easily and rapidly with quite high hygroscopicity (115.4 g/100 g). Adding carrier agents (maltodextrins) into the purified betaxanthins could significantly reduce the hygroscopicity (42.6 g/100 g) and may be helpful for betaxanthin pigment storage, in accordance with the results for betacyanins (amaranthine) (*18*). The color traits of purified betaxanthins (both powders and solutions) were similar between two yellow genotypes of *Celosia* (*C. plumosa* and *C. cristata*). The color parameters in Table 1 indicated that, for the two yellow genotypes, betaxanthin powders had brighter yellow color (mean $H°$= 83.0), higher color purity (C = 43.2), and greater lightness (L^* = 59.1), while betaxanthins in aqueous solution ($A_{474} \approx 0.8$) also presented bright yellow color (mean $H°$ = 100.4, C = 12.6, and L^* = 26.3). Compared to the purified betaxanthins from the two yellow *Celosia* genotypes, crude betalain extracts from the two orange-red *Celosia* genotypes had lower $H°$, C, and L^* values (indicating a more orange shade of red, a lower color intensity, and a darker color) for both powders (mean $H°$ = 33, C = 29.0, and L^* = 49.9) and solutions ($H°$ = 47.3, C = 6.3, and L^* = 23.2). This was because the crude pigments from the orange-red genotypes contained much more nonpigment components and possessed a lower ratio of betaxanthins to betacyanins (~0.62:0.38 estimated by HPLC) than the yellow genotypes (almost 100% betaxanthins). Moreover, the differences in color parameters between the two orange-red genotypes (*C. plumosa* displayed a more orange shade of red than *C. cristata*) (Table 1) also resulted from the differences in the ratio of betaxanthins to betacyanins (~0.66:0.34 for *C. plumosa* and ~0.57:0.43 for *C. cristata*).

The hue angle ($H°$) values of betaxanthin solutions ranged between 95.2 and 98.1 (yellow color) in pH 2.2−7.0, but $H°$ was 215.7 (dark brown color) at pH 9.2

Table 1. Color Characteristics and Storage Stability of Pigment Powders and Solutions from Inflorescences of Four Genotypes in Two *Celosia* Varieties

genotype	form[a]	L^* (±SD)	a^* (±SD)	b^* (±SD)	C (±SD)	H^o (±SD)	pigment retention (%) (±SD)[b] 4 °C	22 °C
Celosia argentea var. *plumosa* (Burvenich) Voss								
yellow	powder	59.8 ± 0.42	5.2 ± 0.13	43.0 ± 0.15	43.3 ± 0.15	83.2 ± 0.12	99.3 ± 0.8	95.6 ± 1.8
	solution	26.7 ± 0.01	−2.2 ± 0.15	12.5 ± 0.19	12.7 ± 0.20	99.8 ± 0.78	76.2 ± 1.3	12.5 ± 2.7
orange-red [c]	powder	50.3 ± 0.50	23.7 ± 0.24	18.2 ± 0.13	29.8 ± 0.21	37.5 ± 0.23	-	-
	solution	23.8 ± 0.02	4.1 ± 0.03	5.2 ± 0.02	6.6 ± 0.06	51.9 ± 0.12	-	-
Celosia argentea var. *cristata* (L.) Kuntze								
yellow	powder	58.4 ± 0.79	5.4 ± 0.16	42.6 ± 0.38	43.0 ± 0.42	82.8 ± 0.17	98.5 ± 1.2	94.3 ± 0.9
	solution	25.8 ± 0.57	−2.4 ± 0.19	12.2 ± 0.27	12.4 ± 0.23	101.0 ± 1.06	74.8 ± 1.5	17.1 ± 1.6
orange-red [c]	powder	49.5 ± 0.43	23.9 ± 0.57	13.0 ± 0.27	28.2 ± 1.32	28.5 ± 0.66	-	-
	solution	22.6 ± 0.26	4.4 ± 0.14	4.0 ± 0.06	5.9 ± 0.06	42.6 ± 1.14	-	-

[a] Pigment solutions were prepared in a model drink (McIlvaine's buffer, pH 5.5) using pigment powders. Color parameters were measured immediately after preparation of the solutions ($A_{\lambda max} \approx 0.8$). [b] Pigment retention (%) was determined after 20 weeks of storage, except the solution samples at 22 °C which were determined just at 10 weeks. SD = standard deviation. [c] Crude pigment extracts were not purified by Sephadex LH-20 column chromatography, and were directly lyophilized, yielding pigment powders.

Table 2. Effect of pH on Color Characteristics of Betaxanthin Solutions[a]

pH	L^* (±SD)	$H°$(±SD)	C (±SD)
2.2	22.1 ± 0.21	95.2 ± 0.55	5.7 ± 0.12
4.0	22.4 ± 0.14	97.6 ± 1.15	7.9 ± 0.13
5.5	21.7 ± 0.12	96.6 ± 0.12	8.8 ± 0.14
7.0	20.6 ± 0.19	98.1 ± 1.75	7.1 ± 0.09
9.2	17.1 ± 0.24	215.7 ± 3.67	1.9 ± 0.03

[a] Betaxanthins from inflorescences of a yellow genotype of *C. plumosa*. Color parameters were tested after the pigment solutions ($A_{474nm} \approx 0.8$) were stored for 24 h at 22 °C. Betacyanin (amaranthine) solution (pH 5.5, $A_{538nm} = 0.85$) as a standard: $L^* = 19.4 \pm 0.02$, $H° = -1.4 \pm 0.12$, and $C = 7.9 \pm 0.09$.

(Table 2). Lightness (L^*) trended downward as pH increased. Chroma (C) had higher values in pH 4.0−7.0 than outside this pH range (especially >7.0). The results revealed that betaxanthin solutions were relatively unaffected by pH, and unchanged in color and more stable at pH 4.0−7.0, unlike the anthocyanins in which color changed markedly with pH (*6*). In this study, it was found that alkaline pH resulted in betaxanthin hydrolysis into betalamic acid and amino acids/amines (lowest L^* and C at pH 9.2), similar to betacyanins (*25*). However, it is not yet known how betaxanthins degrade in lower pH values (<4.0). It is assumed that pH <4.0 may also cause betaxanthin degradation into betalamic acid. Betalamic acid and amino acids/amines may regenerate betaxanthins in acidic pH (see last section). To a certain extent, this perhaps explains why betaxanthins exhibited yellow color in the wider pH range (2.2−7.0), which is slightly different from the betacyanins in which red color was unchanged only in the pH range of 4.0−7.0 (*6, 16*). Although this was so, the color intensity (lower C and higher L^*) and optical density (OD) of betaxanthins at pH 2.2 decreased clearly, as compared with those of pH 4.0−7.0 (Table 2 and Figure 2B).

Storage Stability. The changes in betalain HPLC profiles (Figure 3) of crude extracts from yellow and orange-red genotypes of *C. plumosa* during storage (at 0 week and 5 weeks) at 22 °C reflected pigment stability and degradation patterns. The betalain pigments were characterized, and peak assignments were done based on our previous data (*19*). Five betalain pigments (three betaxanthins, B_1, B_2, and B_3; two betacyanins, A_1 and A_2) were isolated in orange-red inflorescences of *C. plumosa* (Figure 3A), and three betalain pigments (only betaxanthins, B_1, B_2, and B_3) were isolated in yellow inflorescences of *C. plumosa* (Figure 3B). The total betalain content decreased with storage, as shown by reduction in total peak area at 480 nm (Figure 3), indicating that storage caused betalain losses and degradation, which were due to water activity (solution state) and storage temperature (22 °C) (see next section). For the yellow genotype, after 5 weeks the three betaxanthin peaks B_1, B_2, and B_3 decreased 41.4%, 44.5%, and 41.1% in total peak area, respectively. For the orange-red genotype, after 5 weeks the three betaxanthin peaks B_1, B_2, and B_3 reduced 47.7%, 48.7%, and 38.8%, respectively, whereas the two betacyanin peaks A_1 and A_2 decreased 35.7% and 32.2% in total peak area, respectively. Thus, A_1 and A_2 decreased an average of 9.8% more than B_1, B_2, and B_3 in total peak area for the two genotypes, indicating that B_1, B_2, and B_3 seemed to have better storage stability than A_1 and A_2 in this study. In addition, crude pigment extracts from the two genotypes gave rise to more betalamic acid after 5 weeks storage than at 0 week storage (Figure 3). Betalamic acid was reported as one of the key intermediates for betacyanin degradation (*5, 25*). Thus, betalamic acid should also be one of the intermediates of the betaxanthin degradation. The betalamic acid peak in Figure 3 (detected at 480 nm) appears smaller because of its $\lambda_{max} = 424$ nm (not shown).

Pigment retention in the betaxanthin solutions and dried powders (purified by Sephadex LH-20 column chromatography) from yellow genotypes of *C. plumosa* and *C. cristata* stored at room temperature (~22 °C) and at 4 °C was tested for 20 weeks. It was found that there were not obvious differences in pigment retention between *C. plumosa* and *C. cristata* genotypes in the same storage conditions. Dried betaxanthin powders had much higher pigment retention (mean 96.9%) than betaxanthin solutions (45.2%) at two storage temperatures (4 and 22 °C), whereas 22 °C resulted in lower pigment retention (mean 54.9%) than 4 °C (87.2%) in two states (dried powder and solution) (Table 1). Interestingly, the dried powders of the inflorescences of two yellow genotypes had similarly high pigment retention at 4 °C (mean 98.9%) and 22 °C (95.0%) after 20 weeks, whereas the betaxanthin solutions had lower pigment retention (mean 75.6%) at 4 °C but much higher than at 22 °C (14.8%) (Table 1). That means lower water activity (dried state) caused slower degradation of betaxanthins even at higher storage temperature, but higher water activity (solution state) resulted in faster

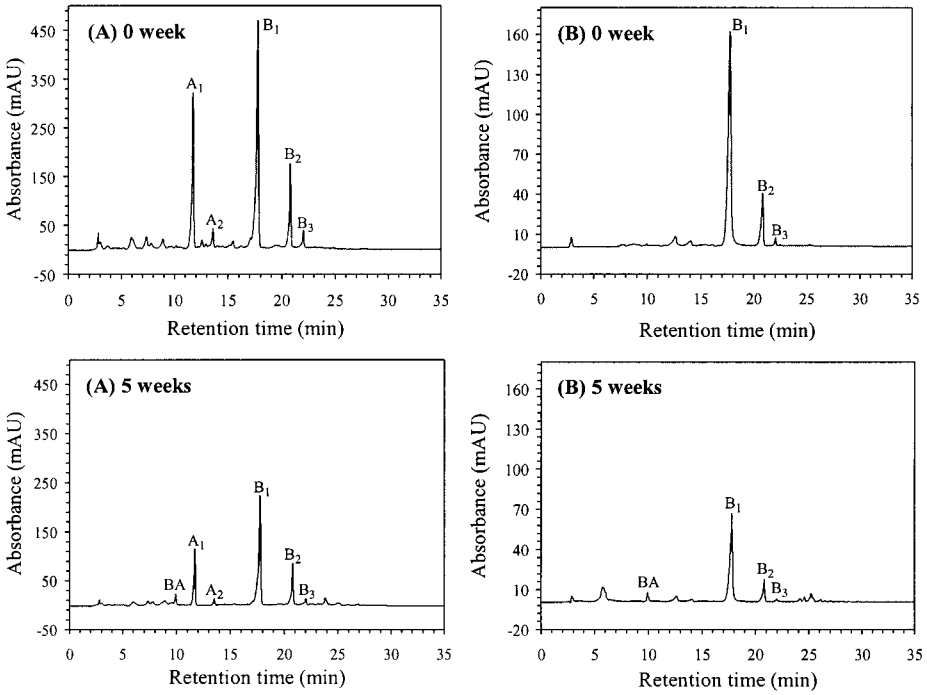

Figure 3. Changes in HPLC profiles of betalain extracts from (A) orange-red and (B) yellow inflorescences of *C. plumosa* before storage (0 week) and after storage (5 weeks) at room temperature (22 °C). Detection was performed at 480 nm. Peak B_1, miraxanthin V; B_2, 3-methoxytyramine−betaxanthin; and B_3, (*S*)-tryptophan−betaxanthin. Peaks A_1, amaranthine; and A_2, isoamaranthine. Peak BA, betalamic acid.

Table 3. Thermal Stability of Betaxanthins and Crude Extracts from Inflorescences of an Orange-Red Genotype of *C. plumosa*

	40 °C [b]			60 °C [b]			80 °C [b]		
betalains[a]	R^2	$k \times 10^2$ (h^{-1})	$t_{1/2}$ (h)	R^2	$k \times 10^2$ (h^{-1})	$t_{1/2}$ (h)	R^2	k (h^{-1})	$t_{1/2}$ (h)
betaxanthins	0.98	1.97 ± 0.53	35.2	0.96	7.26 ± 0.24	9.6	0.92	0.97 ± 0.04	0.71
amaranthine	0.83	2.82 ± 1.05	24.6	0.91	6.53 ± 0.61	10.6	0.95	1.02 ± 0.09	0.68
betanin	0.87	3.12 ± 0.98	22.2	0.92	7.31 ± 0.75	9.5	0.93	0.95 ± 0.07	0.73
crude extracts	0.97	1.70 ± 0.22	40.7	0.93	5.36 ± 0.84	12.9	0.97	0.59 ± 0.05	1.18

[a] Crude extracts contained three betaxanthins (B_1, B_2, and B_3) and two betacyanins (A_1 and A_2) (see Figure 3A). Betaxanthins (B_1, B_2, and B_3) were partially purified from crude extracts. Amaranthine and betanin, as standards, were from *A. tricolor* and red beet powders (no. 3600), respectively. [b] R^2 = R-squared value; k = rate constant (degradation rate); $t_{1/2}$ = half-life time.

degradation of betaxanthins even at lower storage temperature. The results revealed that water activity had a more significant effect on storage stability of betaxanthins than storage temperature. In addition, it was found that the purified betaxanthin solutions (~28% after 5 weeks) seemed to be less stable than crude betaxanthin extracts (> 40% after 5 weeks estimated by HPLC as above) at 22 °C storage.

Thermal Stability. Thermal stability testing of the betaxanthins from an orange-red genotype of *C. plumosa* was carried out at three temperatures (40, 60, and 80 °C) with the exclusion of light and air, as compared to amaranthine and betanin (Table 3). Attoe and von Elbe (*26*) and Huang and von Elbe (*25*) reported that the thermal degradation of betalains deviated from first-order kinetics in the absence of oxygen. However, in this study, when the percentage of pigment retention versus heating time was plotted on a natural logarithmic scale, it still followed a first-order reaction. This was probably due to a different extent of control of oxygen (air or nitrogen) between various studies. Our result was similar to those of some previous studies (*14*, *16*, *24*, *27*) that showed it to follow first-order kinetics. The degradation rate and stability was expressed in terms of the rate constant (k) (higher values of k mean greater degradation speed) and half-life time ($t_{1/2}$). As temperature increased, the degradation of the betaxanthins accelerated, with a k of 1.97×10^{-2}/hr and a $t_{1/2}$ of 35.2 h at 40 °C and a k of 0.97/hr and a $t_{1/2}$ of 0.71 h at 80 °C (Table 3), indicating *Celosia* betaxanthins are susceptible to temperature. Thermal stability between *Celosia* betaxanthins and other betacyanins (amaranthine and betanin) were also compared in the study. Data in Table 3 showed that the betaxanthins ($t_{1/2}$ = 35.2 h) were more stable than amaranthine ($t_{1/2}$ = 24.6 h) and betanin ($t_{1/2}$ = 22.2 h) at lower temperature (40 °C). However, higher temperatures (60 and 80 °C) made betaxanthins, amaranthine, and betanin degrade rapidly and their differences in k and $t_{1/2}$ values were small and not significant, indicating all of them were unstable at higher temperatures. The findings under our experimental conditions differ from those of two previous publications on betaxanthin stability (*14*, *15*). These reported that a betaxanthin from yellow beets, vulgaxanthin-I, was much less stable than betanin in an oxygen-free system from 61.5 to 100 °C. In our study, the betaxanthins (B_1, B_2, and B_3) from *Celosia* show higher stability than amaranthine and betanin at 40 °C and had similar stability at 60 and 80 °C. HPLC analysis provided a direct proof that B_1, B_2, and B_3 were slightly more stable than amaranthine (A_1) and isoamaranthine (A_2) at 22 °C. It was also observed in our experiment that the beta-

xanthins, like other betalains, were sensitive to oxygen and light which obviously influenced stability, but a systematic study was not conducted in comparison to amaranthine and betanin. Moreover, we observed that crude extracts from an orange-red genotype of *C. plumosa* gave lower k and higher $t_{1/2}$ values than purified betaxanthins, amaranthine, and betanin at all three temperatures (Table 3). The result was similar to the above-mentioned result of the purified betaxanthins and crude extracts at 22 °C storage. This might be due to a protective effect on betaxanthins conferred by other natural components (e.g., polyphenols and antioxidants) in crude extracts. Singer and von Elbe (*15*) and Cai et al. (*16*) gave similar explanations in their betalain studies.

The mechanism of betaxanthin degradation has not been elucidated so far. Betanin and amaranthine are known to degrade into betalamic acid and *cyclo*-dopa 5-*O*-glucoside (*28*) as well as betalamic acid and *cyclo*-dopa 5-*O*-(2′β-glucuronosyl)-glucoside (*25*), respectively. In both natural and model systems, betanin and amaranthine may be regenerated via Schiff's base condensation of betalamic acid with *cyclo*-dopa 5-*O*-glucoside and *cyclo*-dopa 5-*O*-(2′β-glucuronosyl)-glucoside. This reaction may also occur in betaxanthin solution and is probably partially reversible. HPLC analysis confirmed this degradation process. Betalamic acid and amino acid/amines (tryptophan, dopamine, and 3-methoxytyramine) in the degraded products of the betaxanthins (B_1, B_2, and B_3) could be detected, respectively, at 424 and 280 nm by HPLC. Conversely, the regeneration of the betaxanthins was also observed under some controlled conditions (e.g., acidic pH), but not investigated thoroughly in this study. In addition, BA may further degrade into brown substances at high temperature (*27*). It is not yet known if there are other degradation pathways.

The differences for stability parameters (k and $t_{1/2}$) of betalains among different studies (*13*−*16*, *24*, *25*, *29*) are significant. These might result from the following: (1) real stability differences between various betalains with different molecular structures; (2) different control of the reaction and storage conditions (temperature, oxygen, light, water activity, pH); and, (3) different systems (natural or model, copigmentation, initial concentration, regeneration, etc.). Therefore, special care is required when data in different reports are compared or cited. Moreover, it was found that crude betaxanthin extracts and partially purified betaxanthin fractions from yellow inflorescences of *Celosia* contained large quantities of dopamine [41.2 μmol/g fr. wt (*C. cristata*)] (*19*). Figure 2A shows the spectrum of dopamine (λ_{max} = 280 nm) by HPLC−DAD. It is necessary to investigate, prior to application of *Celosia* betaxanthin mixtures in the food industry, if its dopamine content has unwanted pharmacological effects.

In conclusion, the novel betaxanthins identified from *Celosia* species in the family Amaranthaceae exhibited bright yellow color characteristics and favorable storage and thermal stability under controlled conditions, as compared to those of well-known red betacyanins from red beets and *Amaranthus* plants, suggesting that they may become a potential new source of water-soluble yellow colorants for use in food processing at low temperature.

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