JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Anthocyanin-Rich Waste from Purple Corncobs (*Zea mays* L.) and Its Application to Color Milk

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Pigment production from anthocyanin-rich purple corncobs generates a deeply colored waste precipitate. Our objectives were to characterize this anthocyanin-rich waste (ARW) and to find a suitable application in a food matrix. Composition and solubility characteristics of ARW were evaluated. Color (CIELAB) and pigment (monomeric anthocyanin and HPLC profiles) stability of ARW in milk (35 mg/100 mL) were evaluated using an accelerated test at 70 °C and phosphate buffer as a control. ARW provided milk an attractive purple hue (324−347°). Monomeric anthocyanin degradation followed zero-order kinetics in skim and whole milk and second-order kinetics in the control, with half-lives of 173, 223, and 44 min at 70 °C, respectively. ARW shows potential as a natural colorant for a pH range unusual for anthocyanin applications. A protective effect of matrix constituents on the stability of anthocyanins was evident. Anthocyanins may interact with different compounds in biological systems when the pH values are close to neutral.

KEYWORDS: Solubility; anthocyanins; macromolecule; phenolics; kinetic

INTRODUCTION

Anthocyanins are the largest and most important group of water soluble pigments in nature, contributing to the attractive orange, red, purple, violet, and blue colors of fruits, vegetables, and flowers. Anthocyanins, which have been consumed for many years without any apparent adverse effects, have bright pH-dependent color (*1*). Interest in anthocyanins has increased due to its color characteristics and health benefits as a promising alternative as a food colorant (*2*). Close to 25% of the population perceives foods without artificial ingredients as desirable, making this very important in their food and beverage purchase decisions (*3*). Recently, anthocyanins have been reported to have various biological activities such as antioxidant (*4*−*7*), antimutagenic (*8*, *9*), and chemopreventive (*10*, *11*), contributing to a reduced incidence of chronic diseases. Researchers have shown that an anthocyanin-based food colorant from purple corn inhibited induced cell mutation (*12*), reduced chemically induced colorectal carcinogenesis (*13*), and contributed to the prevention of obesity and diabetes (*14*).

For centuries, anthocyanin-rich purple corn (*Zea mays* L.) has been cultivated in South America, mainly in Peru and Bolivia, and used to prepare drinks and desserts. A colorant from purple corn is widely used in Asia, South America, and Europe. Cyanidin-3-glucoside is the major anthocyanin in purple corncob (*15*, *17*), although pelargonidin and peonidin glucosides have also been found in maize plants (*16*) as well as their respective malonyl derivatives (*17*).

Large quantities of anthocyanin-rich waste (ARW) are generated during the preparation of commercial purple corn colorant, which are obtained by an easy and economic hot acidified water extraction procedure. This waste has very limited applications in foods due to low solubility in acidified aqueous systems.

Anthocyanin pigments have been traditionally used to provide color to acidic food systems (usually pH below 3) such as fruit beverages and jams. However, our preliminary studies suggested that ARW would be water soluble at a pH near to neutral suggesting that it could find a potential application in foods with a pH level close to neutral such as milk. Nontraditional milk products such as chocolate milk (27%), strawberry (60%), and other flavored milks lead the consumption of school-vended milk, whose market potential is estimated at 130 million 16 ounce (0.47 L) plastic single servings of milk per year (*3*). Therefore, there is a market for natural alternatives to the use of artificial dyes that could provide desired color, stability, and value added for these applications. This alternative must be cost effective, and the use of a waste material, such as ARW, would be ideal.

Our objectives were to characterize the purple corncob ARW generated during purple corn color production and to find a suitable food application. In this study, we evaluated pigment and solubility characteristics of ARW, tested its potential as a natural colorant, and explored the potential interactions between anthocyanins and matrix constituents.

MATERIALS AND METHODS

ARW powder from purple corncob (*Z. mays* L.) was donated by Globenatural International S.A. (Chorrillos-LIMA, Peru). This waste

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10.1021/jf051247o CCC: $30.25
Published on Web 09/30/2005

was obtained by sedimentation and spray drying of the water insoluble portion from anthocyanin extracts of purple corncob.

An ARW stock suspension was prepared by mixing 0.5 g of ARW powder with 50 mL of 0.01% HCl aqueous solution. An ARW stock solution was prepared by mixing 0.5 g of ARW powder with 50 mL of pH 8 phosphate buffer.

Anthocyanidin standards were prepared by acid hydrolysis (*18*) of concentrated anthocyanin extracts from red radishes (pelargonidin) and concord grapes (delphinidin, cyanidin, petunidin, peonidin, and malvidin).

Skim milk (8 g protein, 0 g fat, and 12 g carbohydrate per 240 mL serving) and whole milk (8 g protein, 8 g fat, and 11 g carbohydrate per 240 mL serving) (Richfood, Inc., Richmond, VA) were purchased from a local market.

Folin and Ciocalteau phenol reagent and a standard of gallic acid (crystalline gallic acid, 98% purity) were purchased from Sigma (St. Louis, MO). A Pierce BCA (bicinchoninic acids) protein assay kit was purchased from Fisher Scientific (Fair Lawn, NJ). All high-performance liquid chromatography (HPLC) grade solvents and other chemicals (analytic grade) were from Fisher Scientific.

Solubility of ARW. The solubility of ARW was evaluated in different solutions. The ARW stock suspension was mixed thoroughly with different solutions. Precipitated material was an indicator of lack of solubility, and haze was used as a measure of insoluble material suspended in the liquid. Haze was determined immediately after 30 s of homogenization as described below.

Solubility at Different Ethanol Concentration. The ARW stock suspension (0.5 mL) was added into 9.5 mL of different aqueous ethanol solutions with 0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5, and 100% ethanol (v/v) to a final concentration of 0.5 mg/mL based on ARW dry powder. The haze was then measured as described below.

Solubility at Different pH Aqueous System. A series of buffers were prepared as follows: pH 1 potassium chloride buffer (0.025 mol/L), pH 3 sodium citrate-chloride buffer (0.1 mol/L), pH 3.5 sodium citrate-chloride buffer (0.4 mol/L), pH 4 sodium acetate buffer (0.1 mol/L), pH 4.5 sodium acetate buffer (0.4 mol/L), pH 6 sodium-citrate buffer (0.1 mol/L), pH 7 phosphate buffer (1/15 mol/L), and pH 8 phosphate buffer (1/15 mol/L). The ARW stock suspension (0.5 mL) was added to tubes containing 5 mL of buffer and mixed reaching a final concentration of 0.91 mg ARW/mL buffer. Haze was determined as described below. Samples were centrifuged at 2000 rpm and 4 °C for 10 min in a Beckman J2-21M centrifuge (Beckman Coulter, Inc., Fullerton, CA). The monomeric anthocyanin content and polymeric color were monitored.

Monomeric Anthocyanins and Polymeric Color. The total monomeric anthocyanin content was measured by the pH differential method (*19*). A Shimadzu UV−visible spectrophotometer (Shimadzu Corporation, Tokyo, Japan) was used at 420, 510, and 700 nm. Monomeric anthocyanins were calculated as cyanidin-3-glucoside, using the extinction coefficient of 26900 L cm^{-1} mg^{-1} and a molecular weight of 449.2 g/L. Disposable cuvettes of 1 cm path length were used. Color density and polymeric color were calculated using absorption at 420, 510, and 700 nm before and after bisulfite treatment and used as an index of browning (*19*).

Total Phenolics. Total phenolics were measured using a modification of the Folin−Ciocalteu method for total phenols (*20*). The absorbance of the samples and standards was measured at 755 nm. Total phenols were calculated as gallic acid equivalents based on a gallic acid standard curve.

Protein Analysis. The total protein concentration was measured on a Shimadzu UV−visible spectrophotometer using the Pierce BCA (bicinchoninic acids) protein assay kit. The absorbances at 562 nm of samples, standards of bovine serum albumin (BSA), and controls (samples with deionized distilled water instead of BCA reagent B) were measured. Total protein was quantified as BSA equivalents.

Alkaline and Acid Hydrolysis of Anthocyanins. The ARW suspension (1 mL) was saponified in a screw-cap test tube with 10 mL of 10% aqueous KOH for 8 min at room temperature in the dark (*18*, *21*). The solution was neutralized and acidified by HCl (2 mol/L), and hydrolysate was purified using a C-18 Sep-Pak cartridge (1 g, Waters Corp., Milford, MA) as described by Giusti et al. (*21*).

Anthocyanidins from ARW were obtained by mixing 1 mL of ARW suspension with 10 mL of HCl (2 mol/L) in a 20 mL screw-cap test tube. Tubes were placed in boiling water for 30 min and then cooled in an ice bath. The hydrolysate was purified with a C-18 Sep-Pak cartridge as previously described (*21*).

Analytical Chromatography. An HPLC system (Waters Delta 600 systems) equipped with a photodiode array detector (Water 996), autosampler (Waters 717 plus), and Millennium^{32} software (Waters Corp.) was used. Columns and mobile phase: The reversed phase 5 μm Symmetry C18 column (4.6 mm × 150 mm, Waters Corp.) fitted with a 4.6 mm × 22 mm Symmetry 2 microguard column (Waters Corp.) was used. The solvents used were A, 1% phosphoric acid/10% acetic acid/5% acetonitrile in water, and B, 100% acetonitrile. Solvents and samples were filtered through 0.45 μm poly(tetrafluorothylene) membrane filters (Pall Life Sciences, Ann Arbor, MI) and 0.45 μm polypropylene filters (Whatman Inc., Clifton, NJ), respectively. Separation was achieved by using a linear gradient from 0 to 30% solvent A in 35 min. An injection volume of 50 μL with a 1 mL/min flow rate was used. Spectral information over the wavelength range of 260−600 nm was collected.

Monomeric Anthocyanins, Total Phenolics, and Proteins from ARW in pH 3 Aqueous Environment. ARW stock suspension (10 mg/mL) was diluted by 10 times with pH 3 sodium citrate-chloride buffer. Diluted suspensions (5 mL) were centrifuged at 2000 rpm and 4 °C for 10 min. The pellet was redissolved and taken to 5 mL with pH 8 phosphate buffer. The supernatant was taken to 5 mL with pH 3 sodium citrate-chloride buffer. Monomeric anthocyanins, total phenolics, and proteins were measured on both the supernatant and the redissolved precipitate portions.

Heat Stability in Dairy Products. Purple corn ARW was used to color skim milk and whole milk. Also, pH 6.8 phosphate buffer colored with ARW was used as a control, to match the pH of the milk samples (pH determined with an Accumet pH-meter 25, from Fisher Scientific). Color and pigment stability were evaluated using an accelerated stability test at a temperature close to typical pasteurization temperature (70 °C). The ARW stock solution was mixed with matrices to reach a final concentration of 35 mg ARW/100 mL (containing 3.7 mg monomeric anthocyanins or 44.2 mg total phenolics per 100 mL matrix). A series of 45 test tubes were filled with 20 mL of either pH 6.8 buffer, skim milk, or whole milk (15 test tubes for each matrix), closed with screw caps, and covered with aluminum foil. The tubes were then immersed in a water bath at 70 ± 0.1 °C for 120 min with a shake speed of 80 rpm on a reciprocal shaking bath (Precision, Winchester, VA). Nine tubes (three replicates for each matrix) were collected at regular time intervals (0, 30, 60, 90, and 120 min) and rapidly cooled to room temperature for color analysis.

Color and Haze Analyses. Hue angle, chroma, L^* (CIELAB), and haze were measured with a Hunter ColorQuest XE colorimeter (HunterLab, Hunter Associates Laboratories Inc., Reston, United States) using illuminant C and 10° observer angle. For color measurements of milk, samples were placed in 1 cm path length disposable cuvettes and read using the reflectance specular included mode and covered with light trap while controls were put in the transmittance compartment and measured using total transmittance. For haze measurements, samples were homogenized for 30 s on a Vortex, placed in 2 mm path length disposable cuvettes, and measured using the transmission mode and haze (total transmission and relative transmission).

Anthocyanin Recovery and Purification from Milk. Anthocyanins were recovered from milk by using acidified methanol. Each colored milk (5 mL) was transferred into a Waring Laboratory Blender (New Hartford, CT), mixed with 10 mL of 0.1% acidified methanol for 3 min at low speed and then for 2 min at high speed. The mixture was transferred to a 50 mL chloroform resistant centrifuge tube. Then, 15 mL of 0.1% HCL acidified methanol was used to wash the blender and combined with the previous extract. Chloroform (20 mL) was added into the tubes, mixed well, and centrifuged at 27200*g* and 4 °C for 10 min in Beckman J20-MI (Beckman Coulter, Inc.). The top aqueous phase containing anthocyanins was transferred to a round bottom flask. Residual methanol/chloroform was removed in a rotary evaporator at 40 °C under vacuum. The remaining aqueous extract was made up to 5 mL with acidified deionized distilled water and centrifuged at 14000

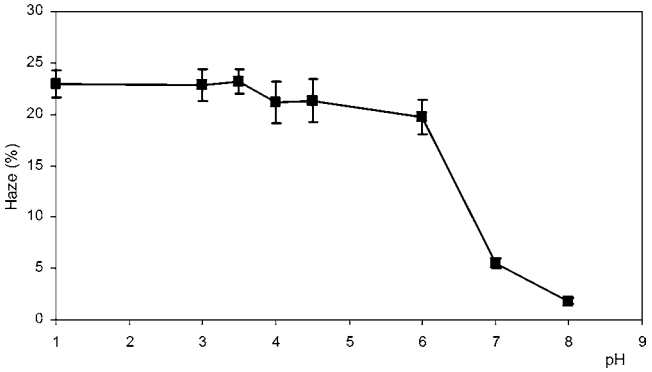

Figure 1. Solubility of ARW at different pH levels (0.91 mg ARW/mL aqueous solution).

rpm and 4 °C for 10 min in an eppendorf centrifuge 5415C (Brinkmann Instruments, Inc., Westbury, NY). The supernatant was refrigerated at 4 °C until analyzed.

Statistical Analysis. The accelerated stability test was carried out according to a completely randomized design (CRD) with three replicates for treatment groups and control. Statistical analysis was done by using the SAS/STAT package (version 8.1, 1999, SAS Institute Inc., Cary, NC). Analysis of variance and PROC MIXED procedure were conducted. Values of $p < 0.05$ were considered significant. Rates for anthocyanin degradation were obtained from linear regression analysis (95% confidence interval).

RESULTS AND DISCUSSION

Solubility Analysis. Solubility characteristics of colorants are very important since they determine the potential application in food matrices. Anthocyanins, water soluble pigments, can usually be used to color acidic foods such as drinks, jams, and jellies. ARW, a byproduct of anthocyanin extraction from purple corncob, showed poor solubility in acidic aqueous systems, maybe due to other compounds bound to anthocyanins. Therefore, evaluation of its solubility characteristics was necessary to evaluate potential applications in food matrices.

The solubility of ARW was different in aqueous solutions of different pH levels (**Figure 1**). ARW showed very limited solubility at low pH value while it was water soluble at pH close to neutral or above. The solubility characteristics of the ARW were very similar to those reported for complexes of protein and tannins. Hagerman and Butler (*22*) reported the formation of tannin−protein complexes that precipitated in a pH-dependent manner. The lowest solubility of the complex was observed within one pH unit of the isoelectric point of the protein (*22*), contradicting an earlier statement by Loomis and Battaile (*23*) that the ability of tannins to precipitate proteins was pH-independent below pH 8 (*23*). These interactions are essentially a dynamic surface phenomenon, generally reversible, which involves hydrophobic effects and hydrogen bonds (*24*, *25*). The tannin's hydroxyl group is an excellent hydrogen donor that can form strong hydrogen bonds with the protein's carboxyl group. Phenolic groups of tannins are ionized and are unavailable for hydrogen bonding at high pH (usually more than 8). In addition, proteins have net negative charges so that precipitates do not occur because proteins exhibit repulsive forces (*23*). Conformation and charge of proteins have an effect on the formation of tannin−protein complexes. Conformationally open proteins, which have a high ratio of proline, readily form complexes with tannins (*26*). The strength of the interactions depends on the nature of both protein and tannin molecule. Tannins have been associated with antinutritional factors in the diet, by decreasing the digestion rate of dietary proteins (*27*).

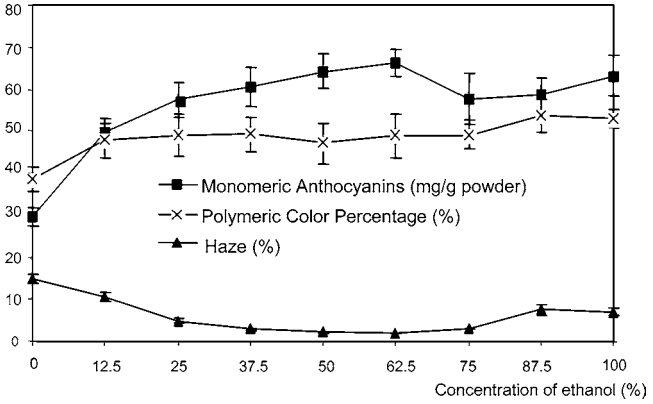

Figure 2. Monomeric anthocyanins, polymeric color, and haze of ARW at different concentrations of aqueous ethanol (0.5 mg ARW/mL).

However, recent studies have found that tannins may counteract these adverse effects by inducing levels of some digestive proteases, lipases, and billary acid (*28*, *29*). Further studies would be needed to elucidate the effects of these interactions on the bioavailability of ARW constituents.

Anthocyanins are highly unstable at pH levels of 5−6, and the color fades rapidly at those pH ranges. However, some acylated anthocyanins have shown high stability and color intensity at pH 7−8 (*30*), suggesting that it may be possible to use them as colorants in food applications in those pH ranges. The high solubility of purple corn ARW at pH close to neutral or above suggested that it was possible to use these waste materials as colorants for food systems around neutral pH.

The solubility characteristics of ARW changed but not proportionally to the ethanol concentration (**Figure 2**). The ARW solubility was higher in a 50 to ~62.5% (v/v) ethanol solution with a final concentration of 0.5 mg ARW/mL in aqueous. The total monomeric anthocyanins concentration was highest (about 66.2 mg/g based on ARW dry powder), while haze was lowest (about 1.9%) when the concentration of ethanol is 62.5% (**Figure 2**), suggesting that anthocyanins had formed complexes with macromolecules that were soluble in a 50% aqueous ethanol environment.

A degradation index of pigments was determined by the ratio between polymerized colored anthocyanin−tannin complexes and color density (*19*). It was very interesting the way that polymeric anthocyanin concentration changed with the increase of ethanol concentration in the solvent (**Figure 2**). The solubility of polymerized colored anthocyanin−tannin complexes may have increased with higher ethanol concentration. When the ethanol concentration in solution increased from 0 to 12.5%, the percent polymeric color increased sharply. However, when ethanol concentration continued to increase from 12.5 to 37.5%, both monomeric anthocyanin and polymerized colored anthocyanin−tannin complexes increased, keeping the percent polymeric color unchanged. The ethanol concentration of 50% exhibited the lowest haze level (1.9%). At this concentration, large quantities of monomeric anthocyanin were released from the complexes so that the percentage of polymeric color slightly decreased. When the ethanol concentration changed from 50 to 100%, the solubility of tannin−proteins decreased trapping more monomeric anthocyanins, resulting in an increased percentage of polymeric color.

Monomeric Anthocyanins, Total Phenolics, and Proteins from ARW in pH 3 Aqueous Environment. The limited solubility of ARW in water was hypothesized to be associated with complexation reactions between anthocyanins and macromolecules present in purple corncob. Therefore, the distribu-

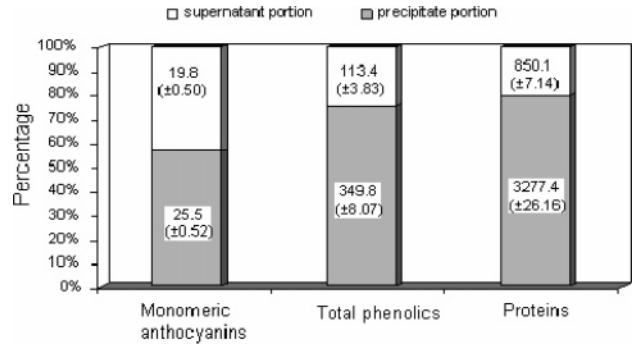

Figure 3. Percentage of monomeric anthocyanins, total phenolics, and proteins in the supernatant and precipitate portions of ARW suspension at pH 3. Monomeric anthocyanins, total phenolics, and proteins were measured as the equivalents of cyanidin, gallic acid, and BSA (mg/L), respectively, and numbers are means of three replications. In parentheses are standard deviations.

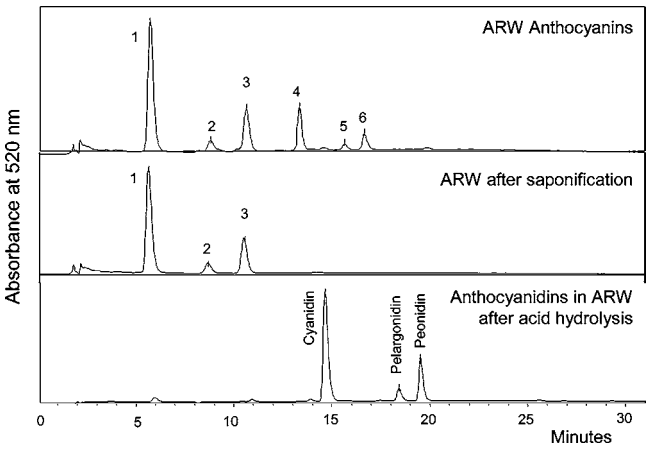

Figure 4. HPLC profiles of anthocyanins in ARW before and after saponification and acid hydrolysis. Key: 1, cyanidin-3-glucoside; 2, pelargonidin-3-glucoside; 3, peonidin-3-glucoside; 4, cyanidin-3-(6″-malonylglucoside); 5, pelargonidin-3-(6″-malonylglucoside); and 6, peonidin-3-(6″-malonylglucoside).

tion of monomeric anthocyanins, total phenolics, and proteins in the supernatant and precipitate portions at pH 3 aqueous environment was investigated.

The monomeric anthocyanins, total phenolics, and protein were distributed in the supernatant portion by 45, 25, and 20%, while they were 55, 75, and 80% in the precipitate portion (**Figure 3**). Combined with the above information on solubility of ARW at different pH values, it suggested that the precipitates in the ARW are rich in the anthocyanin complexes with macromolecules such as protein and polyphenolics. Hagerman and Butler (*22*) pointed out that the low molecular weight phenolics, such as catechin, could bind nonspecifically to the protein−tannin complex (*22*). Anthocyanins, with a similar structure to catechin, possibly contributed to the nonspecific binding to the protein−tannin complex.

HPLC Profiles. The HPLC profiles of ARW were analyzed before and after acid and alkaline hydrolysis (saponification). With published literature (*15*−*17*), six major anthocyanins were identified and shown in **Figure 4**. Three different anthocyanidins (cyanidin, pelargonidin, and peonidin) were found in purple corn, based on the comparison of the anthocyanidins profiles from purple corn and the ones from red radish (*31*) and concord grape (*18*). Cyanidin, pelargonidin, and peonidin in ARW were 69.9, 8.3, and 21.8% of the total peak area at 510 nm, respectively, all glucosylated and present in the acylated and nonacylated forms. The acylating group was reported as malonic acid (*17*), consistent with our data of long retention times of peaks 4−6 but no absorption peak in the 320−360 nm range, typical of cinnamic acid acylation.

Table 1. Color Properties of Matrices Colored with ARW Heated at 70 °C for Different Times[a]

matrix	time (min)	L^*	C^*	h^*	a^*	b^*
pH 6.8 buffer	0	38.3	31.5	29.4	27.5	15.5
	30	48.2	41.2	46.9	28.1	30.1
	60	50.1	43.5	45.6	30.4	31.0
	90	51.0	44.4	51.1	27.9	34.6
	120	51.6	45.7	52.6	27.8	36.3
skim milk	0	56.2	4.0	324.4	3.2	−2.3
	30	59.9	4.2	4.9	4.2	0.4
	60	59.9	4.1	8.4	4.1	0.6
	90	61.2	4.7	10.7	4.7	0.9
	120	61.1	4.5	12.7	4.4	1.0
whole milk	0	65.1	4.1	348.0	4.0	−0.9
	30	67.8	4.7	25.5	4.3	2.0
	60	68.4	4.9	26.7	4.4	2.2
	90	68.1	4.9	28.8	4.3	2.3
	120	68.7	4.9	27.6	4.3	2.3

[a] L^*, lightness; C^*, chroma or saturation $(a^2 + b^2)^{1/2}$; and h*, hue angle, $\tan^{-1}(b^*/a^*)$.

Application in Milk. Milk samples colored with ARW presented an attractive purple hue while the whole milk exhibited a lighter purple color than skim milk. The white color background of milk changed the color appearance of anthocyanins in milk. We chose an accelerated stability test, with a temperature close to that used for typical pasteurization of milk. Application of ARW in dairy products and interaction of anthocyanins with food components were studied with heat treatments at 70 ± 0.1 °C for different times (0, 30, 60, 90, and 120 min). The skim milk and whole milk were studied as matrices in the experiment. The control was pH 6.8 buffer phosphate solution.

Changes in color of the samples revealed that anthocyanins were more stable in the milk matrices than in the control (**Table 1**). Lightness in skim milk or whole milk was higher than the corresponding L^* in the control at time zero. That may be due to the covering of the natural color of milk. Lightness (L^*) values increased from $L^* = 38.29$ (time zero) to $L^* = 51.56$ (after 120 min) in the pH 6.8 buffer, faster than in skim milk and whole milk during the heat treatment, showing that the color in pH 6.8 buffer faded faster than in either of other two matrices. Chroma (C^*) was monitored during storage as an indicator of changes in color saturation during heat treatment. The chroma of the pH 6.8 buffer was highest among three matrices mainly because the white color contribution of milk components counteracted the intensity of purple color. The chroma in skim and whole milk changed slightly with a 20 and 19% increase, respectively, as compared to the 45% increase in pH 6.8 buffer after treatment. The increase of hue angle (h^*) in the control indicating a shift in color from a red ($h^*_{0\text{min}} = 29.37°$) to an orange/brown red ($h^*_{120\text{min}} = 52.61°$) was possibly due to the occurrence of browning reactions with anthocyanins degradation during heat treatment. The h^* value of skim and whole milk shifted from purple ($h^*_{0\text{min}} = -35.65°/-12.01°$) to red ($h^*_{120\text{min}} = 12.65°/27.58°$), a more commercially desirable color over the treatment period.

In **Figure 5**, the monomeric anthocyanin concentration in pH 6.8 buffer decreased sharply with time under heating conditions so that only 52.1% was remaining after heating at 70 °C for 30 min and decreased down to 30.0% after heat treatment for a

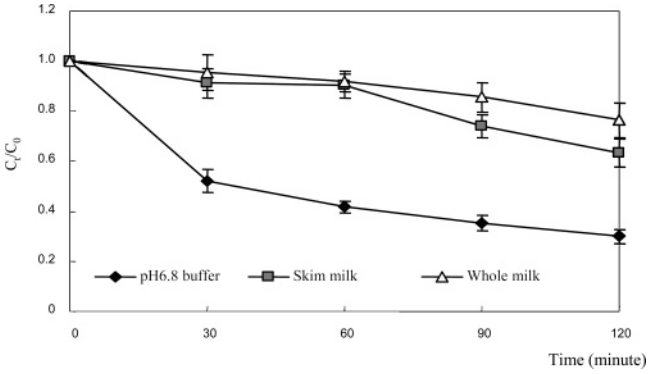

Figure 5. Stability of ARW in different matrices heated at 70 °C for different times. C_0 is the initial anthocyanins content; C_t is the anthocyanin content after t minutes of heating at 70 °C; C_0 and C_t are expressed as cyanidin-3-glucoside equivalents (mg/L).

total time of 120 min. The remaining anthocyanins in the skim milk were 90.3% after heating at 70 °C for 30 min and decreased to 63.4% after 120 min of heating. The whole milk seemed to protect anthocyanins very well. When heated at 70 °C for 30, 60, 90, and 120 min, the residual anthocyanins in the whole milk were 95.5, 92.0, 85.7, and 76.6%. All above suggested that fat in milk and other components such as proteins might protect anthocyanins from degradation during heat treatment. Anthocyanins intermolecular copigmentation with polyphenolics may increase the stability of anthocyanins (*32*, *33*). Anthocyanins in the milk system might form complexes with macromolecules that protect anthocyanins from degradation.

The statistical analysis showed that the anthocyanin retention greatly depended on treatment time ($p < 0.01$). Therefore, linear regression analysis was used to determine the rate of degradation of anthocyanins in each of the milk matrices as well as in the buffer solution. The equations and *R*-square values are presented below. All equations had a *R*-square value of 0.94 or higher, showing that most of the variability was explained by the model. Mean comparisons of the different anthocyanins colored matrices after 120 min of heat treatment revealed that retention of monomeric anthocyanins was significantly different between control (pH 6.8 buffer) and milk matrices at the 0.05 level by least significant difference test (LSD).

The monomeric anthocyanins content decreased during heating treatment at 70 °C (**Figure 5**) and change followed the zero-order kinetics (eqs 1 and 2) in skim milk and whole milk and second-order kinetics (eq 3) in the control. This indicated that anthocyanins degradation rate in skim milk and whole milk (eqs 1 and 2) did not depend on substrate concentration, and as the concentration of anthocyanins decreased, the rate of degradation did not decrease. However, the anthocyanin degradation rate in pH 6.8 control (eq 3) depended on the concentration of anthocyanins. When the concentration of monomeric anthocyanins decreased, the rate of degradation decreased sharply. The equations that described the monomeric anthocyanins degradation in skim milk, whole milk, and pH 6.8 control with time during heating treatment are as follows:

For zero-order kinetics

$$\text{skim milk: } C_t/C_0 = -0.003t + 1.019,\ R^2 = 0.94 \quad (1)$$

$$\text{whole milk: } C_t/C_0 = -0.0021t + 0.969,\ R^2 = 0.96 \quad (2)$$

For second-order kinetics

$$\text{pH 6.8 buffer: } C_0/C_t = 0.019t + 1.180,\ R^2 = 0.97 \quad (3)$$

Table 2. Percentage of Anthocyanins Remaining in Different Matrices after Heat Treatment at 70 °C for 120 min[a]

	matrices		
anthocyanin	pH 6.8 buffer	skim milk	whole milk
Cy-3-glucoside	20.9 ± 0.26 a	64.8 ± 0.48 b	78.4 ± 0.60 c
Pg-3-glucoside	24.5 ± 0.20 a	72.3 ± 0.15 b	79.3 ± 1.3 b
Pe-3-glucoside	28.4 ± 0.38 a	71.5 ± 0.67 b	82.7 ± 0.31 c
Cy-3-(6″-malonylglucoside)	33.4 ± 1.2 a	84.3 ± 1.1 b	91.4 ± 0.62 b
Pg-3-(6″-malonylglucoside)	29.1 ± 1.9 a	84.8 ± 1.3 b	80.7 ± 3.3 b
Pe-3-(6″-malonylglucoside)	32.9 ± 0.52 a	106.3 ± 10.2 b	103.0 ± 4.70 b

[a] Cy, cyanidin; Pg, pelargonidin; and Pe, peonidin. Different superscript letters in the same row denote that the mean difference is significant at the 0.05 level by LSD (mean ± SE, $n = 3$).

where C_0 is the initial anthocyanins content (mg/L of cyanidin-3-glucoside equivalents) and C_t is the anthocyanin content (mg/L of cyanidin-3-glucoside equivalents) after t minutes of heating at 70 °C. The half-lives of monomeric anthocyanins ($C_t/C_0 = 0.5$) at 70 °C were 44, 173, and 223 min for pH 6.8 buffer, skim milk, and whole milk, respectively. Baublis and co-workers (1994) found that the degradation of tradescancia anthocyanins at room temperature and exposed to light showed linearity (zero-order reaction) (*34*). However, most studies on degradation kinetics of anthocyanins have indicated a first-order reaction for red radish anthocyanins (*35*), sour cherries anthocyanins (*36*), blackberry (*37*), concord grape, red cabbage, and ajuga anthocyanins (*34*). Labuza and Riboh pointed out that most quality-related reaction rates are either zero- or first-order reactions and statistical differences between the two types may be insignificant (*38*). The degradation rate of monomeric anthocyanins in close to neutral environment (pH 6.8) followed a second-rate reaction, which means that the degradation rate of monomeric anthocyanins changes sharply as compared to the first-rate reaction as the concentration of anthocyanins decreased. However, the degradation rate of monomeric anthocyanins in milk with the same neutral environment (pH 6.8) followed the zero-order kinetics, a slower reaction, suggesting that milk components protected anthocyanins from heat damage.

Further analysis was done with HPLC. The percentage of anthocyanins remaining in different matrices after heat treatment at 70 °C for 120 min is shown in **Table 2**. The concentration of every individual anthocyanin in pH 6.8 buffer decreased significantly faster than in the milk matrices during heat treatment ($p < 0.05$). The concentrations of individual derivatives of cyanidin, pelargonidin, and peonidin were not significantly different between the skim milk and the whole milk except for the cyanidin-3-glucoside and penonidin-3-glucoside ($p < 0.05$). Cyanidin-3-glucoside was most sensitive to the heat treatment with 20.9, 64.8, and 78.4% remaining after heat treatment in pH 6.8 buffer, skim milk, and whole milk, respectively. Generally, acylated anthocyanins were more resistant than the nonacylated for the heat treatment. Peonidin-3-(6″-malonylglucoside) was most resistant for heat treatment with minimal changes in skim milk and whole milk. The higher concentration of anthocyanins present in skim milk and whole milk after heating treatment as compared to control suggested that food components such as protein and fat might exert a protective effect over anthocyanins from heat degradation.

In conclusion, the limited solubility of ARW from purple corncob in acidified water was attributed to the formation of anthocyanin complexes with macromolecules such as protein and phenolics. However, ARW was easily solubilized at pH close to neutral or above, suggesting the use of these waste

materials as natural colorants for foods with pH values close to neutral.

Purple corn ARW was effective providing color to milk matrices. Milk components such as proteins and fats seemed to protect anthocyanins from degradation when exposed to heat. Acylated anthocyanins such as peonidin-3-(6″-malonylglucoside) were more resistant to heat treatment. The results obtained from this study suggest that it may be possible to use a byproduct of anthocyanin extraction from purple corncob to provide color to products with a pH range unusual for anthocyanin applications and to develop novelty products with new flavors, colors, and health benefits.

ABBREVIATIONS USED

ARW, anthocyanin-rich waste; HPLC, high-performance liquid chromatography; *L**, lightness; *C**, chroma; *h**, hue; BSA, bovine serum albumin.

ACKNOWLEDGMENT

Most of the experiments were carried out at the University of Maryland, College Park.

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Received for review May 27, 2005. Revised manuscript received August 5, 2005. Accepted August 18, 2005. We thank Globenatural International S.A. (Chorrillos-Lima, Peru) for providing samples and funding to carry out this research.

JF051247O