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Influence of *Amaranthus* Betacyanin Pigments on the Physical Properties and Color of Wheat Flours

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The effect of betacyanin pigments from *Amaranthus tricolor* and *Amaranthus cruentus* on chromatic and physicochemical properties of three wheat flours was studied. Addition of *Amaranthus* betacyanins increased the gelatinization temperatures (T_o, T_p, and T_c) of all wheat flours without altering their transition ranges (T_c-T_o). The melting enthalpies (ΔH) were either increased or decreased depending on the types of flour and pigment. *Amaranthus* betacyanins decreased the peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), setback (SB), and pasting time (PT) of all flours and increased the breakdown (BD). Texture profile analysis (TPA) showed that *Amaranthus* betacyanins decreased hardness, and gumminess, and increased cohesiveness of all gels, without altering adhesiveness. Chromatic investigation exhibited that *A. tricolor* and *A. cruentus* pigments imparted gels with red and orange−yellow hues with favorable color stability.

KEYWORDS: ***Amaranthus*** **betacyanin pigments; wheat flours; color; texture; pasting; gelatinization**

INTRODUCTION

Natural pigment sources have been investigated as potential replacement for their synthetic counterparts during the last few decades (*1*). Since synthetic food colorants have been much scrutinized due to the consumers' safety concerns and preferences, it is likely that the volume of currently produced ecofriendly natural colorants will be insufficient to meet future demands (*1, 2*). Major traditional natural food colorants are carotenoids, chlorophylls, and anthocyanins (*2*). Betalains, though much less investigated compared to the traditional ones, have been gaining in popularity recently (*3*).

The water-soluble betalains, whose presence in plants is mutually exclusive to anthocyanins phytochemically, occur only in 10 families in the order Caryophyllales, such as Amaranthaceae, Cactaceae, and Chenopodiaceae (*4*−*6*). Chromatically, betalains can cover a broad color range from yellow to purple (e.g., violet-red betacyanins and yellow betaxanthins) and complement the widespread application of anthocyanins for foodstuff coloring (*6*). Compared with anthocyanins, betalains showed a broader pH stability from around pH 2 to 7 (*5*), which is suitable for acid food systems. However, their stability could be significantly affected by different food matrix and composition (*6*). Apart from their chromatic functions, betalains exhibited strong antioxidant, antiradical, and anti-inflammatory activities (*6*). It was proposed that regular use of betalains containing red beet products in the diet might provide protection against certain oxidative stress-related disorders in humans (*7*). Our previous study showed that different betalains from plants in the family Amaranthaceae possessed a range of antioxidant capacity (*8*). Despite the above-mentioned merits and varied botanical sources, only betalains from red beet have been commercialized for food production (*3*).

Amaranth, as an ancient crop with great agricultural significance, has gained in popularity especially in China and is used in food industry, such as *Amaranthus* flours as cake coating (*5*). Some *Amaranthus* genotypes produce a particularly high biomass and contain high levels of betacyanins (mostly amaranthin) (**Figure 1**) (*5*). *Amaranthus* betacyanins, as a promising natural colorant, have great potential for use in the food industry. The stability of *Amaranthus* betacyanins is governed by various factors. It was reported that *Amaranthus* pigments are particularly suited for low temperature use at pH 5.6 and showed different stabilities in different model food systems (*5*). However, most of the studies have been focused on the phytochemistry, isolation, and characterization of betalains instead of using them as food quality enhancers (*6*).

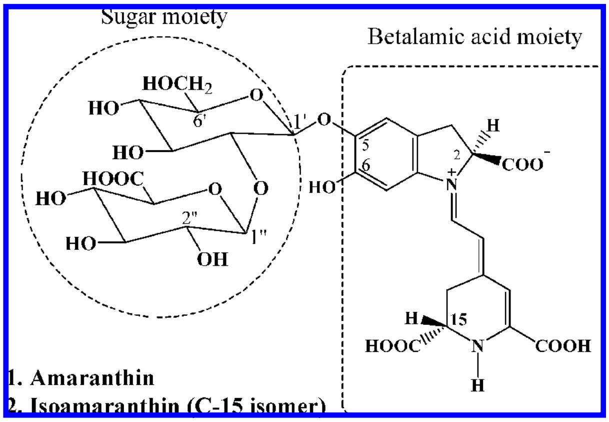

Figure 1. Chemical structures of amaranthin and its isomer (isoamaranthin).

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Wheat flour is one of the most important food ingredients. Besides the composition of its major constituents (starch and protein), its functional properties and the quality of resulting food products can be significantly affected by various additives (*9, 10*). It was reported that different polyhydroxyl compounds could change the gelatinization properties of starch to different extents (*11*). Acid treatment could increase the gelatinization temperatures and change the enthalpies of both gelatinization and retrogradation of various starches (*12, 13*). Use of *Monascus* pigments in bread production significantly changed the mechanical properties, microstructure, and color tones of the crumb and improved the overall sensory qualities of bread (*14*). However, there has been no report on the effects of *Amaranthus* betacyanins on the functional and chromatic properties of wheat flours.

The main purpose of this study was to examine the effects of *Amaranthus* betacyanin pigments from two different species (*A. tricolor* and *A. cruentus*) on the chromatic and functional properties of three commercial wheat flours with different production purposes. This research may provide a basis to expand the applications of betalains as natural quality enhancers of wheat-based foods.

MATERIALS AND METHODS

Wheat Flours. Three commercial wheat flours, i.e., Golden Statue, Red Bicycle, and American Roses, were obtained from Hong Kong Flour Mills Limited (Kowloon, Hong Kong). They differed greatly in chemical composition and recommended use. Golden Statue, high in proteins (13.6 ± 0.2%) and wet gluten index (38−40), was for baked bread production; Red Bicycle, with medium proteins (11.0 ± 0.2%) and wet gluten index (28−31), was for production of Shanghai-style noodles; American Roses, low in proteins (7.8 ± 0.2%) and wet gluten index (20−22), was for production of steamed breads, cakes, and Chinese dumplings.

***Amaranthus* Betacyanin Pigments.** The leaves of two *Amaranthus* genotypes from *A. tricolor* (Tr35) and *A. cruentus* (Cr72) grown in Wuhan, Hubei, China were collected. Pigment extraction followed our previous methods (*15, 16*) with minor modification. Briefly, the collected fresh leaf samples were washed, chilled, cut into small pieces, and extracted, followed by centrifugation, concentration, and freeze-drying. The dried pigment extracts were sealed and stored at 4 °C in the dark until use. The betacyanins in these two pigment extracts were identified as amaranthin and its isomer (*15, 16*). On the basis of spectrophotometer analysis, total betacyanin concentration of *A. tricolor* pigment extract was 86.5% (dry basis), higher than that of *A. cruentus* (75.3%). Their chemical structures are illustrated in **Figure 1**.

Differential Scanning Calorimetry (DSC). Gelatinization of wheat flour−water−pigment mixtures was measured using a TA 2920 Modulated DSC Thermal Analyzer differential scanning calorimeter equipped with a thermal analysis data station (TA Instruments, Newcastle, DE) following Gunaratne and Corke (*13*) with some modifications. Solution of *Amaranthus* betacyanins was prepared by dissolving 10 mg of pigment powder in 1 mL of double deionized water before vortexing to obtain homogeneity. Wheat flour (ca. 2.5 mg, dry basis) was weighed directly into an aluminum DSC pan, and either pure double deionized water or pigment solution (ca. 7.5 μL) (i.e., flour/water (solution) = 1:3, w/v) was added using a microsyringe. Pans were sealed and allowed to stand for 24 h at room temperature (23 °C) for even distribution of water. The scanning temperature range and the heating rates were 30−90 and 10 °C/min, respectively. An empty pan was used as reference for all measurements. All tests were performed in triplicate.

Pasting Properties. The pasting properties of different wheat flour−water−pigment mixtures were studied using a Rapid Visco-Analyzer (RVA) model 3 D (Newport Scientific, Warriewood, NSW, Australia). Suspensions of wheat flour (4 g, dry basis) in pure double deionized water (control) or in double deionized water with addition of pigment powder (150 mg) (a concentration of 0.54% (w/w) for betacyanins pigment) were prepared in the RVA canisters to obtain a total constant sample weight of 28.0 g. The slurry was then quickly manually homogenized using the plastic paddle to avoid lump formation right before the RVA run. A programmed heating and cooling cycle was set for 23 min, where it was first held at 50 °C for 1.0 min, heated to 95 °C in 7.5 min, further held at 95 °C for 5 min, cooled to 50 °C within 7.5 min, and held at 50 °C for another 1 min. The viscosities were presented in Rapid Visco Units (RVU). All tests were conducted at least in duplicate.

Table 1. Characteristic Temperatures [Onset (T_o), Peak (T_p), Conclusion (T_c), and Transition Range (T_c-T_o)] and Enthalpies (ΔH) of the Gelatinization Endotherm of Wheat Flours in the Presence of Betacyanins from *A. tricolor* (Tr35) and *A. cruentus* (Cr72) as Compared with Native Flours in Pure Water[a]

flour	pigment	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	T_c-T_o (°C)
American Roses	control	6.7 A	58.6 A	64.4 A	72.4 A	13.8 A
	Tr35	6.4 B	59.6 B	65.5 B	73.5 B	13.8 A
	Cr72	6.8 A	59.0 A	64.7 AB	72.3 A	13.4 A
Red Bicycle	control	5.0 A	57.4 A	63.9 A	72.3 A	15.0 A
	Tr35	5.5 B	58.7 B	64.9 B	74.1 B	15.4 A
	Cr72	4.9 A	58.3 B	64.4 C	72.4 A	14.2 B
Golden Statue	control	4.5 A	57.3 A	64.0 A	71.6 A	14.3 A
	Tr35	5.0 B	57.7 A	64.8 B	72.9 B	15.3 A
	Cr72	5.1 B	57.3 A	64.1 A	72.3 A	15.0 A

[a] Different upper case letters indicate the significance of difference among mean values within each column of the same flour samples at $p < 0.05$.

Gel Texture Analysis. Gel textural properties were determined on the wheat flour gels made from the RVA testing using a TA-XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, England) under Texture Profile Analysis (TPA) mode. After RVA testing, the paddle was removed immediately, and the flour paste in the canister was covered by Parafilm and stored at 4 °C for 24 h before testing. The testing speed was 1 mm/s to a distance of 10 mm with a 5 mm cylindrical probe. The maximum force peak during the first cycle represented gel hardness, and the negative area of the curve during retraction of the crosshead was termed adhesiveness. Cohesiveness was defined as the ratio of the positive force areas during the second compression to that during the first compression. Gumminess was defined as hardness × cohesiveness. All tests were performed in triplicate.

Color Measurement. Color development and stability during storage for 4 weeks were determined on the wheat flour gels made from the RVA testing. Gels were transferred into screw-capped and transparent cylinder plastic cups, which were sterilized beforehand to prevent microbial growth. Samples were put in the dark at 4 °C. Color was determined through the bottom of the plastic cups with a CR-300 Chroma Meter colorimeter (Minolta Camera Co., Osaka, Japan). Color results were expressed as tristimulus parameters as follows: lightness (L^*, 100 = white and 0 = black), redness−greenness (a^*, positive = red), and yellowness−blueness (b^*, positive = yellow) of the CIE LABORATORY color space (CIE 1986) were analyzed. Chroma ($C^* = (a^{*2} + b^*(2))^{1/2}$, hue angle ($H^o = \tan^{-1}(b^*/a^*)$), and color difference $\Delta E^*\mathrm{ab} = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$ were calculated. All measures were conducted in triplicate.

Statistical Analysis. The results were calculated as the mean. Data were compared by using the least significance difference (LSD) test. Statistical software was the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Thermal Analysis. Addition of betacyanins from *A. tricolor* significantly influenced the gelatinization of all wheat flours to different degrees with a similar trend, whereas the effects of *A. cruentus* were generally marginal (**Table 1**). Among the native wheat flours, melting enthalpy (ΔH) was greatest with American Roses (6.7 J/g) and least with Golden Statue (4.5 J/g). This could be attributed to the different chemical compositions of different flours (*17*), especially the starch present in flours, which largely

Table 2. TPA Textural Properties of Wheat Flour Gels in the Presence of Betacyanins from *A. tricolor* (Tr35) and *A. cruentus* (Cr72) as Compared with Native Flours[a]

flours	pigment	hardness (g)	adhesiveness (g·s)	cohesiveness	gumminess (g)
American Roses	control	29.4 A	61.4 A	0.507 A	14.9 A
	Tr35	20.8 B	74.1 A	0.594 B	12.4 B
	Cr72	13.4 C	36.7 B	0.603 B	8.1 C
Red Bicycle	control	27.6 A	48.1 A	0.536 A	14.8 A
	Tr35	15.6 B	55.9 AB	0.596 B	9.3 B
	Cr72	18.5 C	62.6 B	0.604 B	11.2 C
Golden Statue	control	22.3 A	44.1 A	0.526 A	11.7 A
	Tr35	15.9 B	45.8 A	0.544 A	8.7 B
	Cr72	13.1 C	46.4 A	0.577 A	7.6 B

[a] Different upper case letters indicate the significance of difference among mean values within each column of the same flour samples at $p < 0.05$.

Table 3. Pasting Properties of Wheat Flours in the Presence of Betacyanins from *A. tricolor* (Tr35) and *A. cruentus* (Cr72) as Compared with Native Flours in Pure Water[a]

flours	pigment	PV (RVU)	HPV (RVU)	BD (RVU)	CPV (RVU)	SB (RVU)	PT (min)
American Roses	control	418 A	170 A	247 A	366 A	195 A	8.9 A
	Tr35	394 B	121 B	272 B	261 B	139 B	8.8 A
	Cr72	373 C	92 C	281 B	203 C	111 C	8.7 A
Red Bicycle	control	388 A	194 A	193 A	398 A	203 A	9.4 A
	Tr35	370 AB	130 B	240 B	284 B	153 B	9.3 A
	Cr72	343 B	100 C	243 B	226 C	126 C	9.1 B
Golden Statue	control	357 A	167 A	191 A	347 A	180 A	9.3 A
	Tr35	341 A	123 B	218 B	270 B	147 B	9.2 AB
	Cr72	321 B	102 C	210 B	227 C	125 C	9.1 B

[a] Pasting properties: PT, time from the initial to the peak viscosity; PV, peak viscosity; HPV, hot paste viscosity; BD, breakdown (PV-HPV); CPV, final viscosity; SB, setback (CPV-HPV). Different upper case letters indicate the significance of difference among mean values within each column of the same flour samples at $p < 0.05$.

determines the thermal properties of wheat flour and the quality of some food products (*18*). Considerable difference in gelatinization temperatures [onset (T_o), peak (T_p), conclusion (T_c), and transition range ($T_c - T_o$)] were not observed between the native samples, with the exception of T_o for American Roses, which was significantly higher than that of the other two ($P < 0.05$).

Different *Amaranthus* betacyanins elevated wheat flour gelatinization temperatures (T_o, T_p, and T_c) to various extents. In general, the effect of betacyanins from *A. tricolor* was greater than that from *A. cruentus* and varied among different wheat flours. For example, addition of betacyanins from *A. tricolor* and *A. cruentus* into Red Bicycle increased peak temperatures (T_p) by 1.0 and 0.5 °C, respectively. For Golden Statue, the increases were 0.8 and 0.1 °C, respectively, and the latter was statistically insignificant from the control. The transition ranges ($T_c - T_o$) were maintained except for Red Bicycle, which was narrowed by 0.8 °C.

Amaranthus betacyanins from *A. tricolor* and *A. cruentus* are amaranthin and its isomer as depicted in **Figure 1**. They are the condensation products of betalamic acid and cyclo-Dopa 5-*O*-2′-*O*-(β-glucuronic acid)-β-glucose (*5*). When subjected to 50 °C or above, betacyanins can be degraded, hydrolyzed, and transformed (e.g., isomerization) to betalamic acid, various amines, and other products such as cyclo-Dopa 5-*O*-2′-*O*-(β-glucuronic acid)-β-glucose through the hydrolytic cleavage of the aldimine bond, and deglycosylation can be generated at high temperatures (*6*). The stability and the balance of degraded and transformed products can be influenced by factors such as pH, water activity, and food matrix where the pigments were added (*19*). Different levels of diverse degraded and transformed products might be achieved due to the different food matrix systems (i.e., different types of flours used). These three flours significantly differed in their protein composition (protein contents (7.8−13.6%) and wet gluten index (20−40)), which resulted in varied textural properties (**Table 2**) in the suspensions during heating or resulted in gels during storage. *Amaranthus* betacyanins themselves and the degraded product amines often contain several hydroxyl groups and two glucose moieties. These two glucose moieties could be released into solution during heating (*5, 6*). The hydroxyl groups from various additives can be important to gelatinization properties of starch and could increase gelatinization temperature (*20*). Addition of glucose and sucrose to wheat starch increased both gelatinization temperatures and endothermic enthalpies (*13*). The stabilizing effect of betacyanins and the related polyhydroxy compounds from their degradation and transformation on starch gelatinization could be attributed to either competition for water between starch and polyhydroxy compounds by altering water activity or to interaction between polyhydroxyls and starch chains to stabilize the amorphous regions of starch granules known as the sugar-bridge effect or plasticization (*20, 21*). It was also proposed that increased enthalpies might be partially explained as some extra crystallization has been formed by specific interaction between wheat starch and hydroxyl groups (*20, 22*) of betacyanins or its degraded and transformed products.

However, *Amaranthus* betacyanins and their degraded and transformed products possess the betalamic acid moiety (**Figure 1**); thus, the betalamic acid and betanidins degraded from betacyanins might acidify the flour suspension. Acid-thinned starch has been reported to show an altered gelatinization behavior (*12, 13*). Also, it was reported that the thermal properties of starch were pH dependent (*23*). Acid attacks both amylose and amylopectin during the early stages of lintnerization but tends to hydrolyze the amorphous sections of the starch granules, resulting in a relative higher crystallinity (*12, 23−25*). Amorphous regions of starch granules play an important role in the thermodynamics of gelatinization (*12*). Thus, higher temperatures would be required to break down the starch granules, resulting in enhanced DSC gelatinization temperatures. Therefore, the effect of *Amaranthus* betacyanins on thermal properties of wheat flours might be the combination of polyhydroxyl groups and acid-thinning caused by acid molecules. The increased or decreased enthalpies thus could be determined by which of these two factors dominates in the system.

Pasting of Wheat Flours. Addition of betacyanins from two *Amaranthus* species significantly ($P = 0.05$) influenced the pasting behavior of all wheat flours to various extents with a similar trend (**Table 3**). The pasting curves of wheat flours were very similar to that of wheat starch as previously observed, and the pasting and rheological properties of wheat flour were, to a large extent, determined by the starch in the flours (*26*). Among the native wheat flours, most pasting parameters showed considerable differences. For example, American Roses had the highest PV (418 RVU) compared to that of Red Bicycle and Golden Statue (388 and 357 RVU, respectively). Red Bicycle had the greatest CPV (398 RVU) followed by American Roses and Golden Statue (366 and 347 RVU, respectively). These differences could be ascribed to different chemical compositions of wheat flours such as the characteristics of wheat starch, proteins, lipids, and phosphorus present, and α-amylase activity, all of which affect the swelling and rheological behavior of wheat starch (*27, 28*).

In general, *Amaranthus* betacyanins decreased PV, HPV, CPV, SB, and PT of all the wheat flours and increased BD (**Table 3**). For example, betacyanins from *A. cruentus* decreased peak viscosities of American Roses, Red Bicycle, and Golden Statue by 45, 45, and 36 RVU, respectively, and decreased PT by 0.2 (statistically insignificant), 0.3, and 0.2 min, respectively. CPV of American Roses, Red Bicycle, and Golden Statue flour was dramatically decreased by betacyanins from *A. tricolor* by 105, 114, and 77 RVU, respectively. In general, betacyanins from *A. cruentus* caused greater effects than betacyanins from *A. tricolor*. For instance, the addition of betacyanins from *A. cruentus* increased the BD of American Roses by 34 RVU, greater than that from *A. tricolor* (25 RVU). *A. cruentus* betacyanins decreased the CPV of Golden Statue by 120 RVU, greater than that from *A. tricolor* (77 RVU).

When subjected to heat treatment in the presence of water and shear force, the starch in wheat flour swells with water molecules penetrating into starch granules and amylose leaching out of the granules, increasing the viscosity of suspension. On reaching a certain degree of swelling, the granules collapsed with a rapid reduction in the viscosity (*29*). It was reported that polyhydroxyl compounds could significantly alter the pasting properties of wheat starch and that their effects depend on the type and concentration (*13*). pH can also be important to pasting (*23*). Acid treatment could decrease both peak viscosity and peak time of rice starch (*12*). This is roughly in agreement with the influence of betacyanins on wheat flours in this study. Because of both the sugar (glucose) and acid moieties present in the molecule of amaranthin (**Figure 1**), the structural characteristics of *Amaranthus* betacyanins combine both the aspects of polyhydroxyl compounds and acids as stated in the DSC discussion session, changing the pH and interacting with macromolecles of amylose and amylopectin, thus resulting in altered pasting behaviors. Addition of glucose to wheat starch could increase the peak viscosity and cold paste viscosity because of the influence of sugar on the close packing concentration of swollen starch granules during heating and creating junction zones on amylose chains, facilitating the realignment of amylose during cooling when amylose molecules reorder themselves to start short-term retrogradation in the initial few hours (*11, 30*). However, it was reported that the pasting properties of starch or flour were pH dependent (*22, 31*). Furthermore, the slight erosion of the amorphous region of starch granules by acid molecules could result in weaker starch granules. Weaker granules are more deformable in shearing, thus resulting in reduced peak and cold paste viscosities (*13*). The effect of betacyanins thus might be a combination of the influences of both the sugar and acid and the effect of acid outweighs that of sugar in our situation.

Texture Profile Analysis (TPA). Addition of betacyanins affected the textural parameters of all wheat flour gels stored at 4 °C for 24 h (**Table 2**). For native flours, significant differences in textural parameters were observed except for cohesiveness. For example, American Roses showed the greatest hardness (29.4 g) and Golden Statue the lowest (22.3 g). These differences could be attributed to differences in chemical compositions of native flours (*26*).

Amaranthus betacyanin presence significantly decreased the hardness of all flour gels. The increase of gel hardness after pasting was mainly due to the retrogradation of gelatinized starch. The hardness reduction effects indicated that *Amaranthus* betacyanins might be used as a softening agent. Betacyanins from *A. tricolor* had the greatest effect on Red Bicycle (12.0 g) and the least effect on Golden Statue (6.4 g), whereas betacyanins from *A. cruentus* had the greatest effect on American Roses (16.0 g) and the least effect on Red Bicycle (9.1 g).

Table 4. Color Characteristics of Three Wheat Flours Gels with Betacyanins from *A. tricolor* (Tr35) and *A. cruentus* (Cr72) as Compared with Native Gels at 2 h Storage Time[a]

flours	pigment	L^*	a^*	b^*	C	H^o	ΔE^*
American Roses	control	60.7 A	−1.8 A	−0.8 A	2.0	202.8	
	Tr35	37.3 B	17.8 B	4.0 B	18.2	12.6	30.9
	Cr72	46.0 C	6.4 C	7.5 C	9.9	49.7	18.8
Red Bicycle	control	64.1 A	−1.7 A	1.0 A	2.0	150.0	
	Tr35	39.1 B	20.1 B	5.2 B	20.7	14.5	33.4
	Cr72	48.4 C	5.9 C	9.3 C	11	57.6	19.5
Golden Statue	control	63.9 A	−1.7 A	2.1 A	2.7	128.0	
	Tr35	38.8 B	20.3 B	4.9 B	20.9	13.5	33.5
	Cr72	48.2 C	6.7 C	10.0 C	12	56.3	19.4

[a] Different upper case letters indicate the significance of difference among mean values within each column of the same flour samples at $p < 0.05$.

Gumminess is mutually exclusive with chewiness (*32*) and is often employed to characterize the energy to disintegrate semisolid foods (chewiness is for solid products) and could nicely correlate to sensory evaluations by a trained texture profile panel (*33*). Since gumminess is dependent on hardness, similar influence trends were observed. The greatest reduction (6.8 g) was observed with *A. cruentus* betacyanins added into American Roses.

Adhesiveness is a surface property and depends on a combined effect of adhesive and cohesive forces, and other factors such as viscoelasticity (*34, 35*). Introduction of betacyanins from two *Amaranthus* species generally had little influence on adhesiveness of the wheat flour gels. However, adhesiveness exhibited the greatest standard deviations, compared with other parameters. This is consistent with previous reports employing TPA mode (*35, 36*).

Cohesiveness is a measure of the degree of difficulty in breaking down the gel's internal structure (*37*). Addition of two types of *Amaranthus* betacyanins to Golden Statue had little effect on its cohesiveness, but dramatically increased that of both American Roses and Red Bicycle. The increased cohesiveness indicated that more energy was required to break down the remaining gel matrix after the first bite, compared with the control, so that the gels may be perceived as being tough when chewed (*37*).

Color Development and Stability of Betacyanins from Two *Amaranthus* Species Applied in Three Wheat Flour Gels. Introduction of two *Amaranthus* betacyanin pigments at a concentration of 0.54% (w/w) imparted different color characteristics to different wheat flours (**Table 4**). Two hours after gel formation prior to storage, different flours gels treated with the same *Amaranthus* pigments exhibited similar color characteristics. All wheat flour gels treated with betacyanins from *A. tricolor* were red ($H^o = 12.6-14.5$), whereas all samples containing betacyanins from *A. cruentus* appeared similarly orange−yellow ($H^o = 49.7-57.6$). Gels with betacyanins from *A. tricolor* possessed higher color purity (more saturated due to higher betacyanin concentration) ($C = 18.2-20.9$) compared with that of *A. cruentus* ($C = 9.9-12.0$). Gels with betacyanins from *A. tricolor* appeared darker ($L^* = 37.3-39.1$) than those from *A. cruentus* ($L^* = 46.0-48.4$). The total color difference (ΔE^*) between the each control and its colored samples was similar. ΔE^* values between the native and flour gels treated with betacyanins from *A. tricolor* were higher ($\Delta E^* = 30.9-33.5$) than that between the control and the *A. cruentus* treated ones ($\Delta E^* = 18.8-19.5$), indicating higher efficiency of total coloring by *A. tricolor* pigments. Lower concentration of betacyanins could be more easily degraded and hydrolyzed

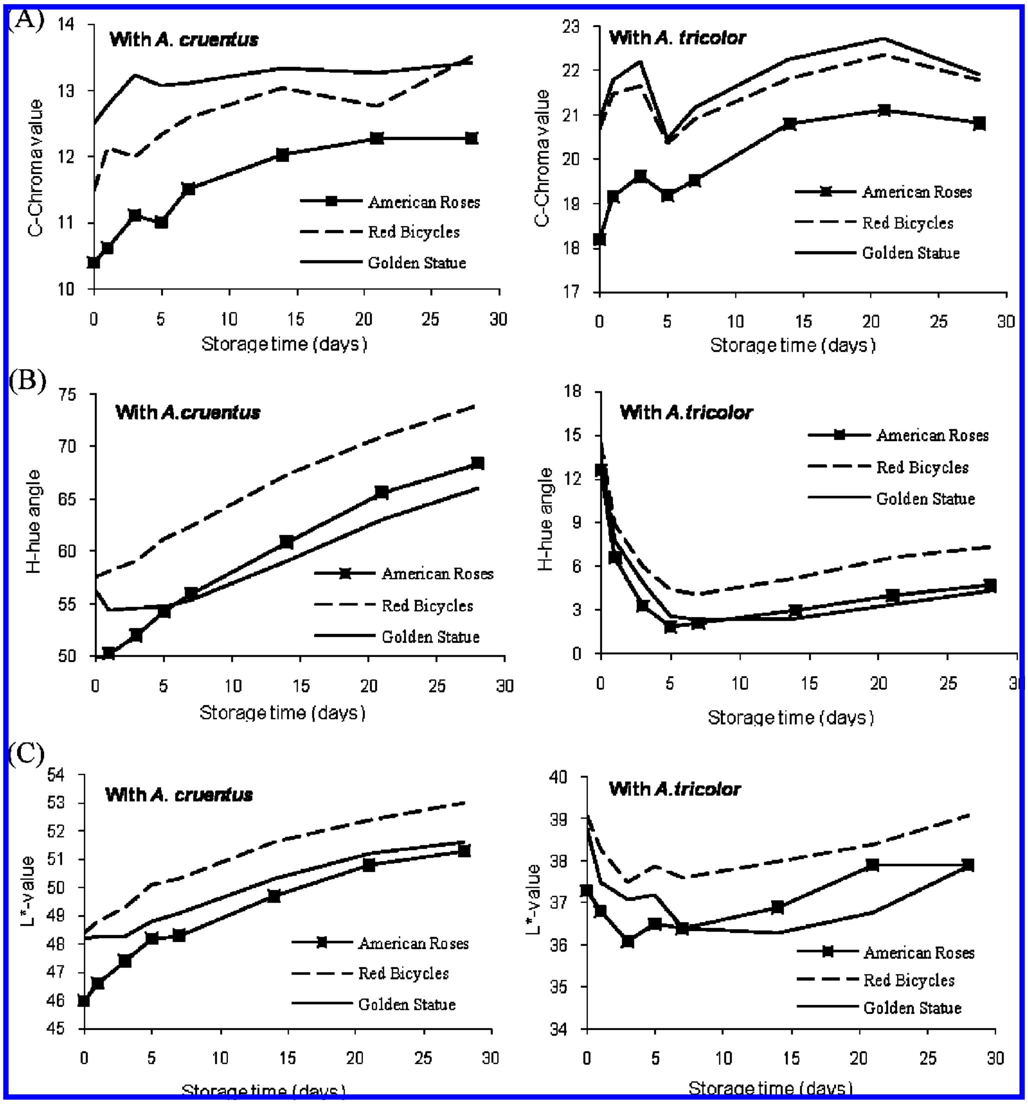

Figure 2. Color stability (C, H^o, L^*) of betacyanins from *A. tricolor* and *A. cruentus* applied in three different wheat flour gels during 4 weeks of storage at 4 °C in the dark. (**A**) Chroma value (C); (**B**) hue angle (H^o); (**C**) lightness (L^*).

during thermal treatment (*6*). Compared to *A. tricolor*, *A. cruentus* pigments had lower concentration of betacyanins (*5*). Sample gels treated with pigments from *A. cruentus* appeared yellowish ($H^o = 49.7-57.6$), with the higher L^*, indicating more bright yellow betalamic acid, and other degraded compounds might have dominated the color characteristics.

The color parameters of flour gels treated with *Amaranthus* pigments during 4-week storage were generally stable with some fluctuations (**Figure 2**). Similar trends were observed for gels of each flour sample treated with the same pigment. In general, C increased gradually in the first few days, with a drop at around day 5, and then peaked at week 3 for *A. tricolor*, or later for *A. cruentus* (**Figure 2A**). For gels treated with *A. cruentus* pigments, H^o increased gradually during the storage; for those with *A. tricolor* pigments, H^o dramatically decreased in the initial 5 days to greater redness and then slightly and gradually increased during the next 3 weeks of storage (**Figure 2B**). Trends of L^* appeared to combine the effects of H^o and C (**Figure 2C**).

Amaranthus betacyanins could regenerate to certain degrees in the first few hours after thermal degradation and hydrolysis, particularly at low temperatures and higher betacyanin concentration (e.g., cyclo-Dopa 5-*O*-2′-*O*-(β-glucuronic acid)-β-glucose and betalamic acid could recondense to form amaranthin in the water phase of flour gels) (*6, 38, 39*). For gels with *A. tricolor* pigments, the decreasing H^o and increasing C for the first few days indicated the partial regeneration of betacyanins. However, gels with *A. cruentus* pigments did not show such trends. This might be attributed to the lower concentration of degraded products of betacyanins dispersed in the matrix of flour gels. Food products treated with *A. tricolor* pigments may be stored for a few days for optimal betacyanin regeneration to achieve the desired tonality before consumption, whereas those with *A. cruentus* pigments should be consumed as soon as possible before losing color.

Despite some fluctuation of several color parameters during storage, the overall stability was good for the *Amaranthus* pigments applied in all the flours. Thus, *Amaranthus* betacyanins might be suitable for coloring wheat-based foods. Since food production and preservation often requires thermal treatment for the raw material to ensure food quality and safety, balance between heating conditions and coloring effectiveness should be well understood. These two *Amaranthus* pigments varied in the concentration of betacyanins and exhibited different coloring strength and storage stability. Therefore, optimization of pigment concentrations in food production could be easily realized to meet quality requirements.

In conclusion, *Amaranthus* betacyanins contribute both with acid and sugar moieties. On heating, betacyanins could degrade into betalamic acid and various amines containing glucose and betalamic acid. The effect of betacyanins on wheat starch thus could be the combined effects of both the acids and polyhydroxyl compounds. These two factors may be either synergistic or antagonistic depending on reaction conditions and the food matrix. In general, the addition of *Amaranthus* betacyanins generally delayed the gelatinization of wheat flours without altering the gelatinization transition ranges, whereas both increases and decreases of melting enthalpies were recorded. *Amaranthus* betacyanins significantly decreased the peak viscosity, hot paste viscosity, cold paste viscosity, and pasting time of all wheat flours, and significantly softened the gels. *Amaranthus* betacyanins could contribute to useful red color to wheat flour gels, with good color stabilities during a 4-week storage test. However, other components present in the flours such as protein and lipids may also contribute to the interactions. The effect of concentrations of pure betacyanins on physical properties and color of wheat flours is worth further investigation for its commercial potential. Additionally, since betacyanins can greatly benefit human health because of the antioxidant properties and red-colored foods may have potential markets, this study may provide the basis for the production of colored wheat-based foods using *Amaranthus* betacyanins as functional ingredients.

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